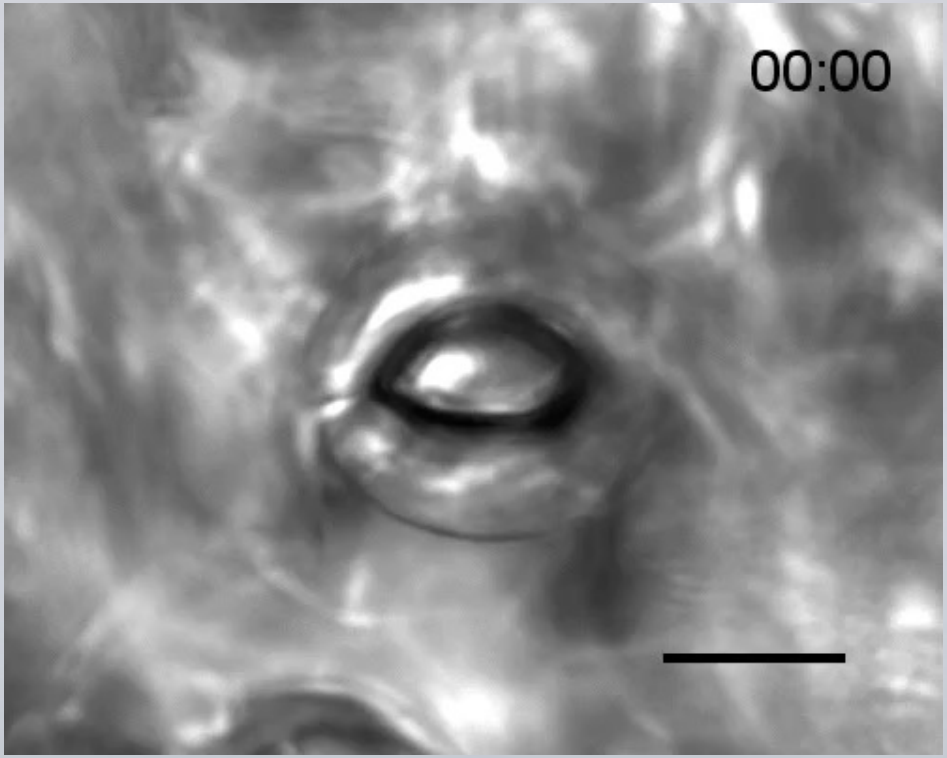
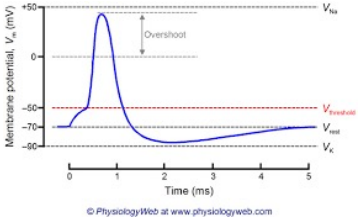
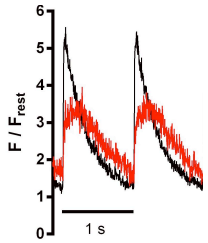
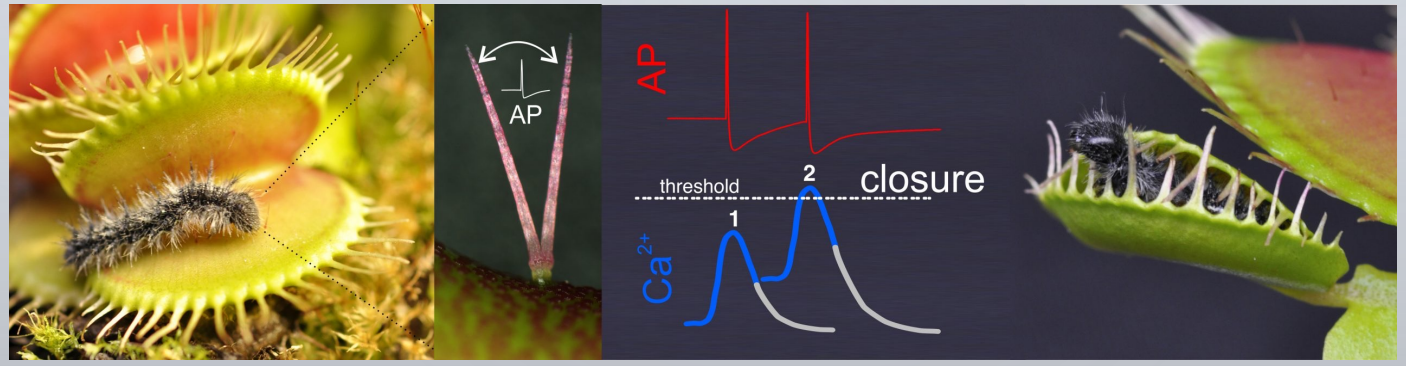
FOKUS Life Sciences

Single cell methods to study ion-transport in plant cells



Comparison of movements in animals and plants

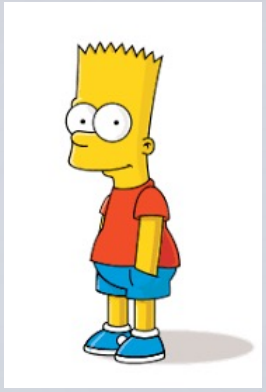
Species	Trigger	Electrical signal	Ca^{2+}-Signal	Movement
				



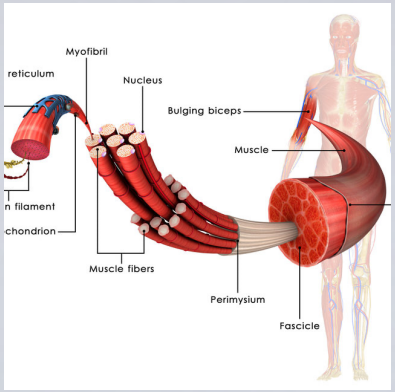
https://www.research-news.org/2020/10/05/how-the-venus-flytrap-counts/

Comparison of movements in animals and plants

Animals

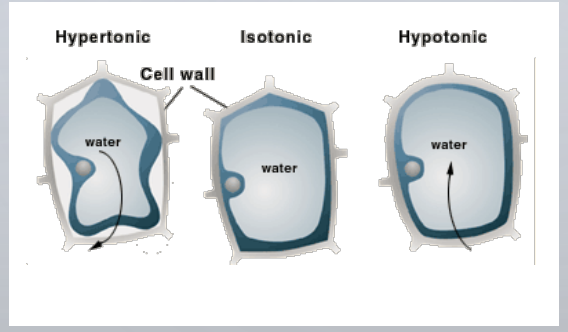


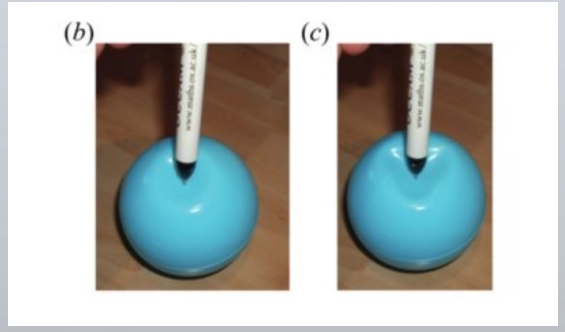
Muscle driven movement



Plants

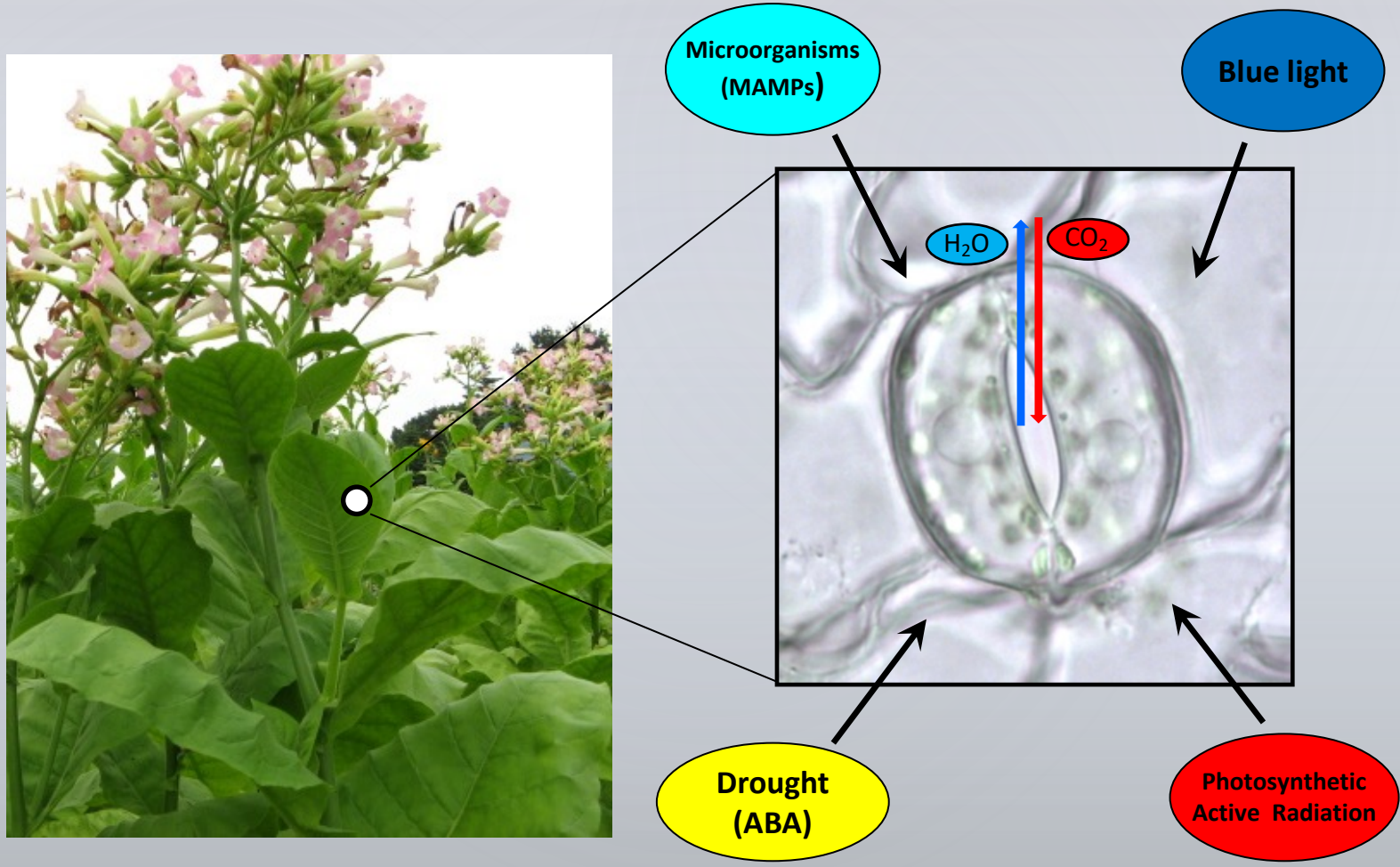
Osmotically driven movements ,or … Buckling driven movement



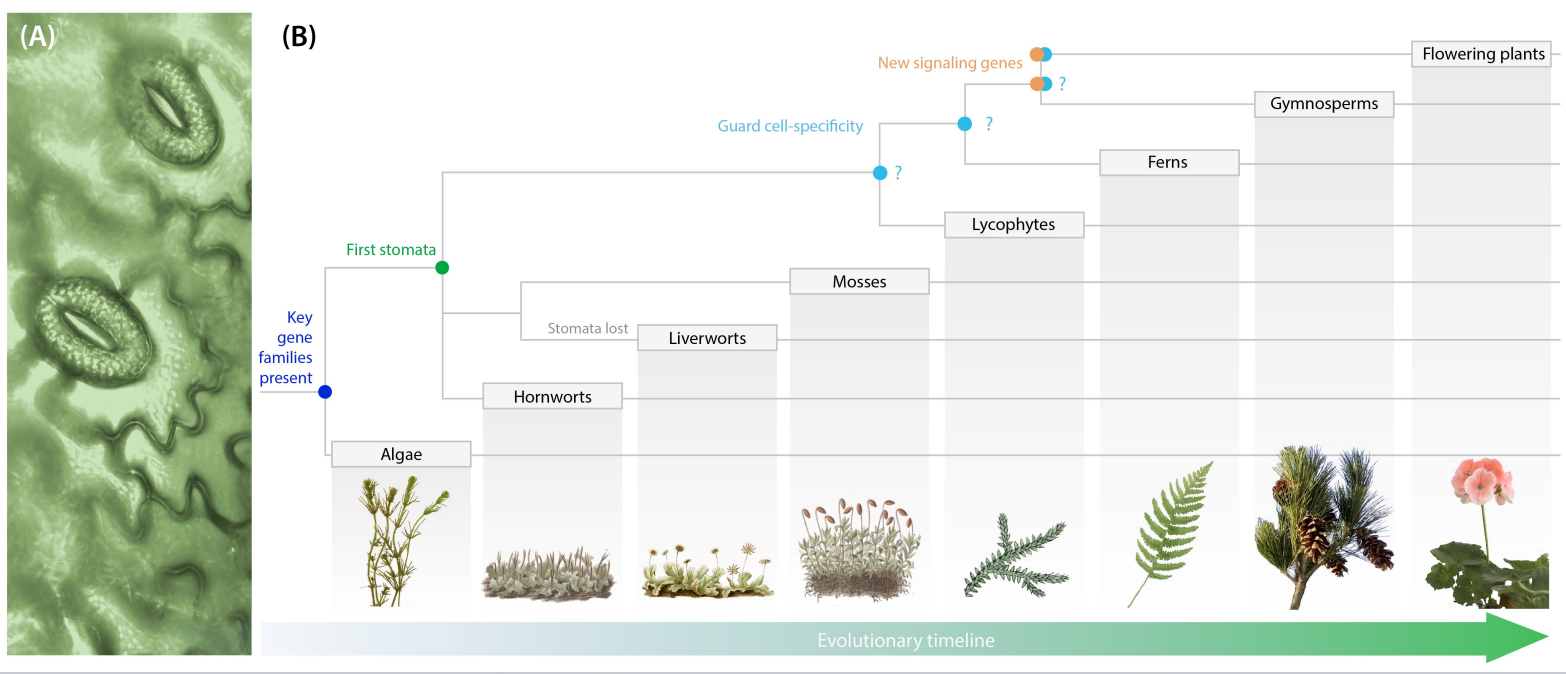


Light-induced stomatal opening in tobacco leaves

Stomata enable uptake of CO_2 and release of water vapor to the atmosphere



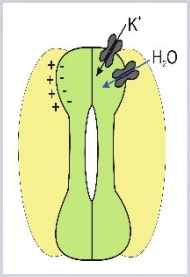
The development of stomata was important for land plant evolution



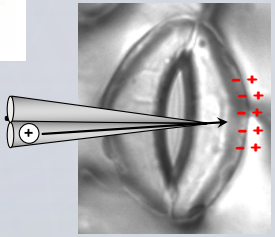
Light-induced stomatal opening in *Asplenium scolopendrium*

Control of stomatal movements

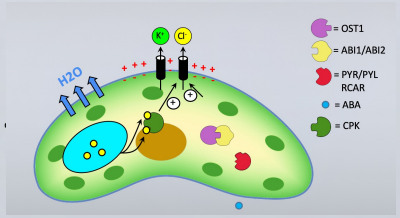
1. Biophysical background of stomatal movements…..



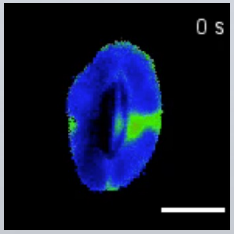
2. Voltage clamp with double barreled electrodes……



3. ABA and anion channels ……

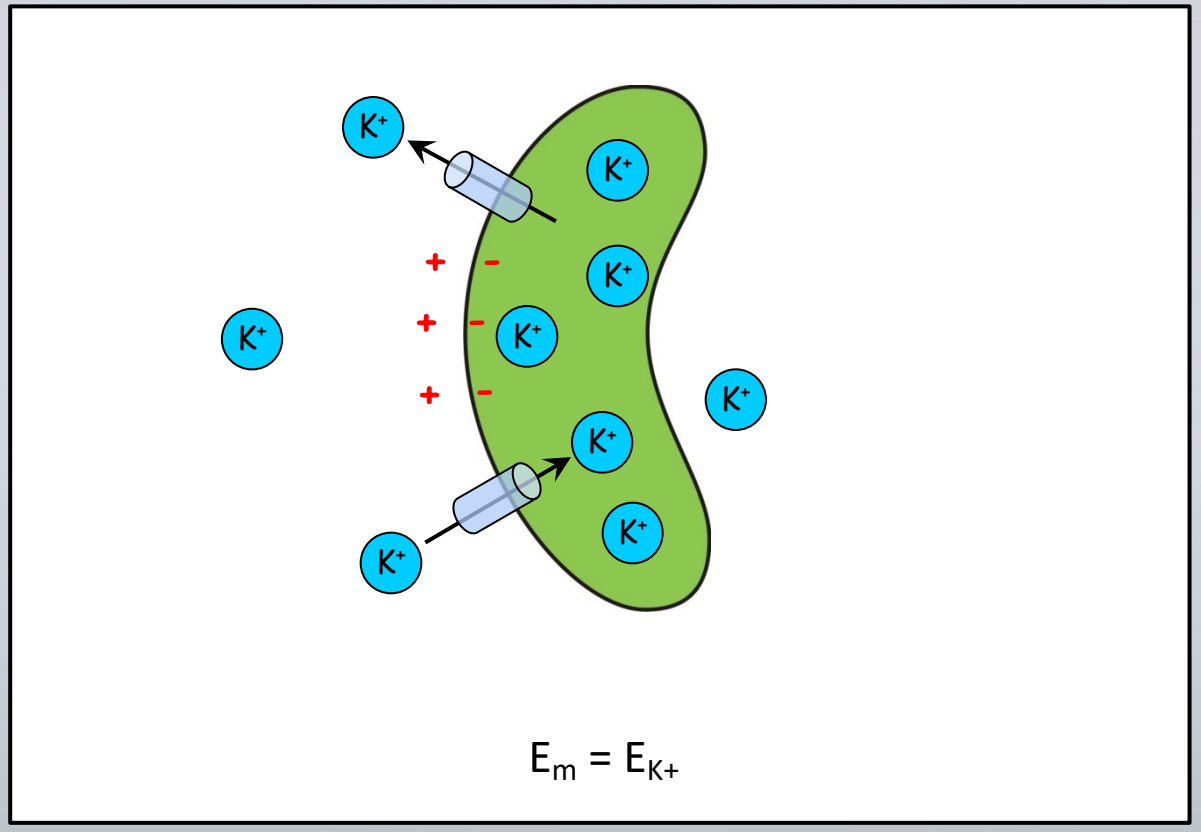


4. Ca^{2+}-signals and ABA response……..

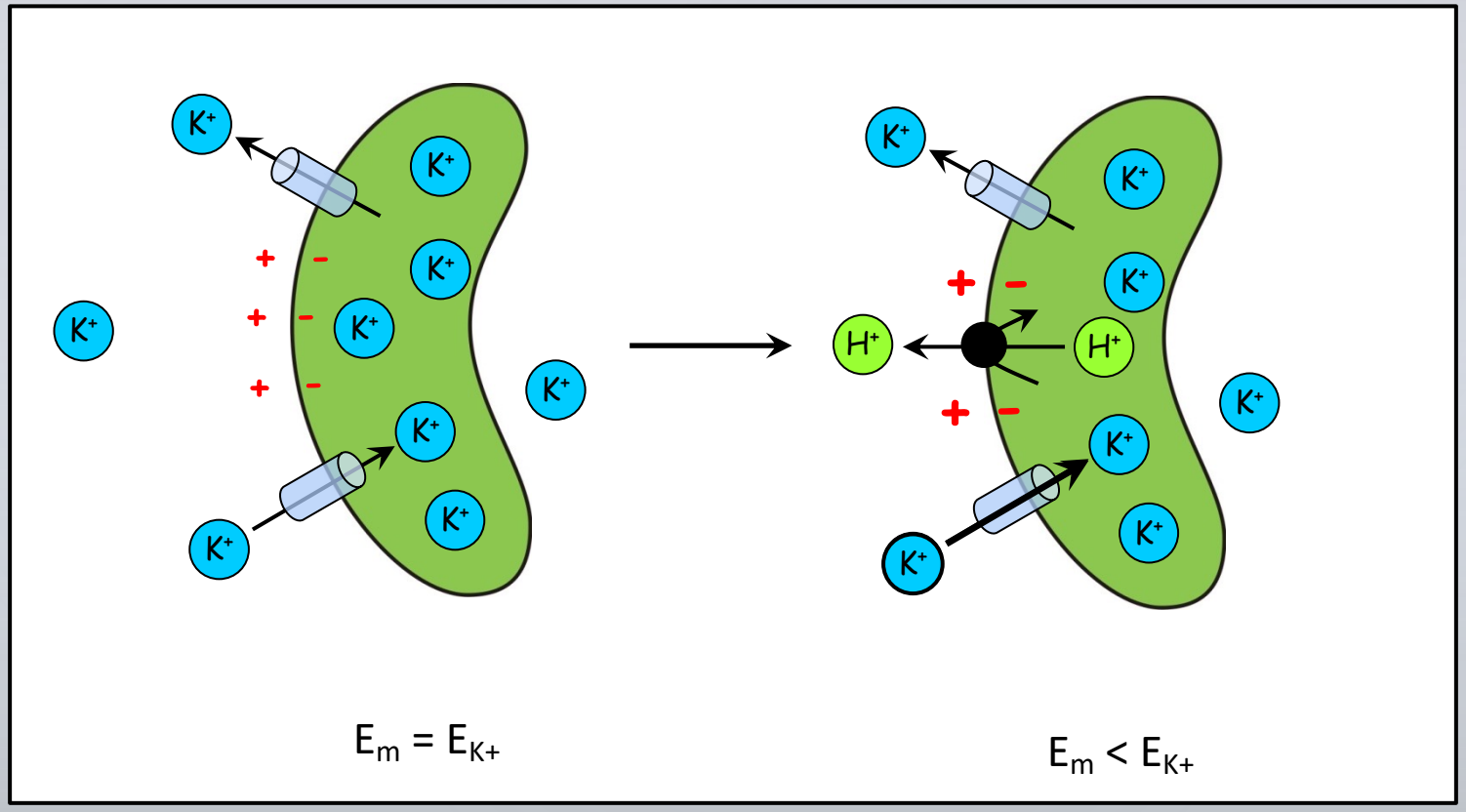


Uptake of K^+ is facilitated by K^+-channels, but

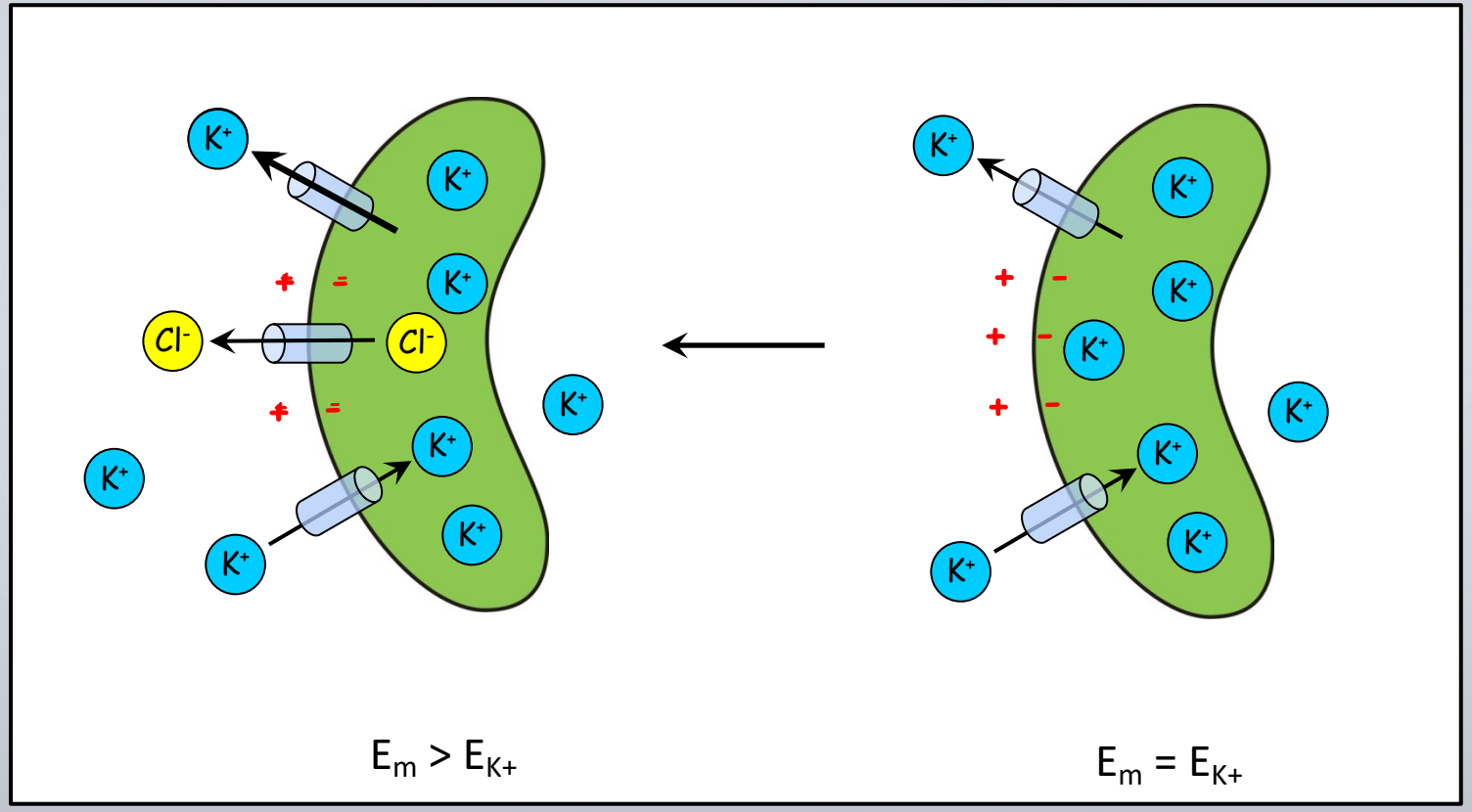
K^+ channels do not determine the direction of K^+ flow



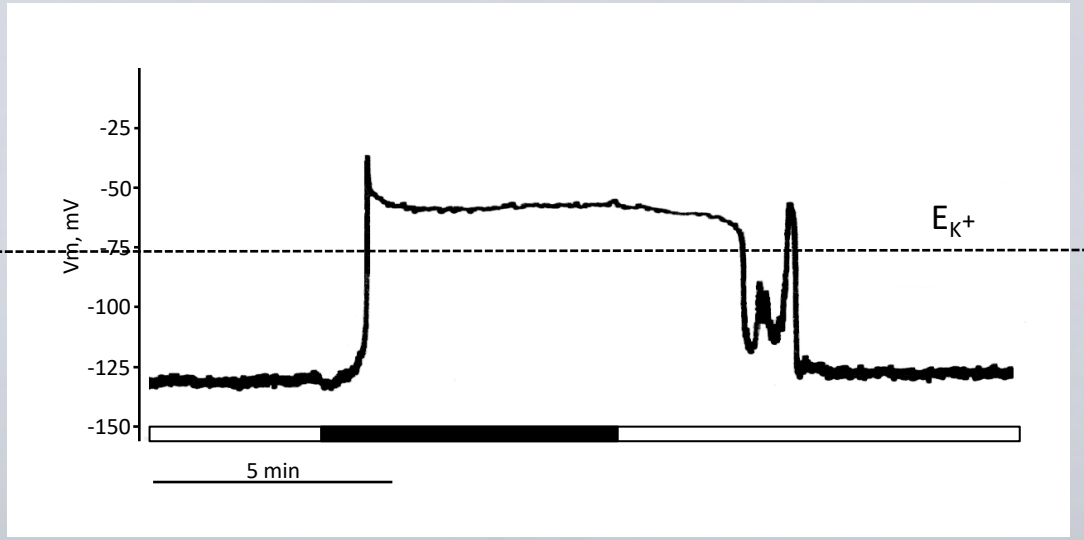
Activation of H^+-ATPases drives the uptake of K^+

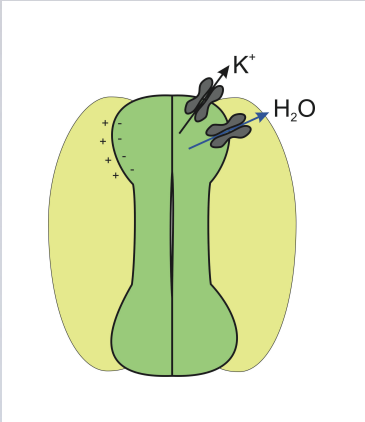


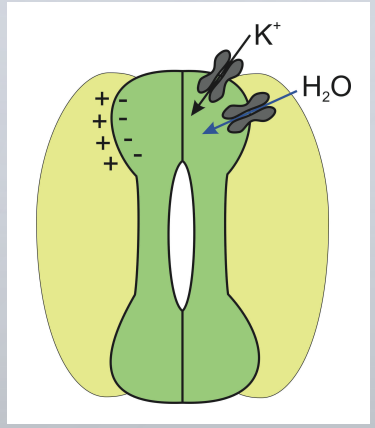
Opening of anion channels causes the extrusion of K^+



Light stimulates stomatal opening

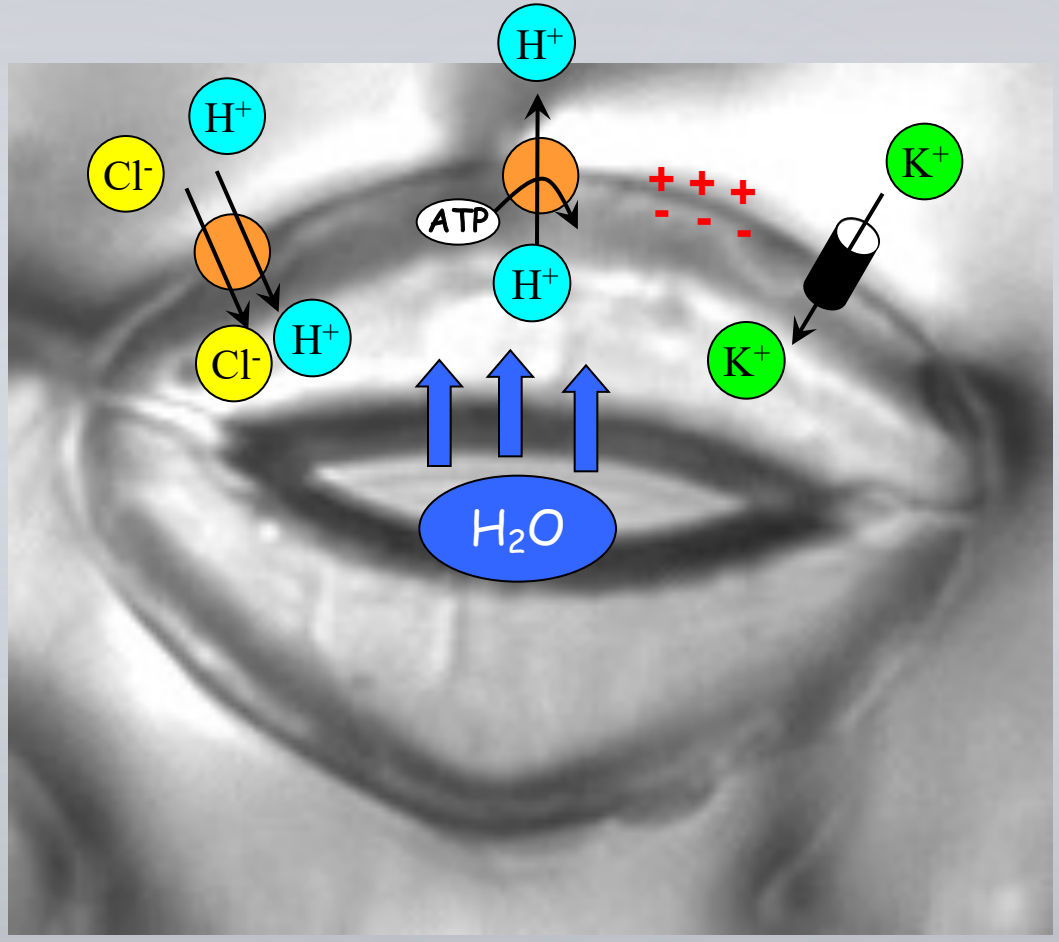




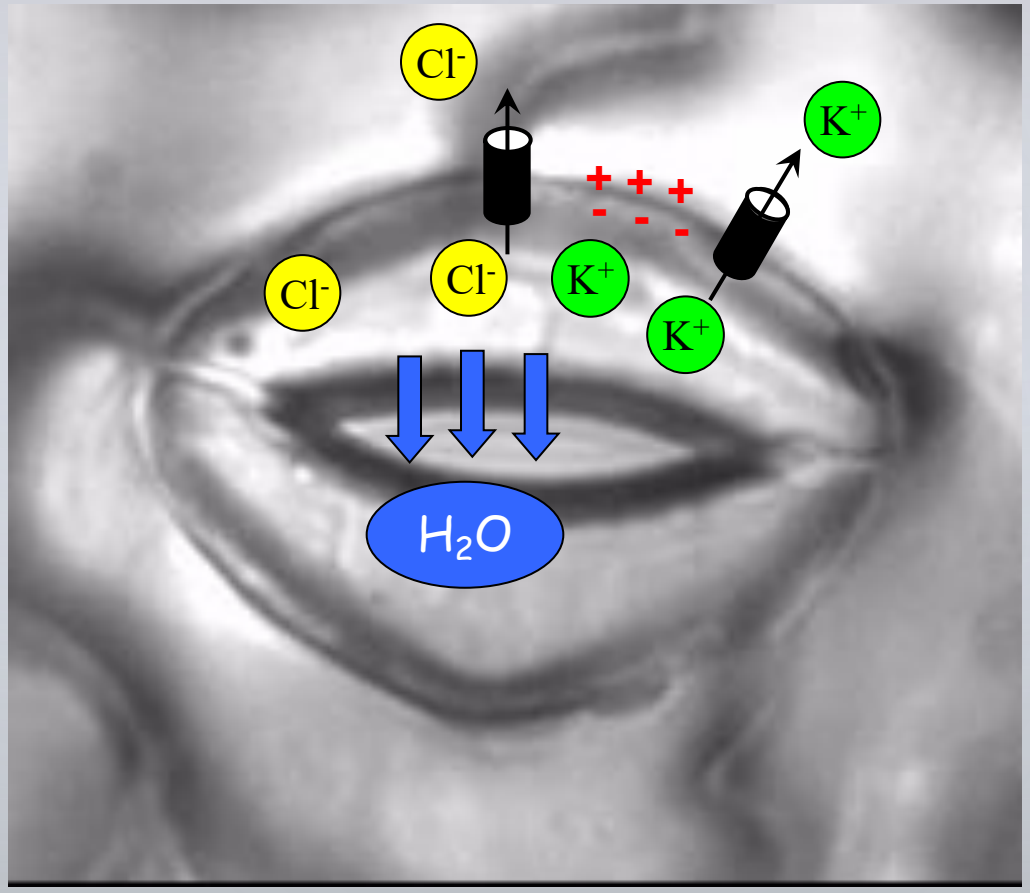


Kollist et al., 2014

Ion uptake during stomatal opening

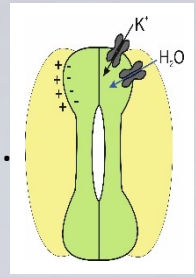


Ion release during stomatal closure

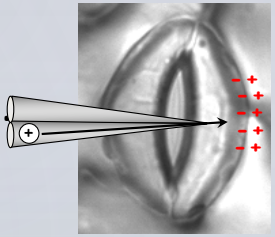


Guard cell anion channels control stomatal movements

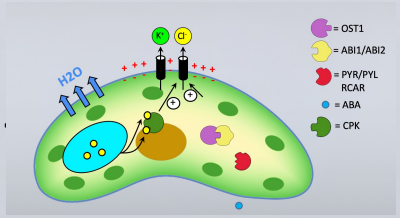
1. Biophysics of stomatal movements……



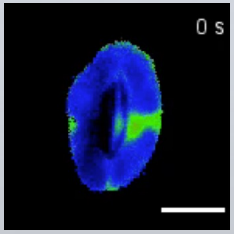
2. Voltage clamp with double barreled electrodes……



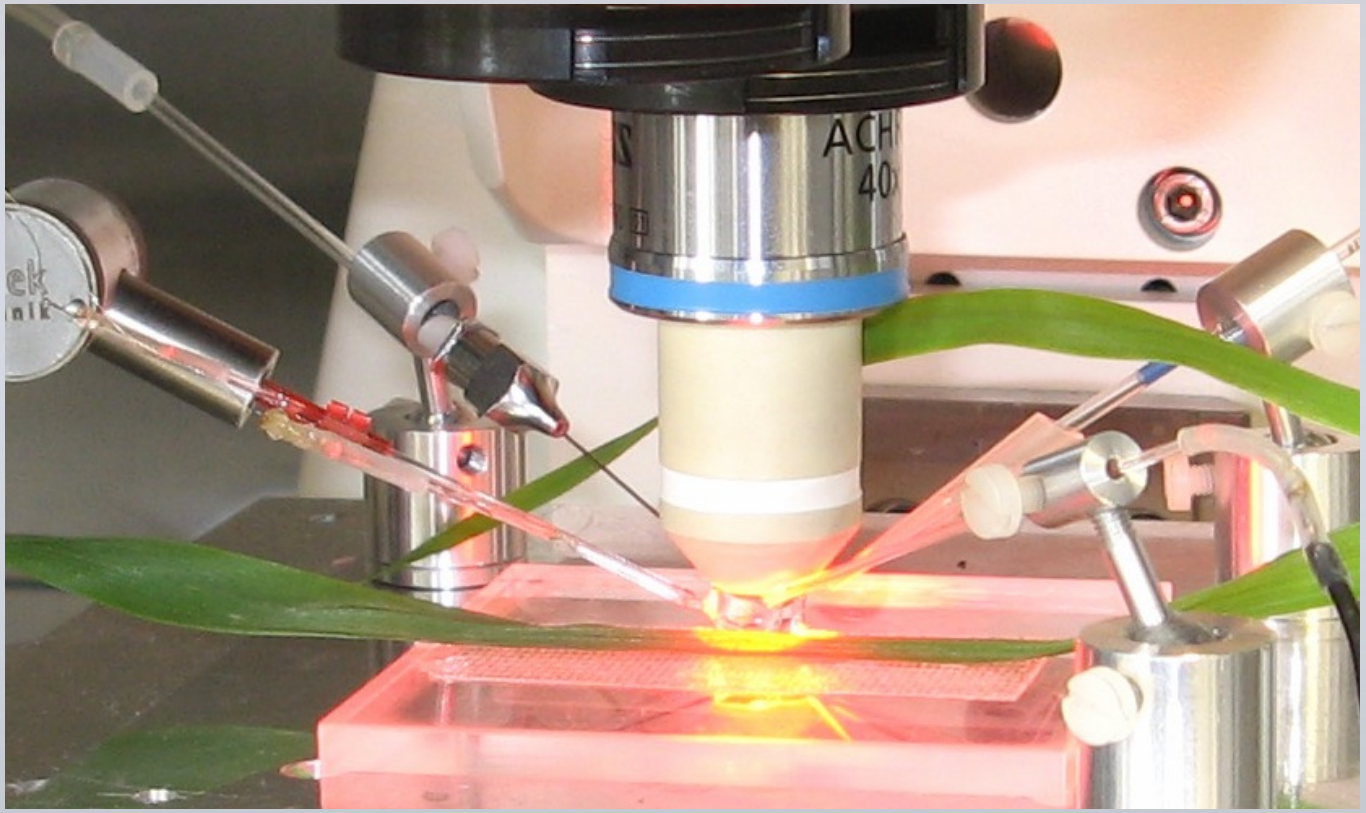
3. ABA and anion channels ……



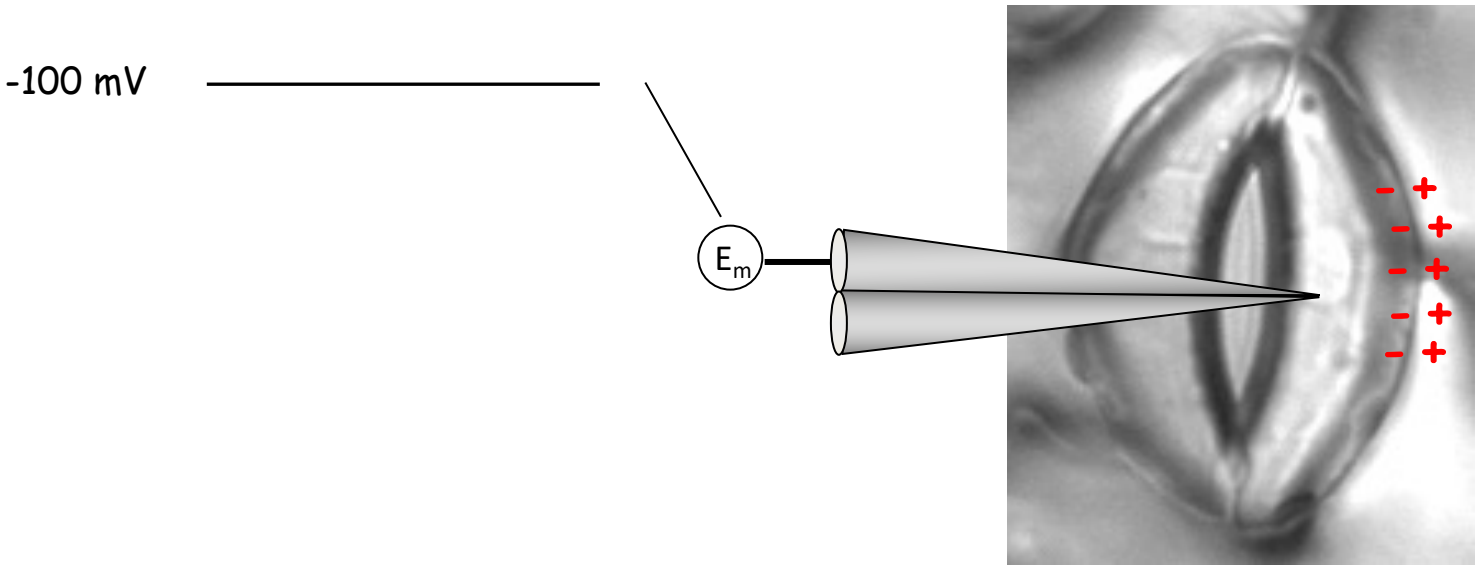
4. Ca^{2+}-signals and ABA response……..



Impalement experiments with double barreled micro electrodes



Recording the free running membrane potential (E_m)



Recording the free running membrane potential (E_m)

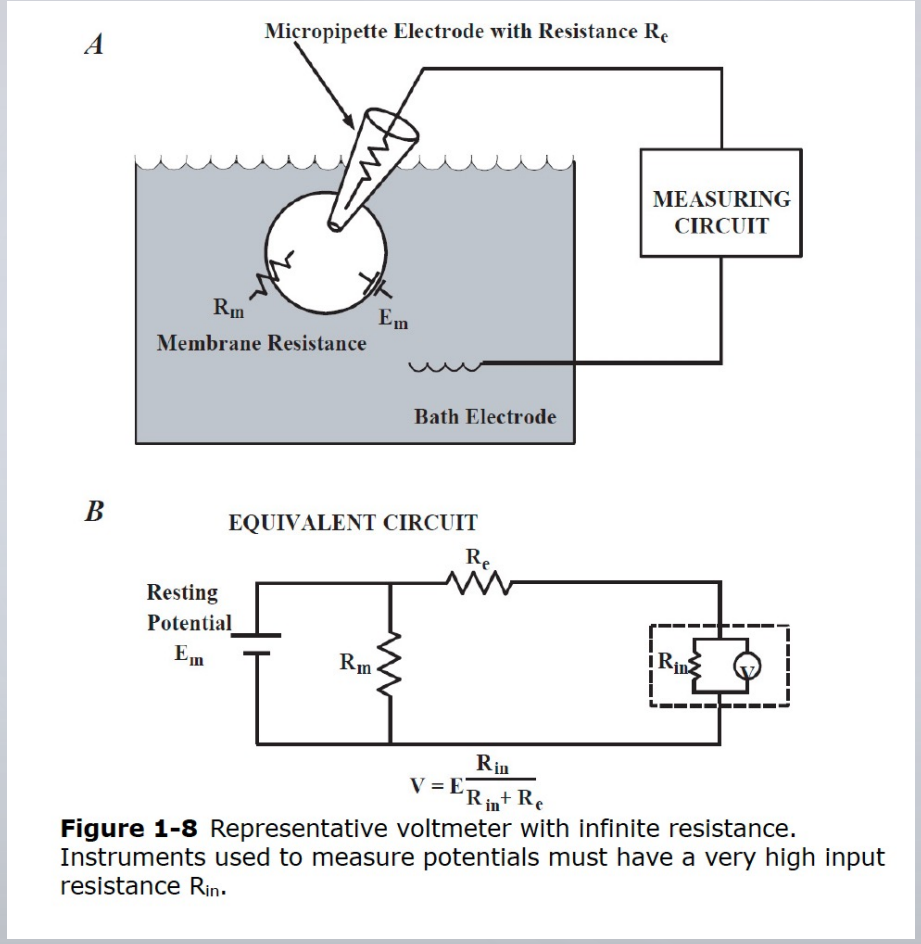


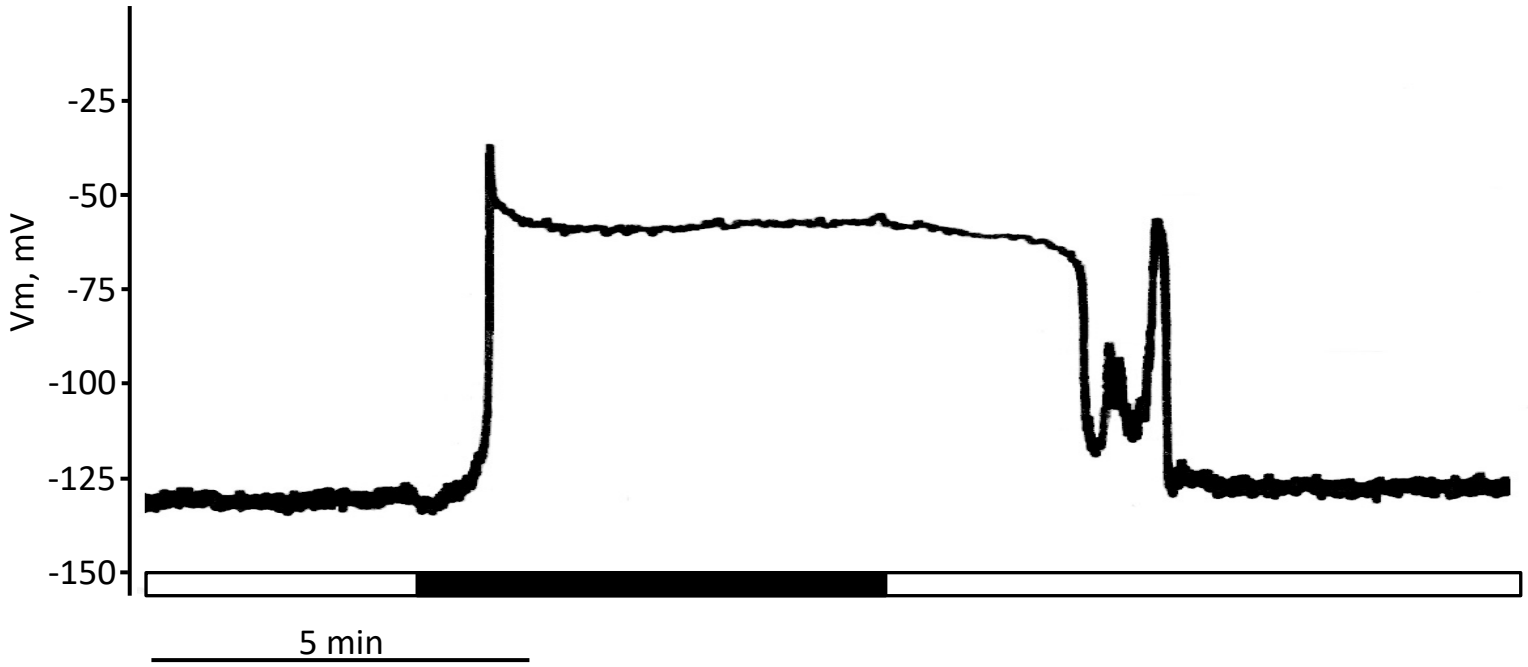
Figure 1-8 Representative voltmeter with infinite resistance. Instruments used to measure potentials must have a very high input resistance R_{in}.

The Axon guide 3rd edition, Molecular devices

Measuring the free-running membrane potential:

The resistance of the amplifier has to be much higher (approximately 100 times), as the series resistance of the electrode and cell membrane.

Light-induced membrane potential changes



Kollist et al., 2014

Two-electrode voltage clamp technique

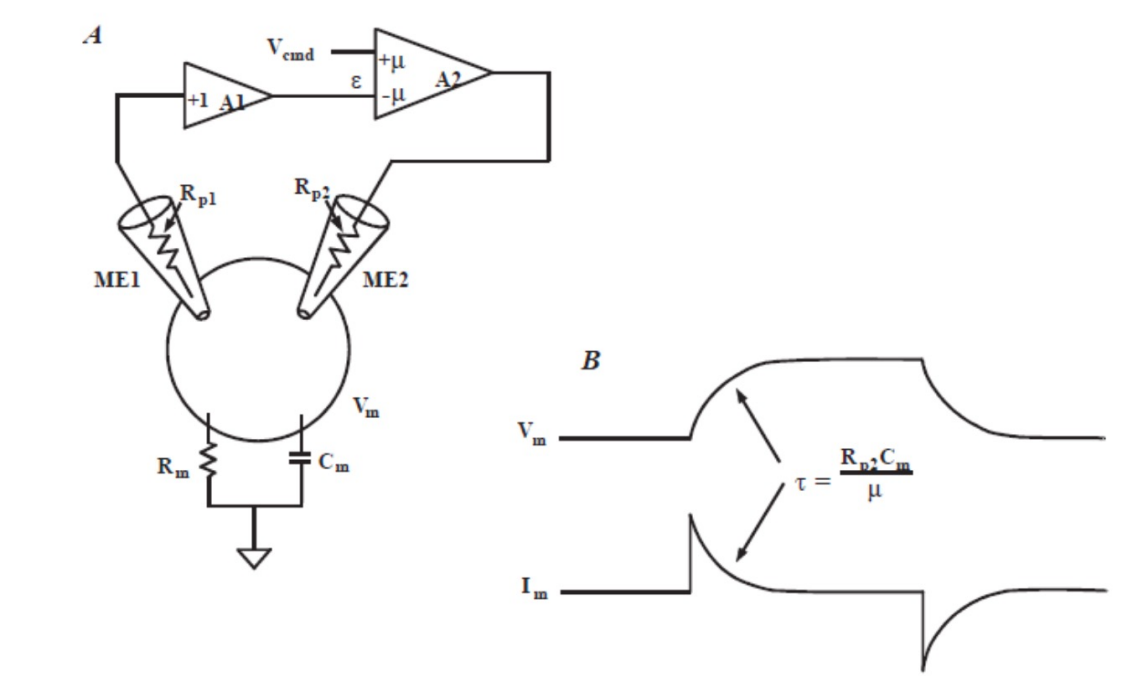
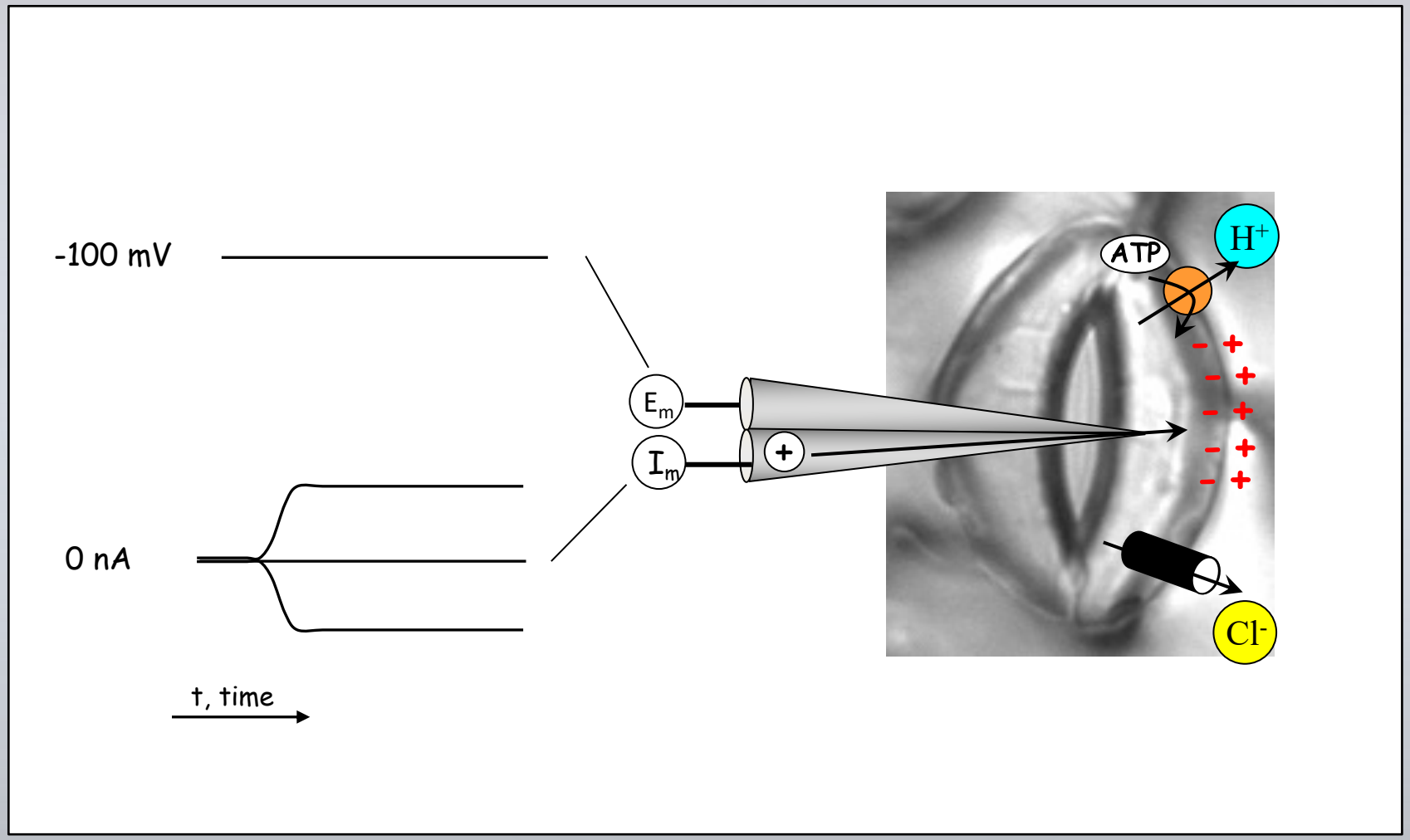


Figure 3-11 Conventional two-electrode voltage clamp.

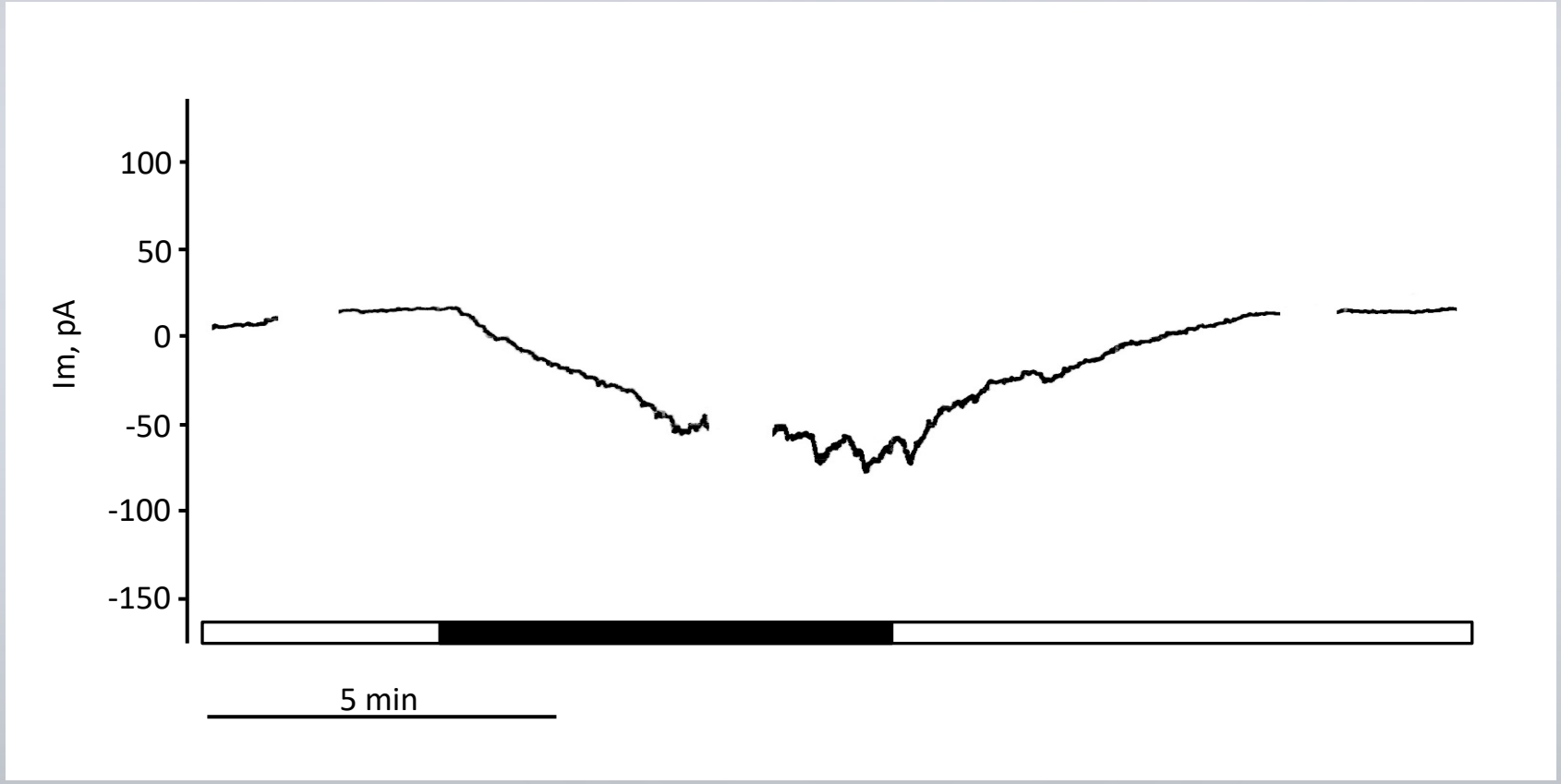
The Axon guide 3rd edition, Molecular devices

Conventional voltage clamp experiments are carried out with two electrodes, since the electrode resistance (ME1 and ME2) are unknown and can change during the experiment.

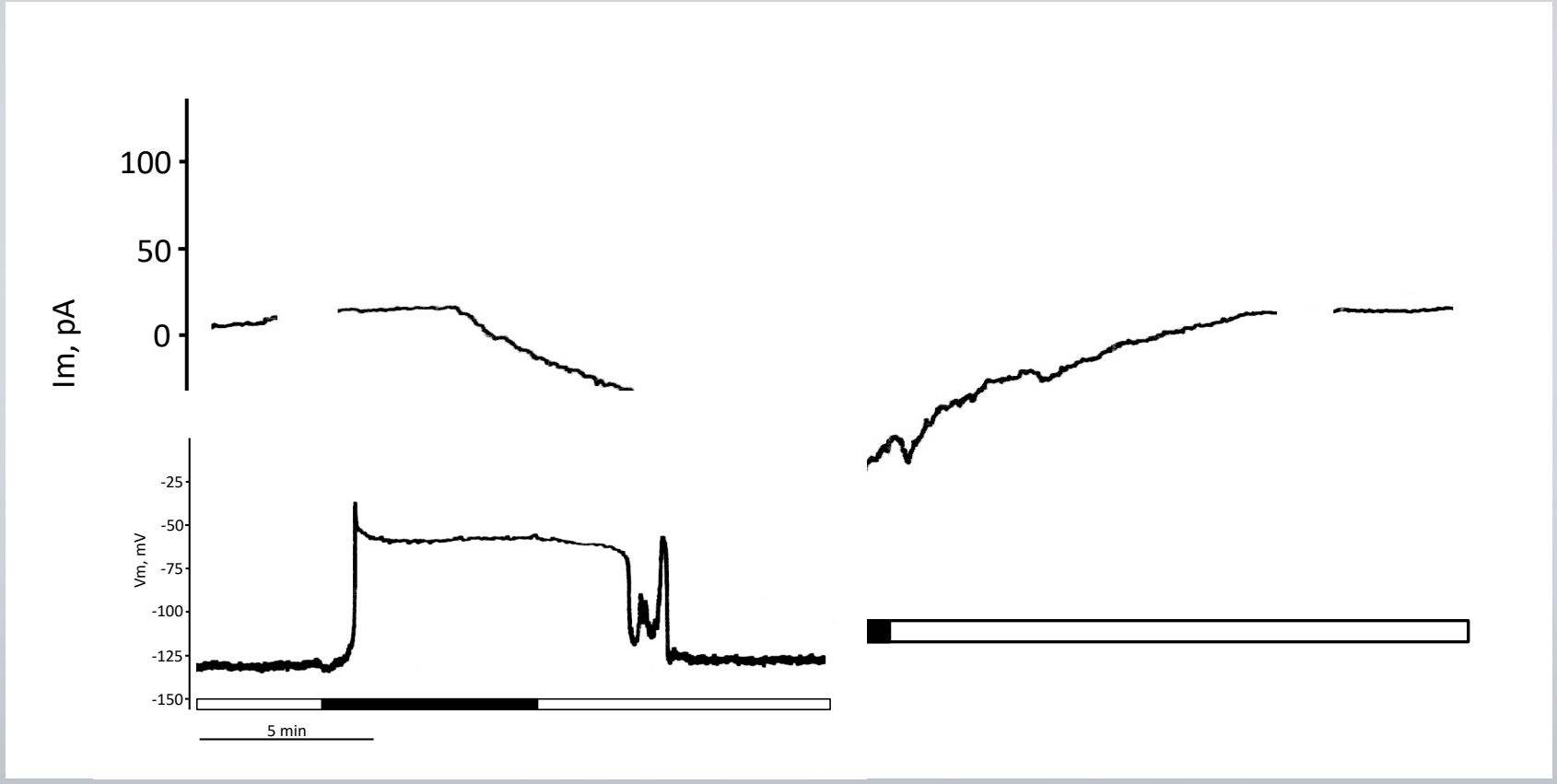
Voltage clamp with double barreled micro electrodes



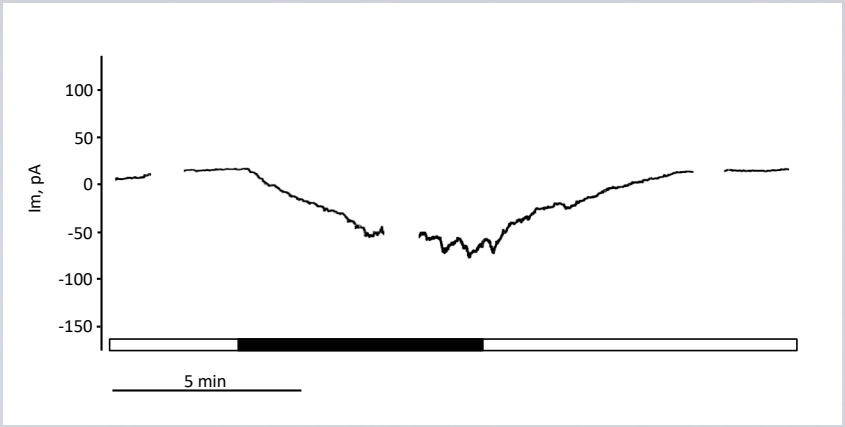
Light-induced membrane current changes (V_m = -100 mV)

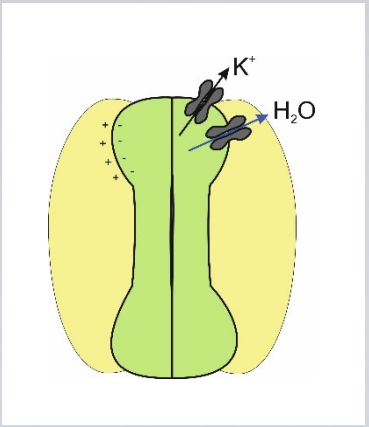


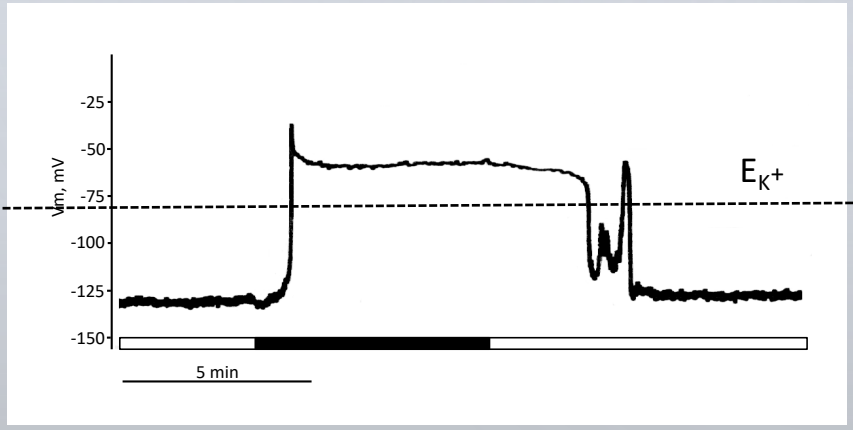
Light-induced membrane current changes (V_m = -100 mV)

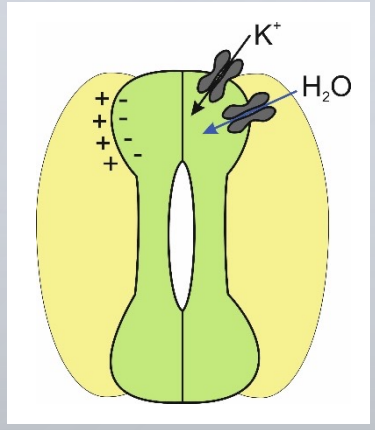


Light-induced membrane current changes (V_m = -100 mV)

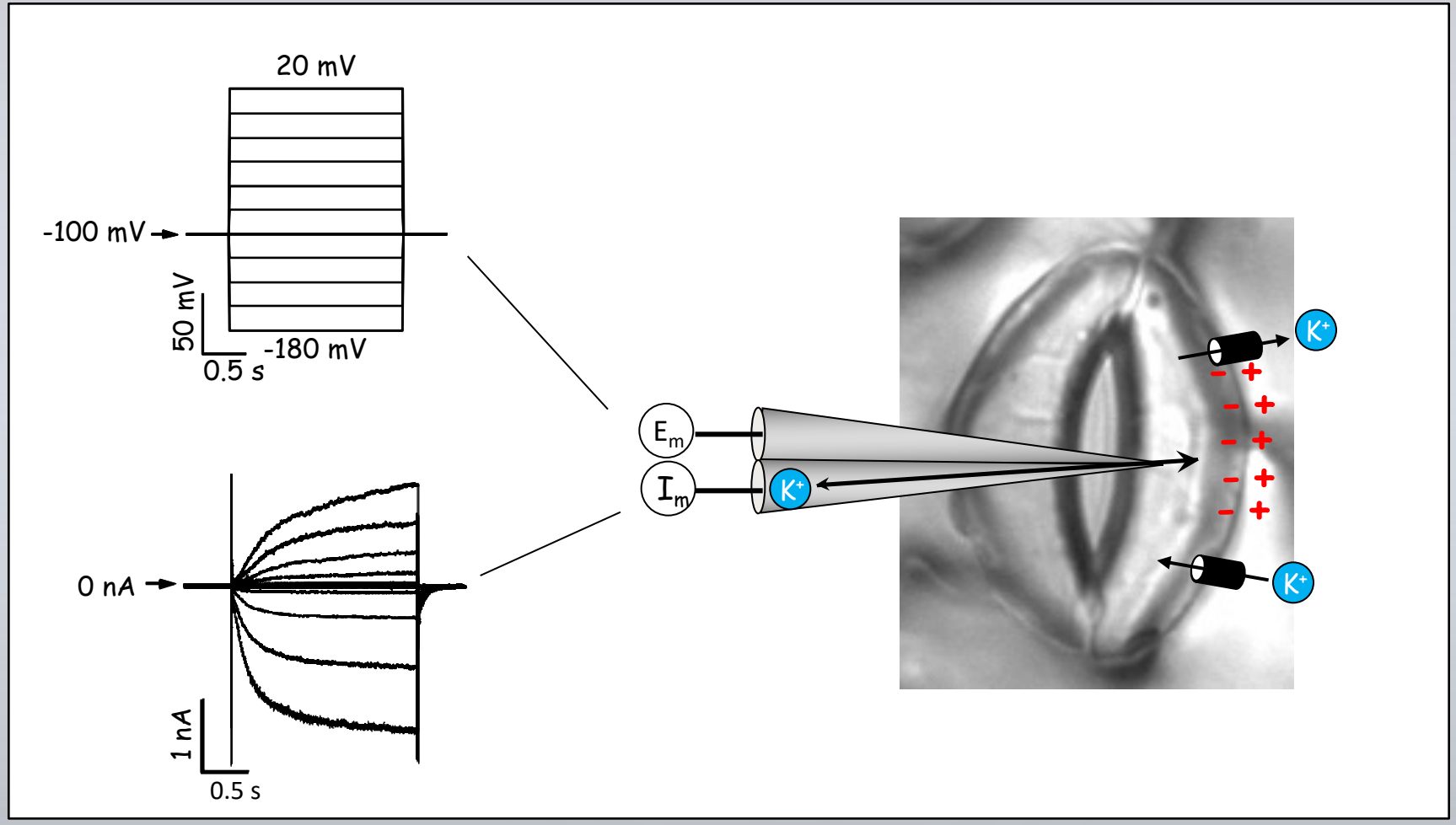






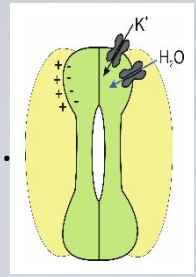


Voltage-clamp pulses to test ion channel activity

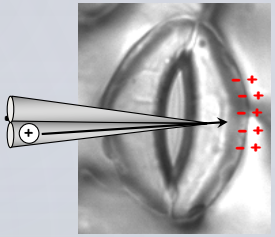


Guard cell anion channels control stomatal movements

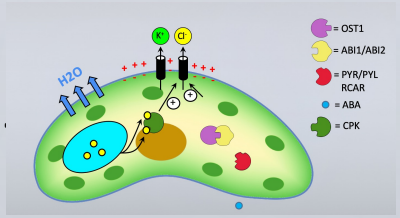
1. Biophysics of stomatal movements……



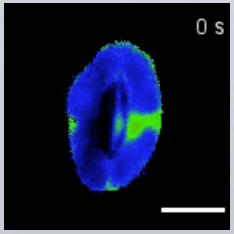
2. Voltage clamp with double barreled electrodes……



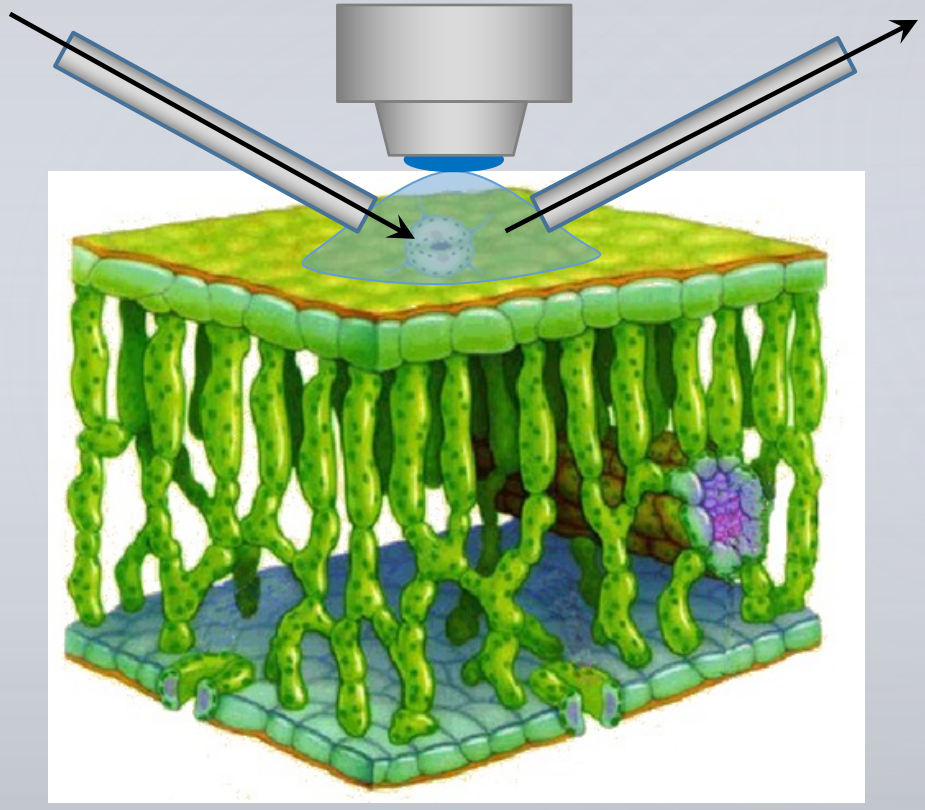
3. ABA and anion channels ……



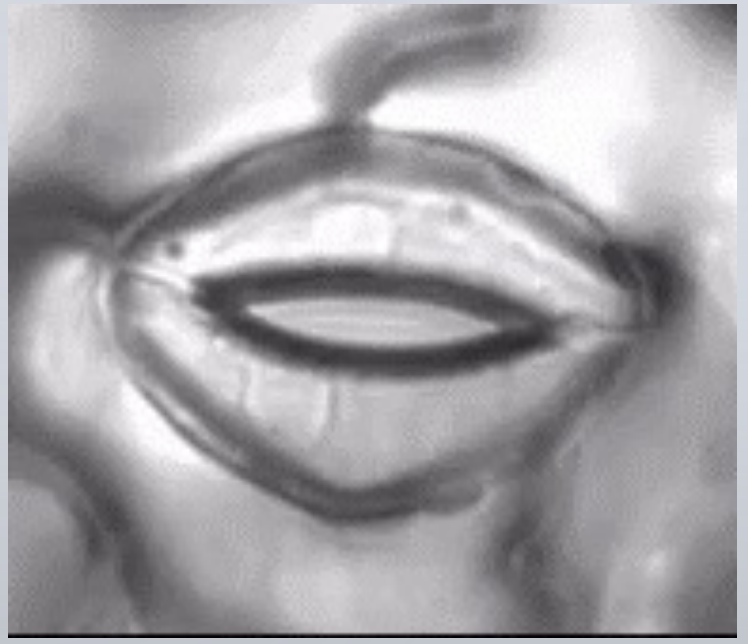
4. Ca^{2+}-signals and ABA response……..



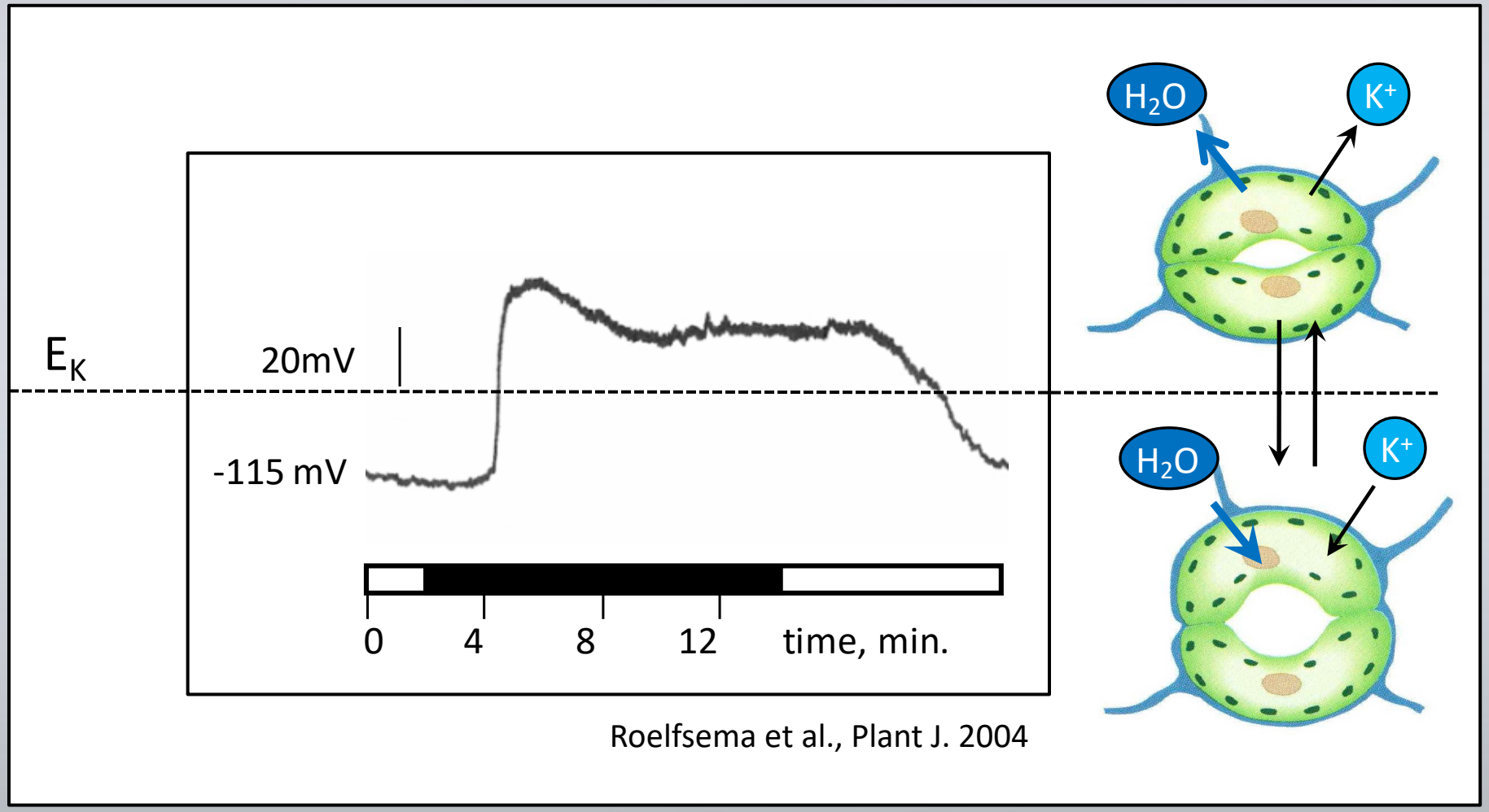
Abscisic acid-induced stomatal closure



Real time 30 min.

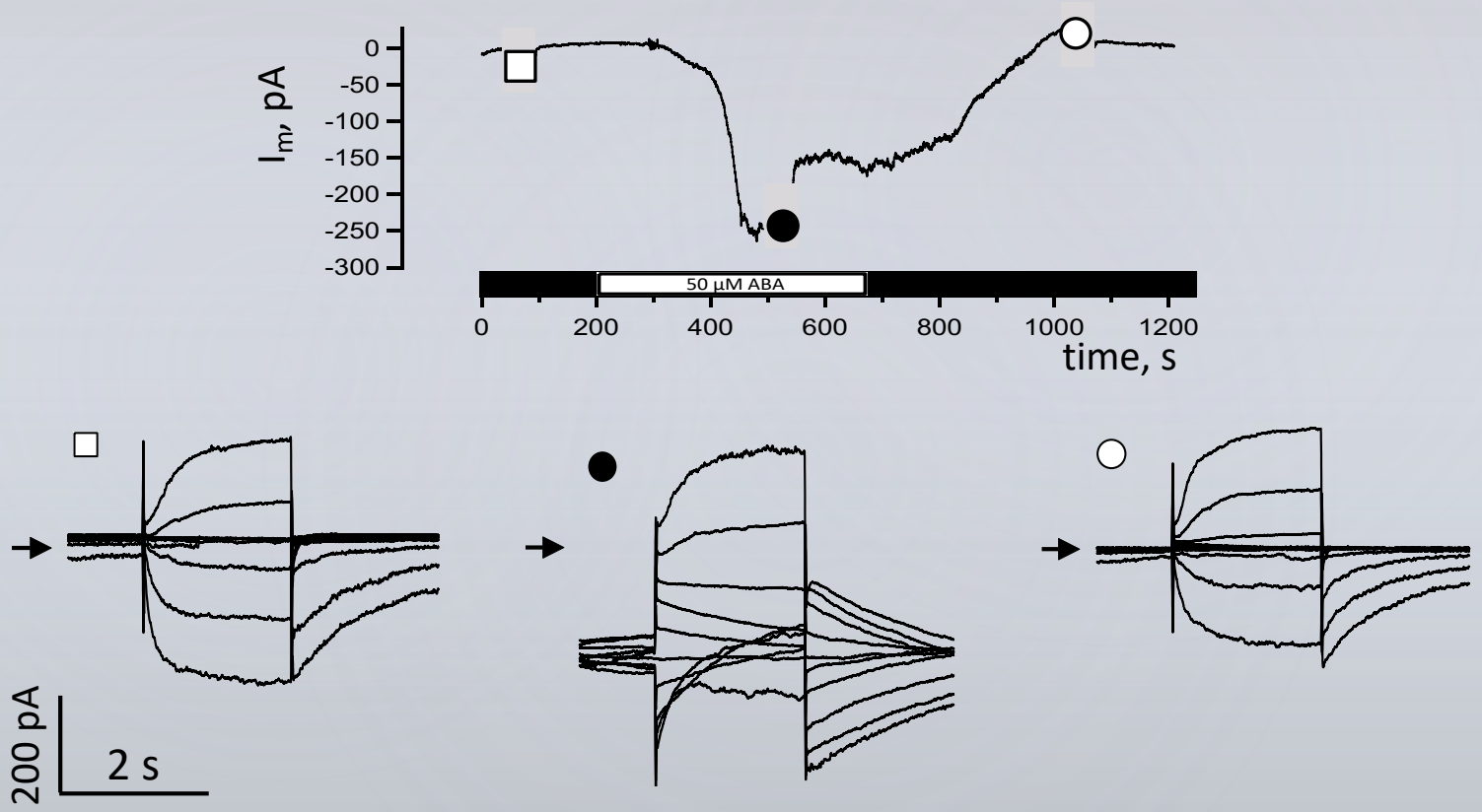


ABA-induced membrane potential changes in guard cells



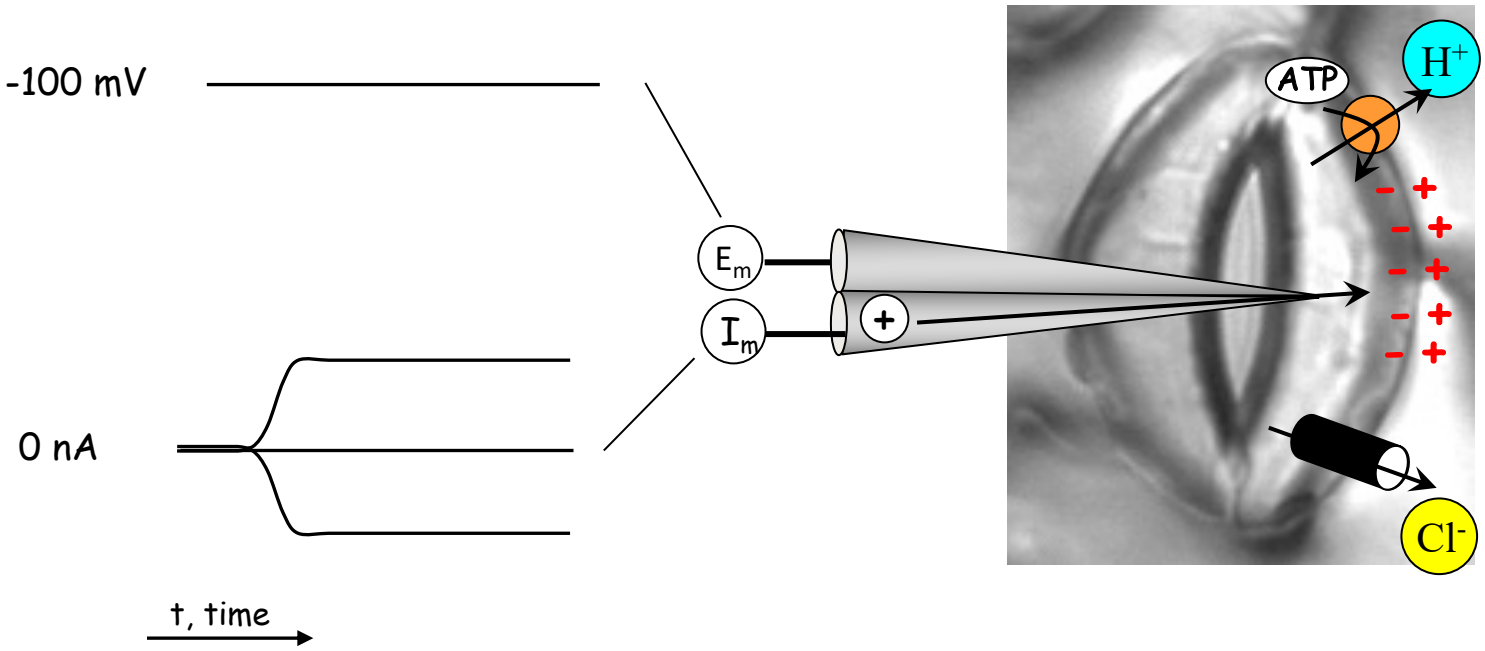
Roelfsema et al., Plant J. 2004

ABA activates S-type anion channels in guard cells

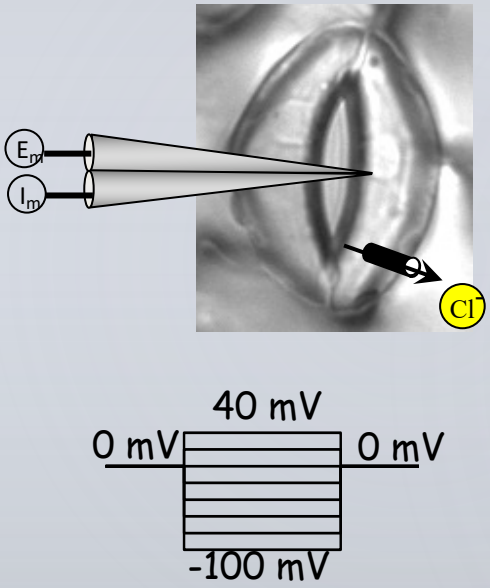


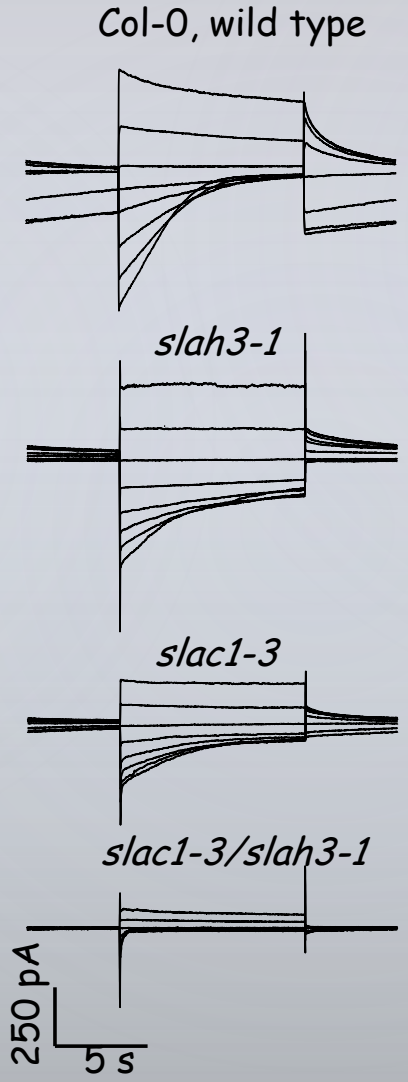
Marten et al. Plant Phys. 2007

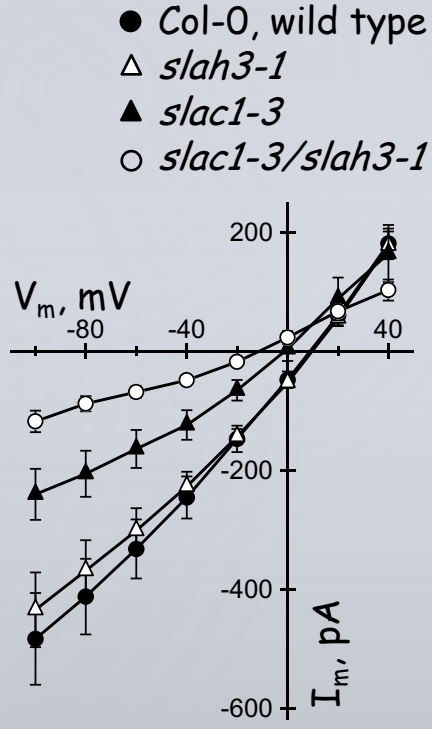
Voltage clamp with double barreled micro electrodes



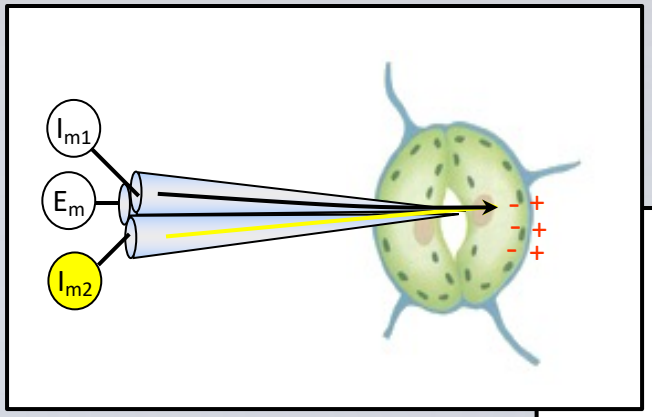
Two S-type anion channels are active in guard cells



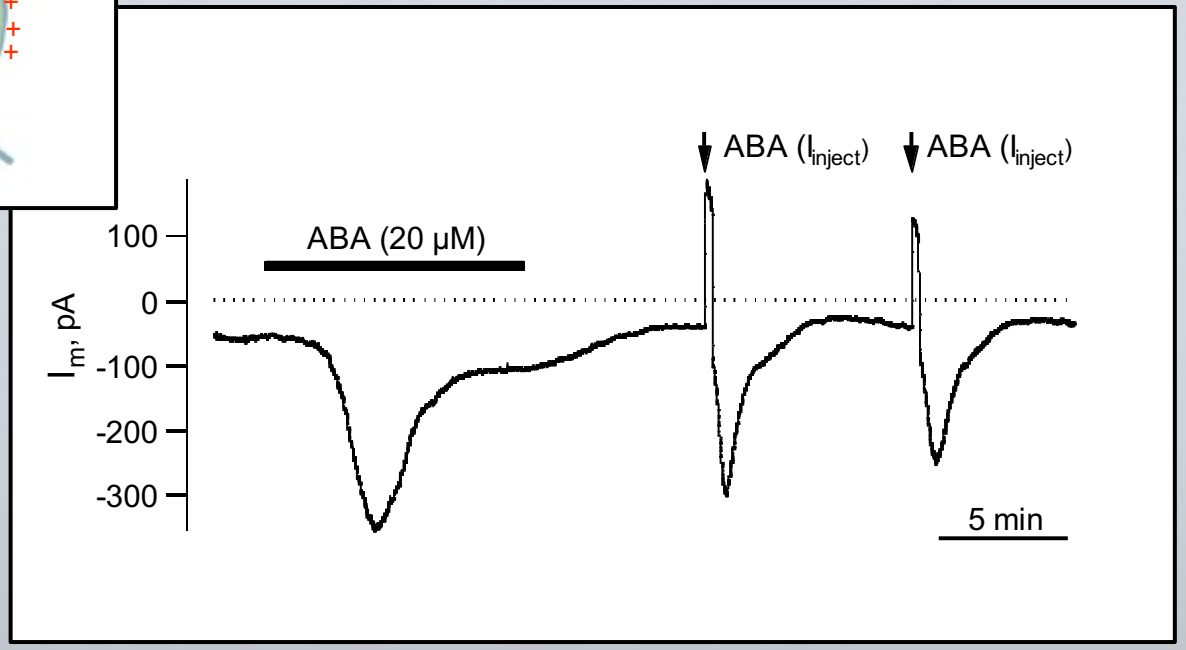




Electrical responses of a guard cell to the stress hormone ABA

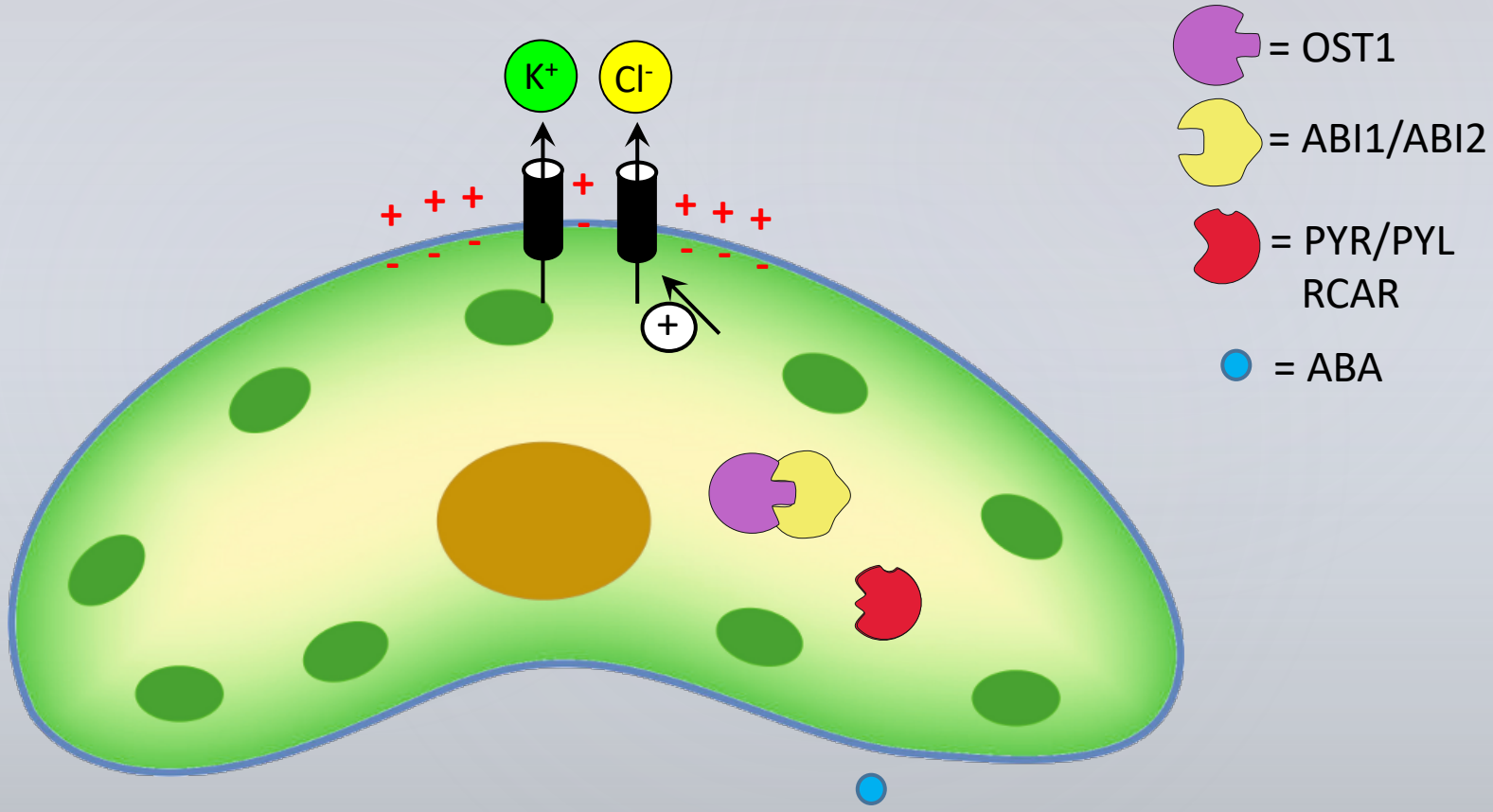


Victor Levchenko

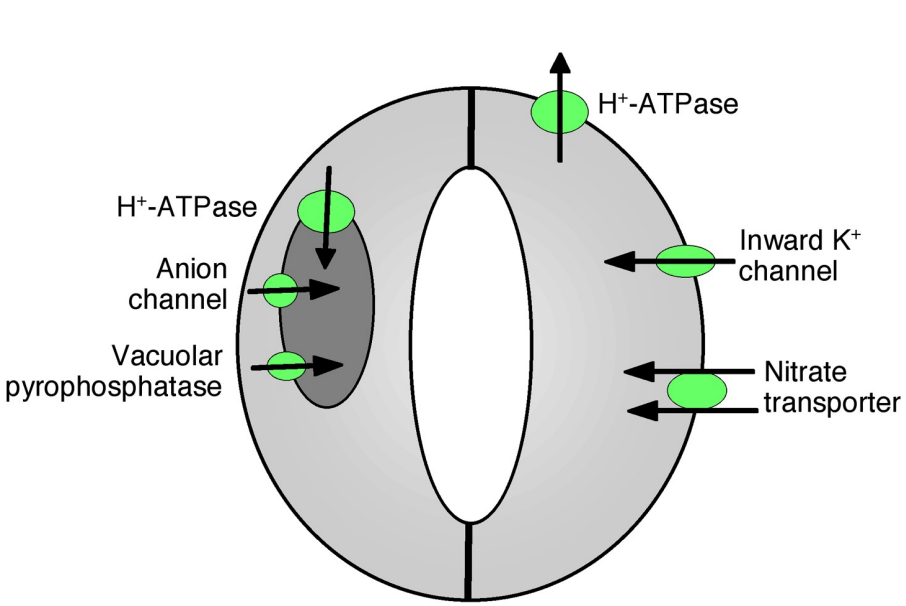


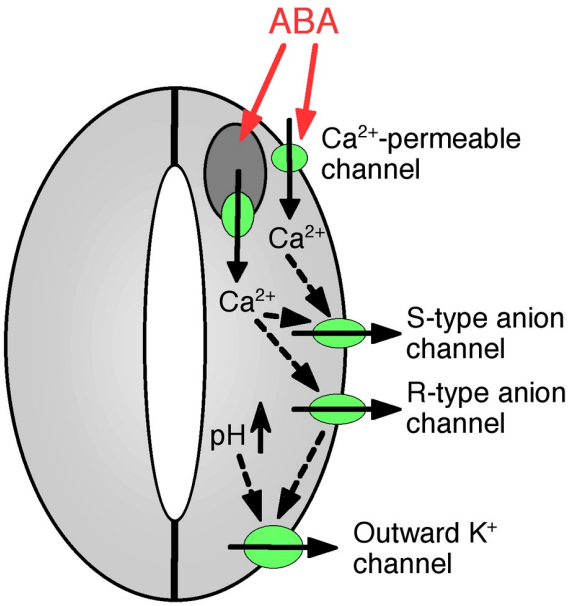
Levchenko et al. PNAS, 2005

ABA-dependent activation of SLAC1 and SLAH3



What is the role of the cytosolic free Ca^{2+} concentration in ABA-responses?

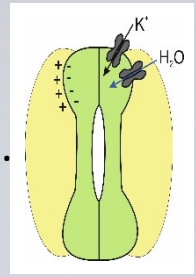




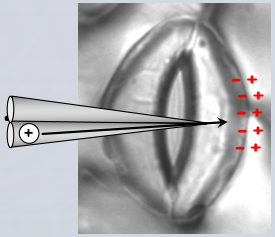
https://en.wikipedia.org/wiki/Guard_cell (Jan2020)

Guard cell anion channels control stomatal movements

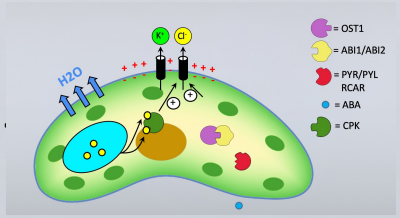
1. Biophysics of stomatal movements……



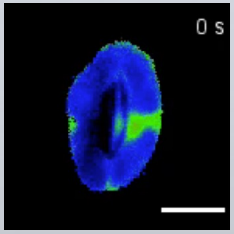
2. Voltage clamp with double barreled electrodes……



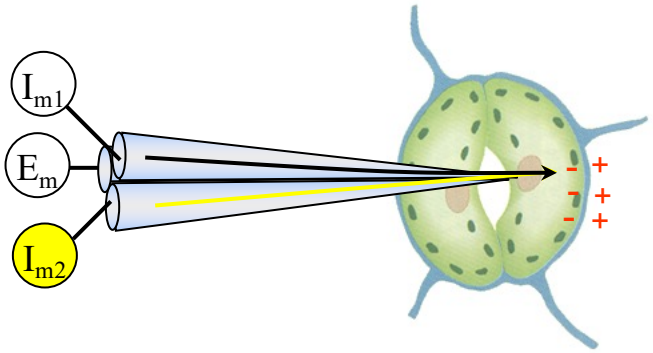
3. ABA and anion channels ……

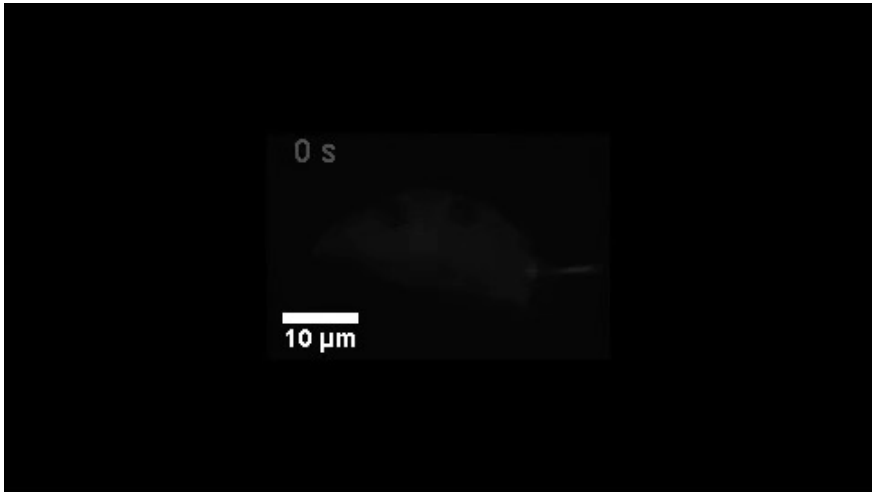


4. Ca^{2+}-signals and ABA response……



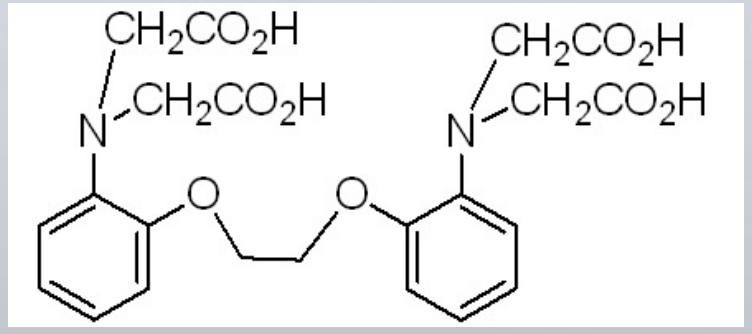
Loading of guard cells with fluorescent dyes



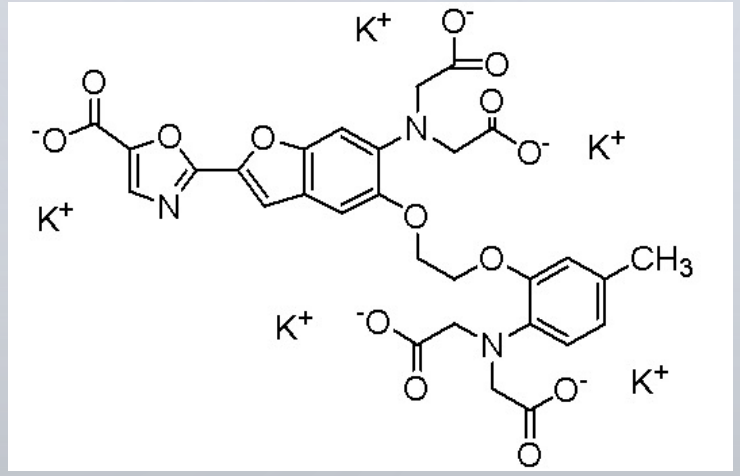


Fluorescence based Ca^{2+}-measurements with FURA2

Roger Tsien 1952-2016
Nobel prize 2008

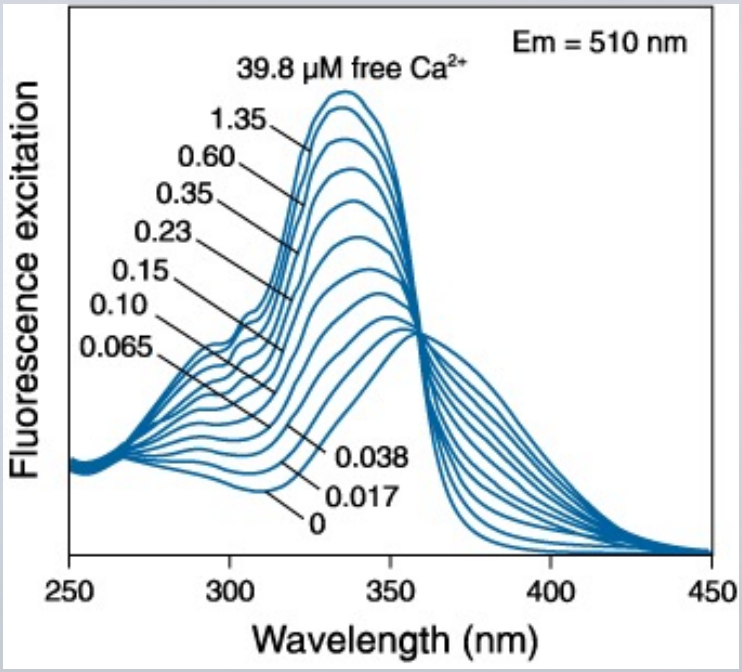


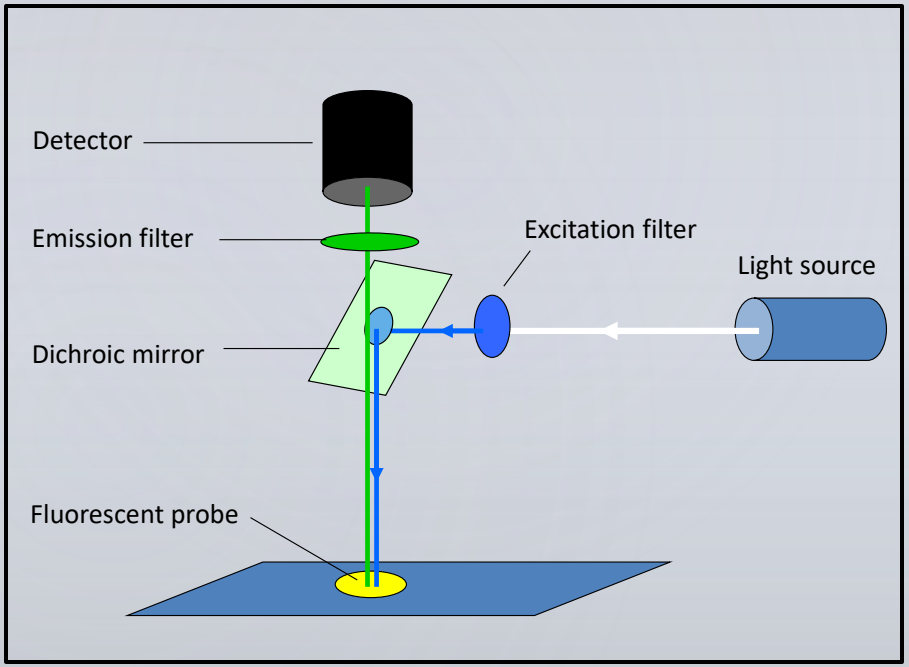
BAPTA



FURA2

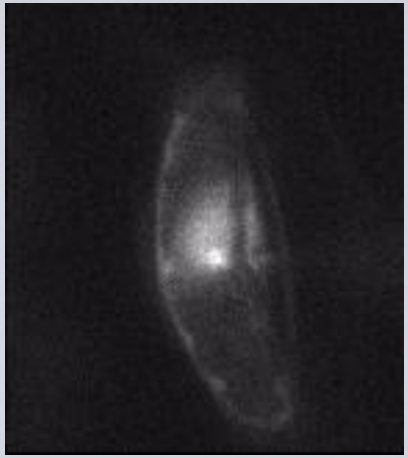
The excitation spectrum of changes when FURA2 binds Ca^{2+}





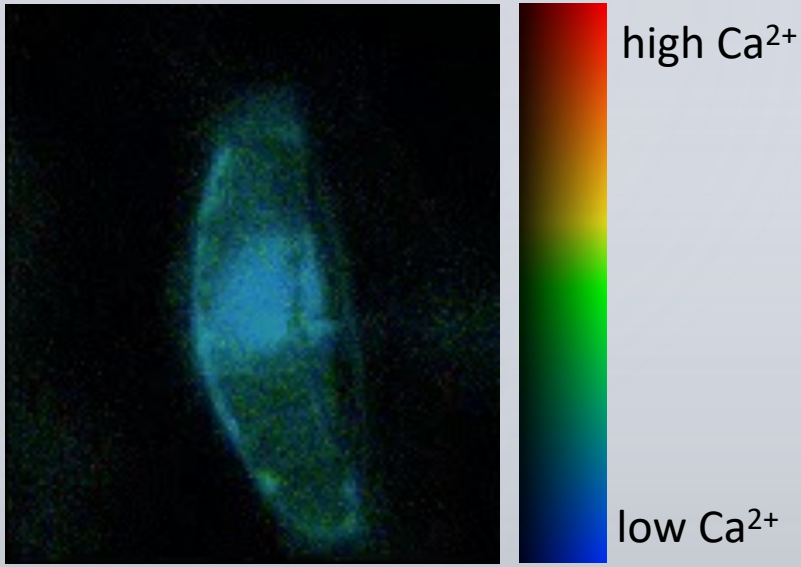
Mechanically-induced changes in the cytosolic [Ca^{2+}]

Excitation 355 nm



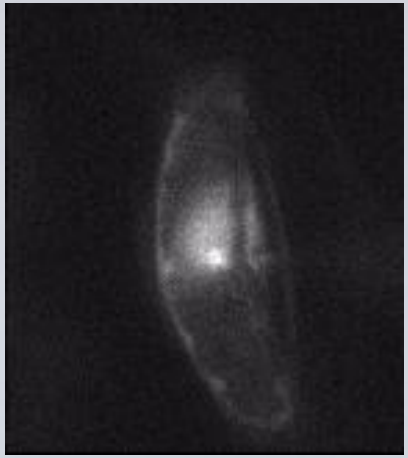
Excitation 380 nm

FURA2 ratio



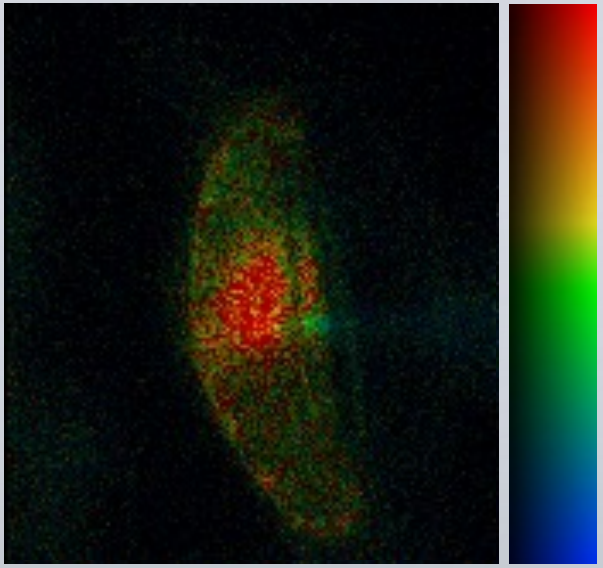
Mechanically-induced changes in the cytosolic [Ca^{2+}]

Excitation 355 nm

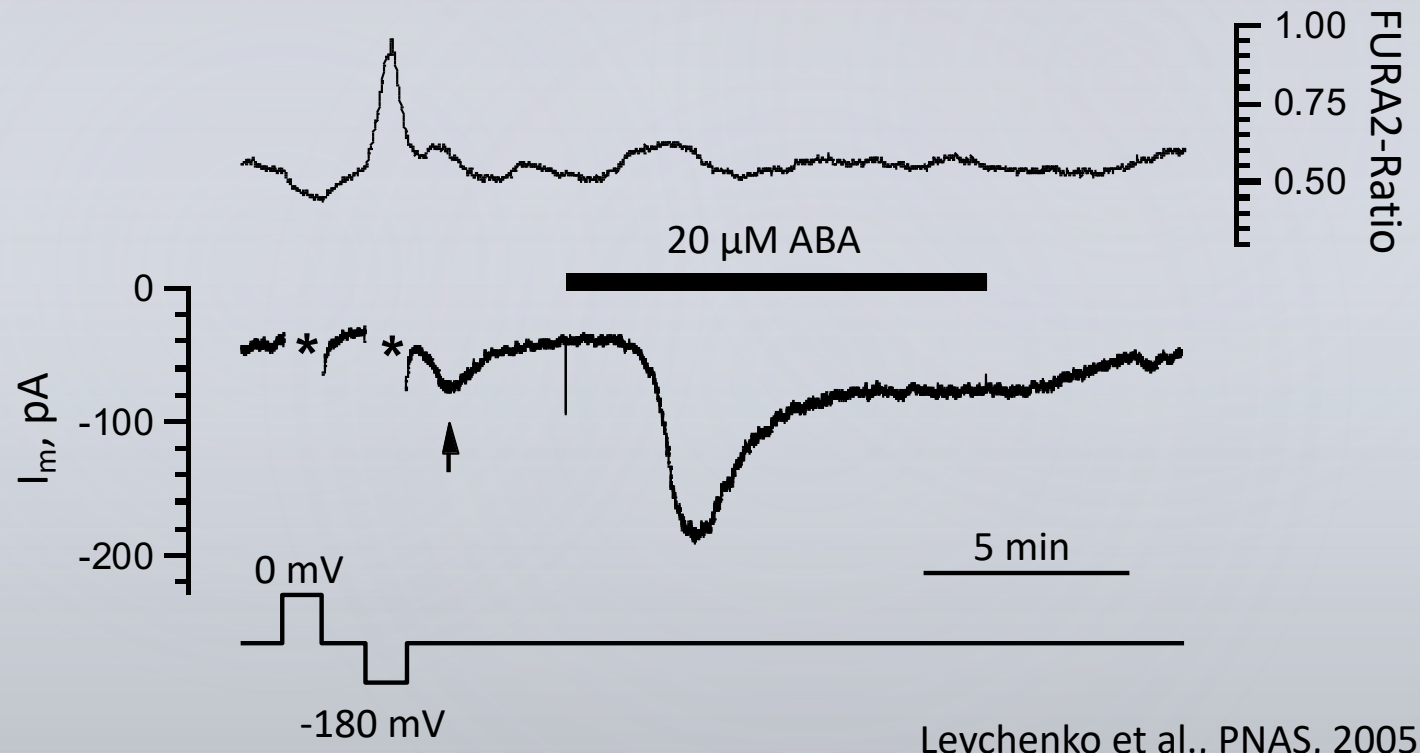


Excitation 380 nm

FURA2 ratio

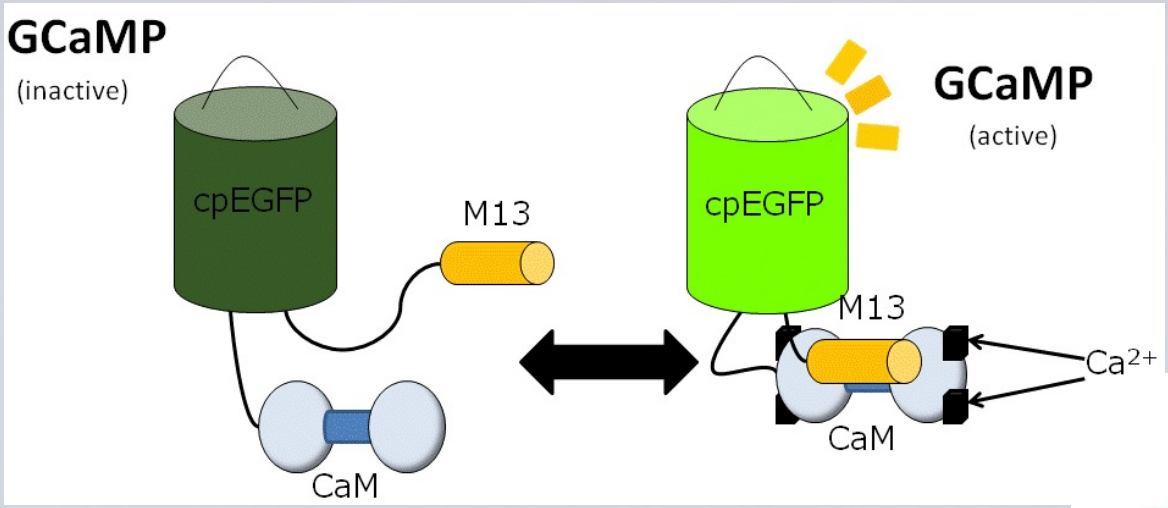


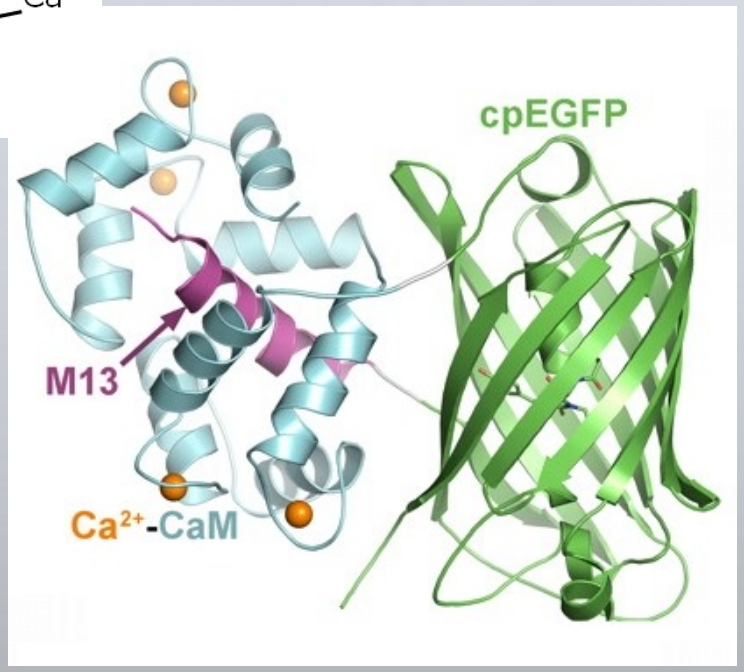
In *Vicia faba*, ABA activates S-type anion channels independent of cytosolic Ca^{2+} signals



Levchenko et al., PNAS, 2005

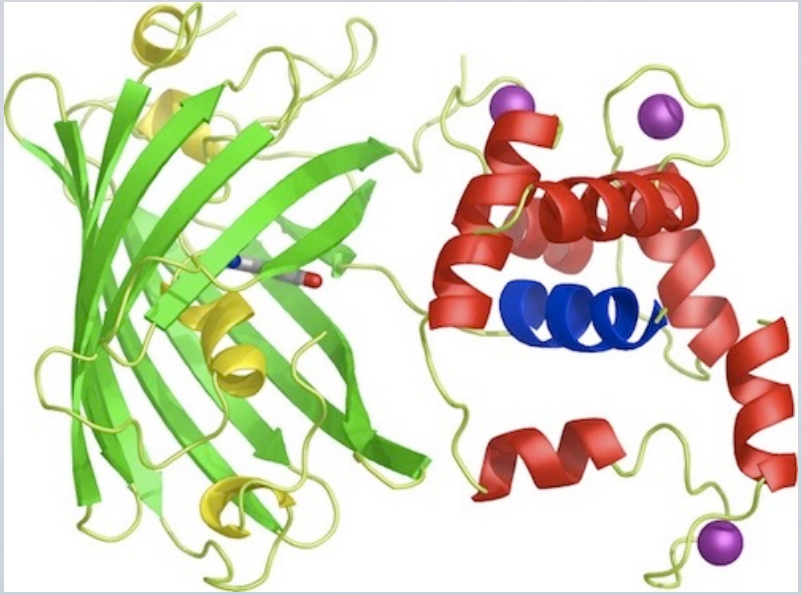
Cytosolic Ca^{2+} signals detected with fluorescent proteins



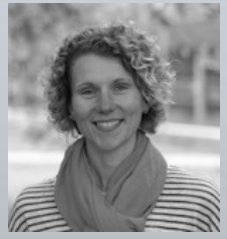


M13 fragment from myosin light chain kinase (M13) was attached to a circularly permutated EGFP (cpEGFP) and calmodulin (CaM)
Nakai et al., Nat Biotech. 2001

Cytosolic Ca^{2+} signals measured with R-GECO1



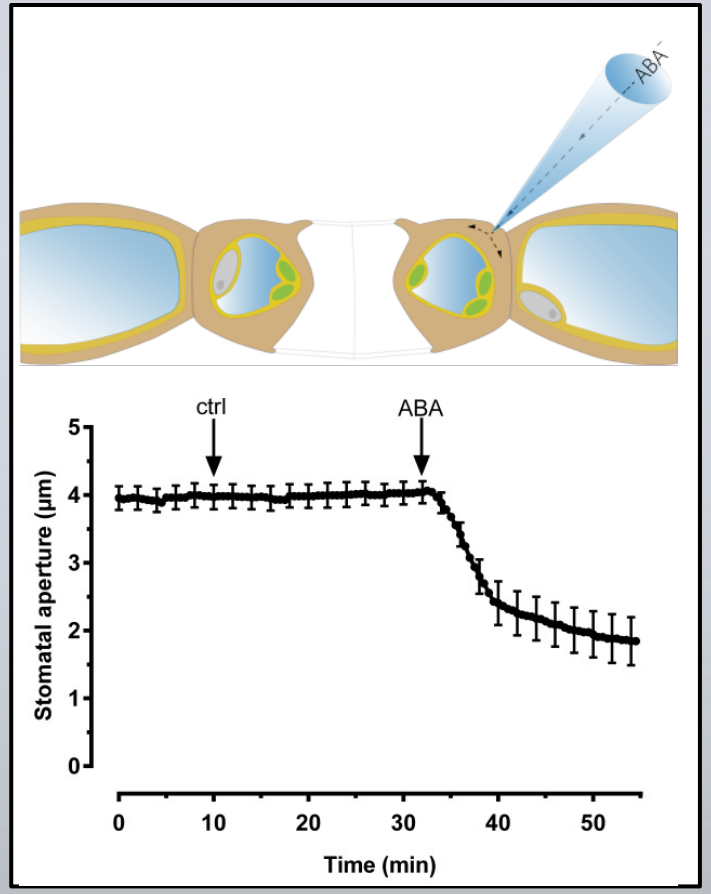
cpEGFP was replaced by mApple red FP and fluorescence properties were enhanced
Zhao et al., Science 2011

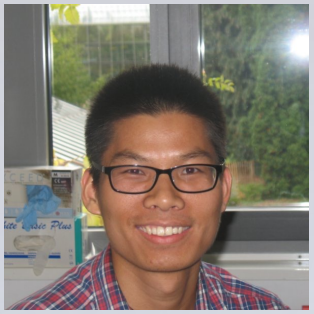


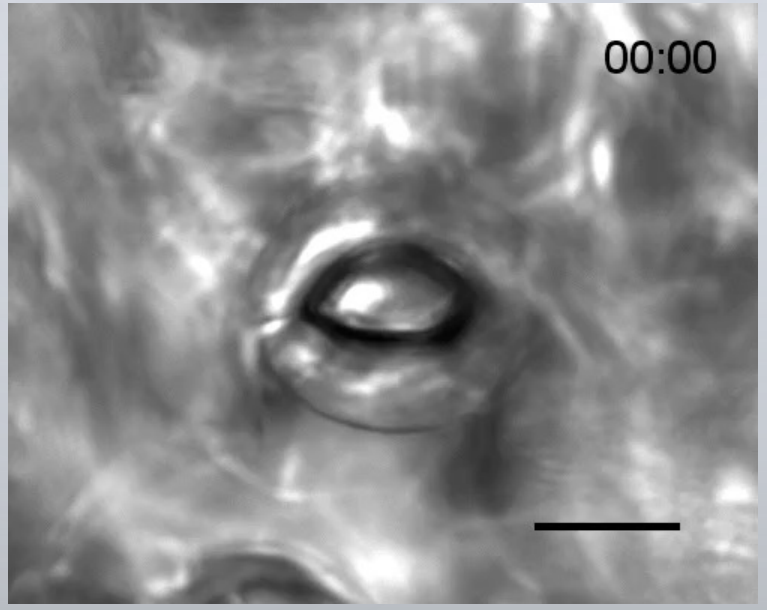
Arabidopsis plants generated by:
M. Krebs, R Waadt and K. Schumacher,
University of Heidelberg

ABA-induced Ca^{2+} signals in guard cells

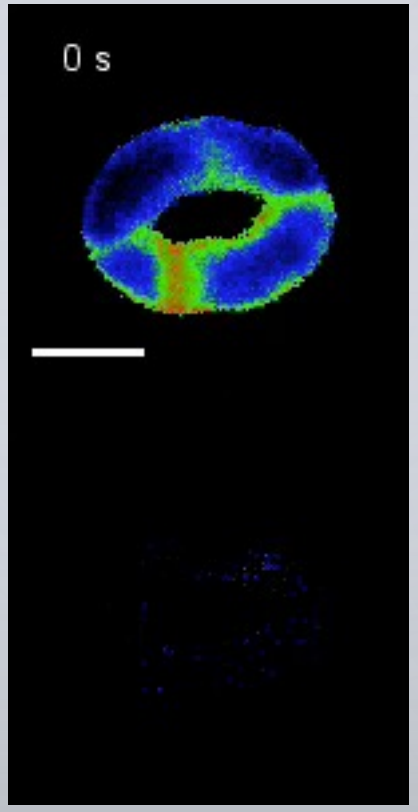
Shouguang Huang, New Phytologist 2019

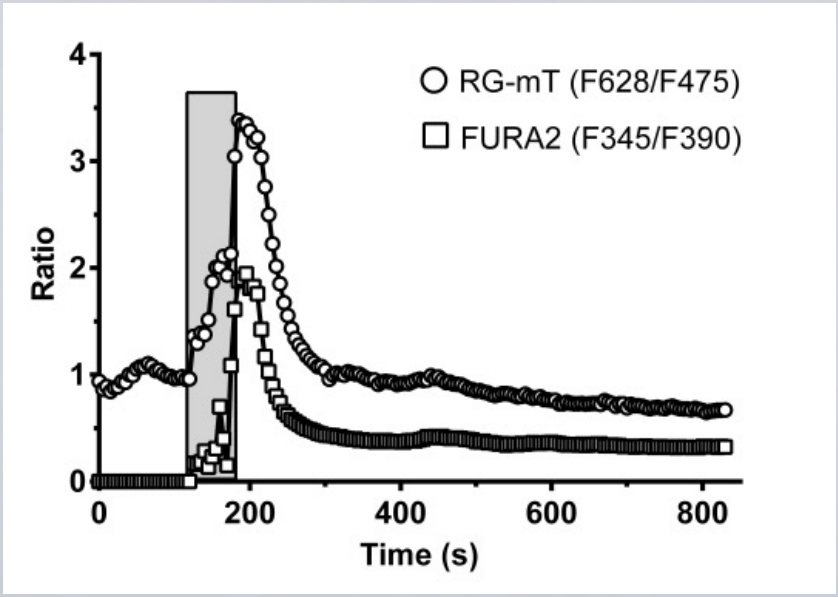






Imaging of cytosolic [Ca^{2+}] simultaneously with R-GECO1-mTurquoise and FURA2

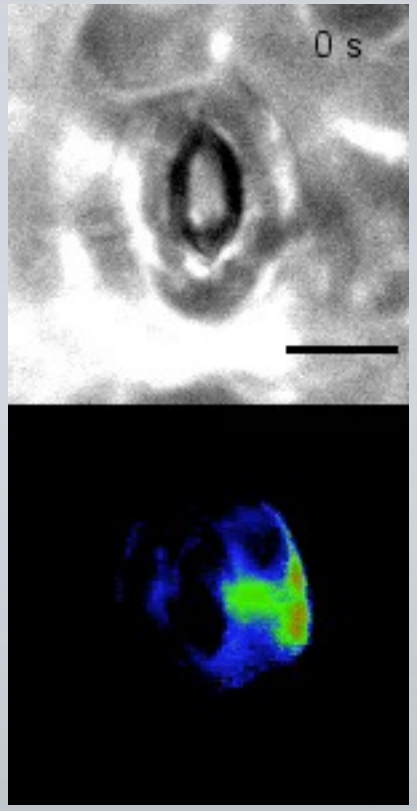


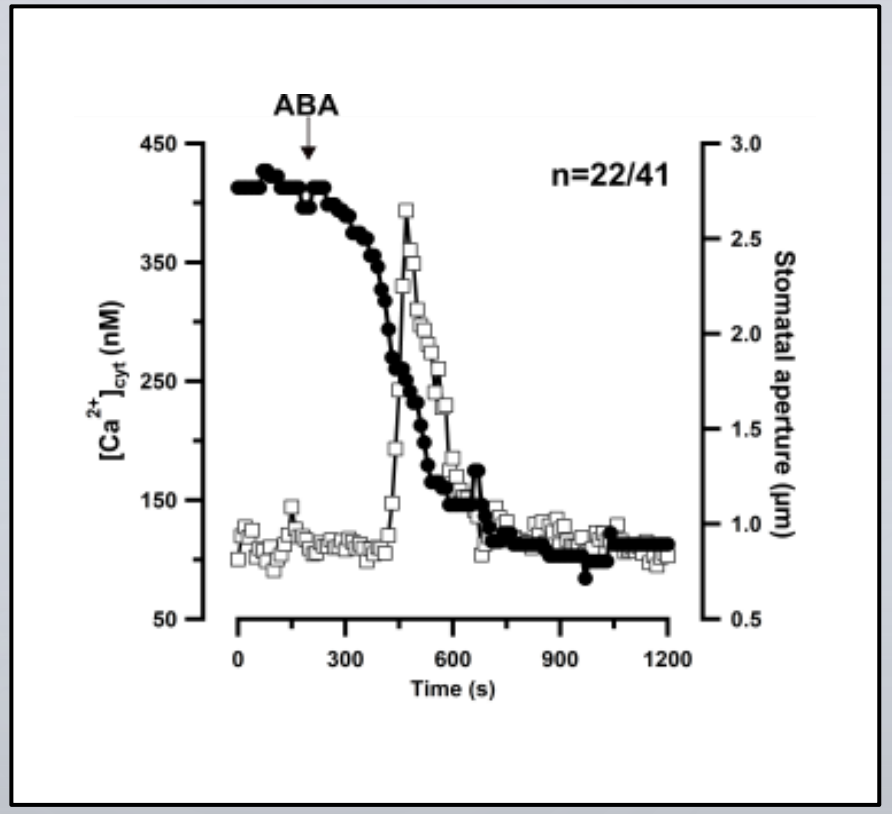


Collaboration with Rainer Waadt, Univ. of Heidelberg

Huang et al., New Phytologist, 2019

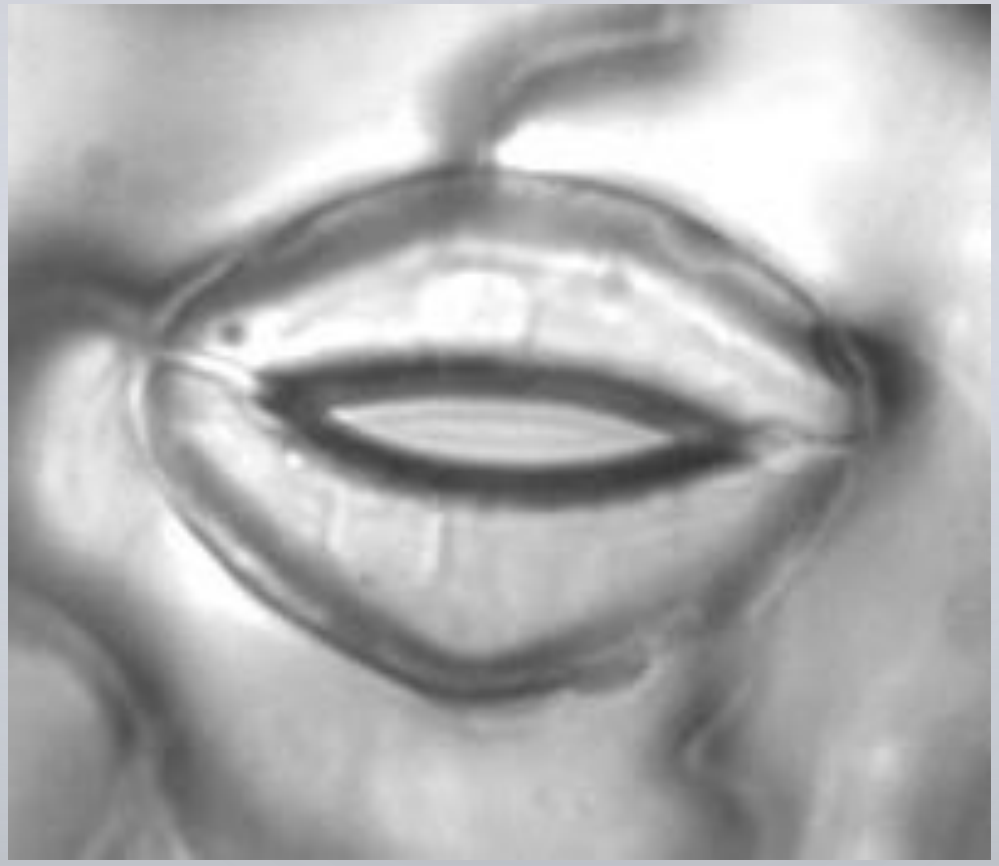
In most guard cells ABA causes Ca^{2+} signals during stomatal closure



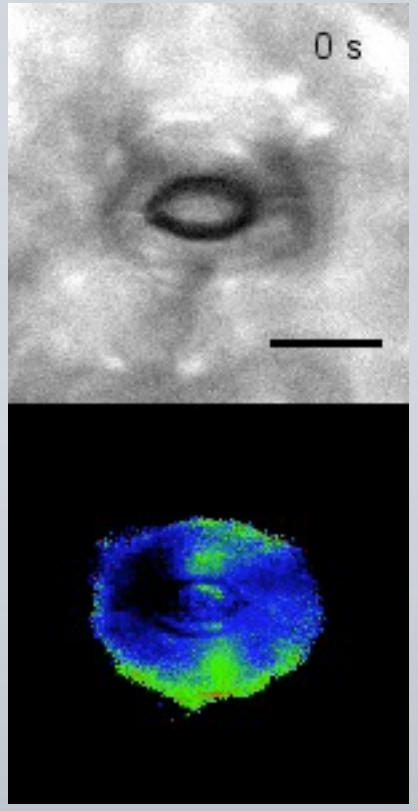


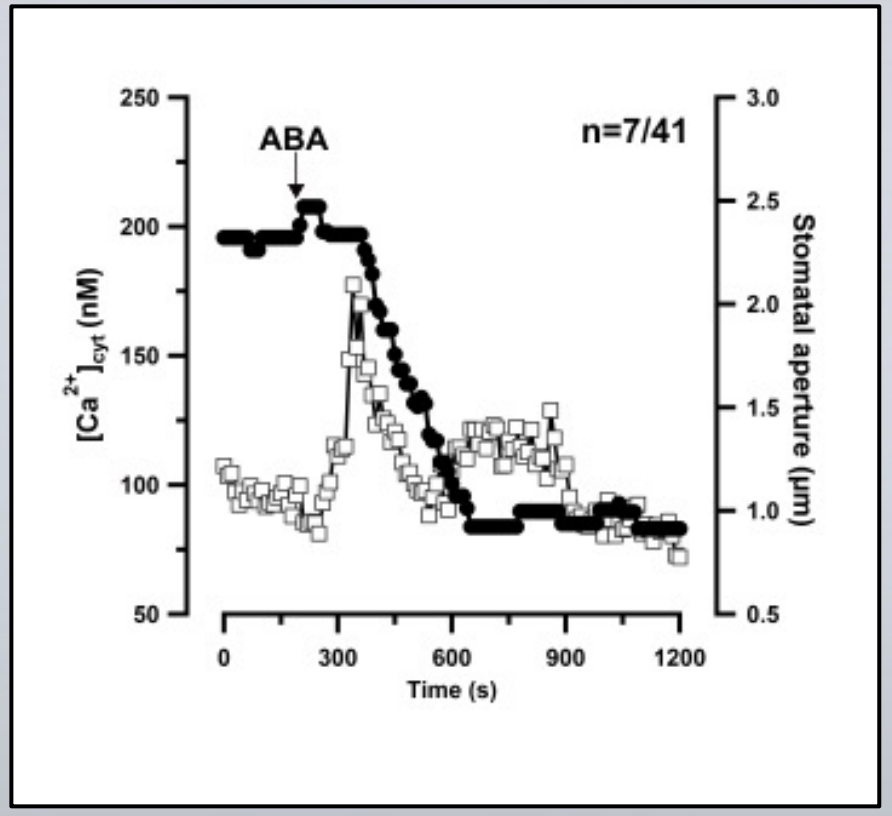
Huang et al., New Phytologist, 2019

Are ABA-induced Ca^{2+} signals due to changes in the osmotic value of the cytosol, during stomatal closure?



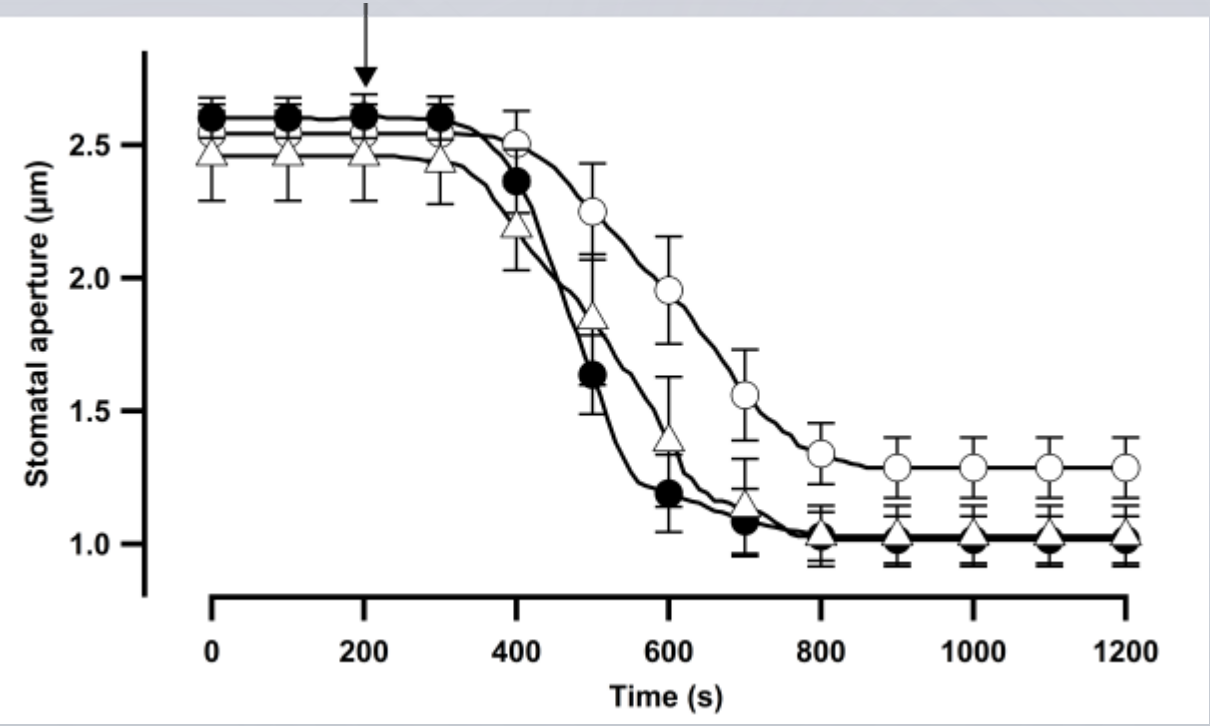
In 1 to 4 guard cells ABA causes Ca^{2+} signals before stomatal closure





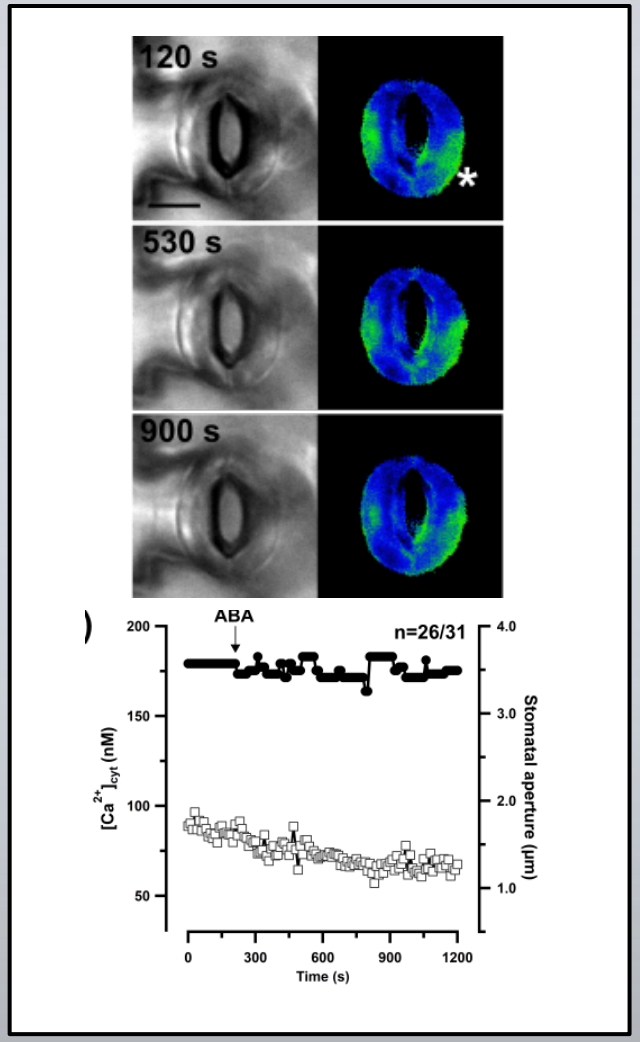
Huang et al., New Phytologist, 2019

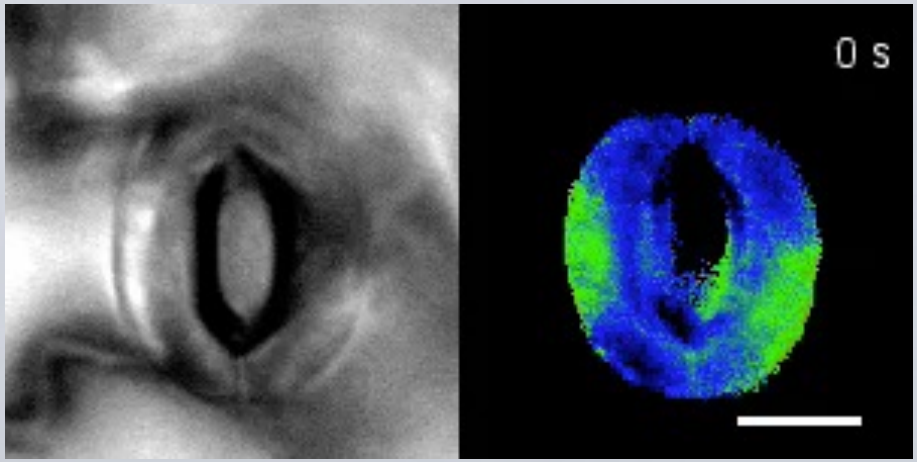
Are Ca^{2+} signals important for ABA-induced stomatal closure?



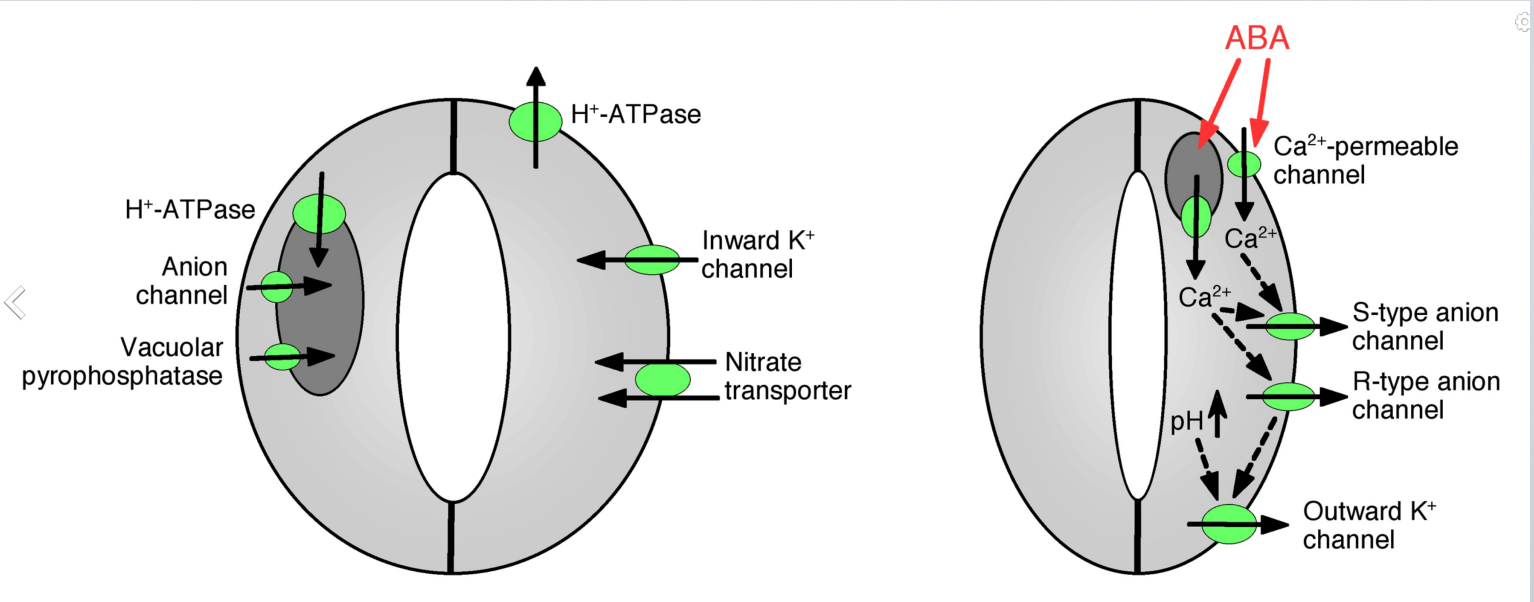
Huang et al., unpublished data

ABA neither triggers stomatal closure, nor Ca^{2+} signals in guard cells of the *ost1* mutant



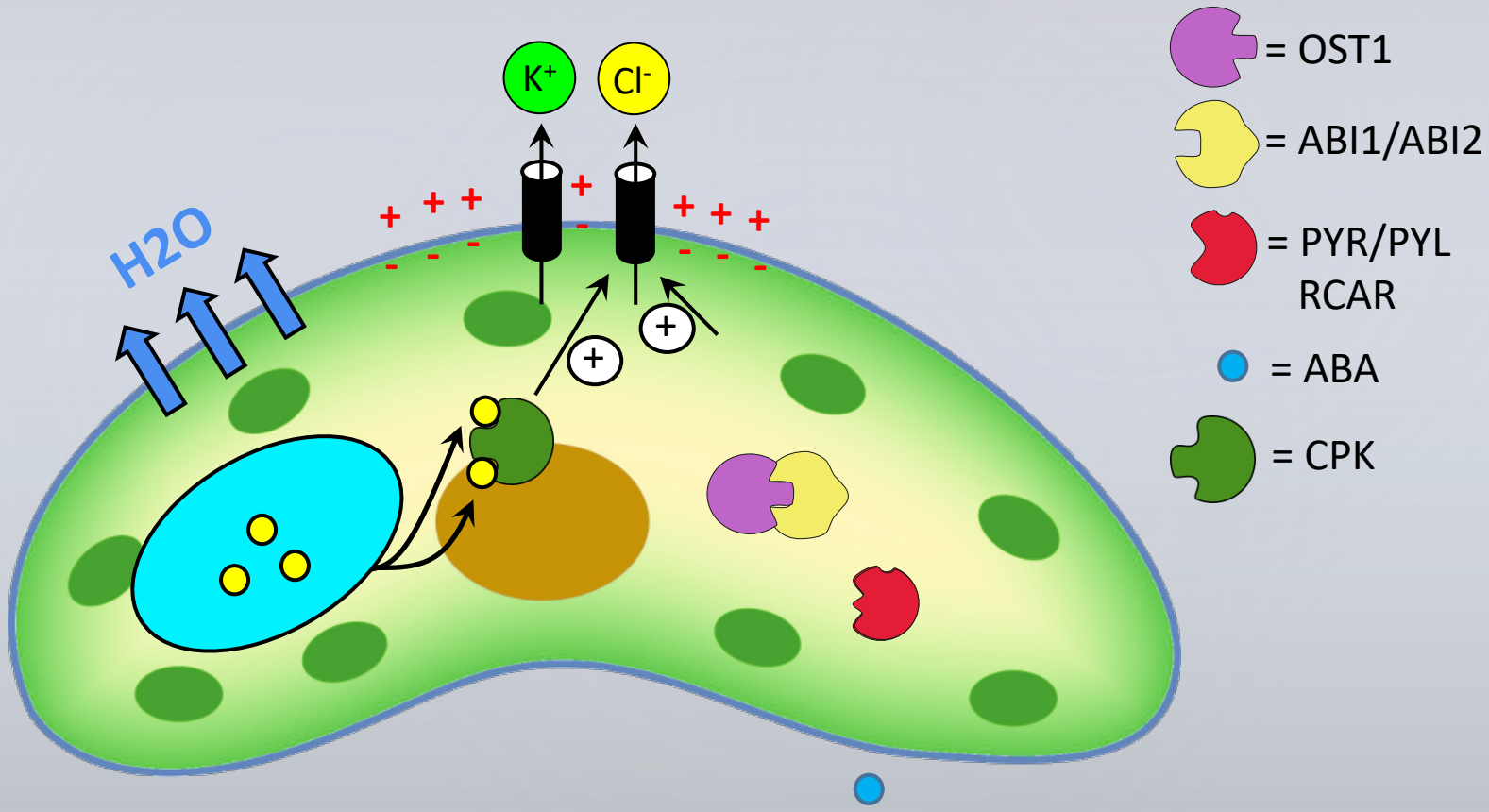


Ca^{2+} signals enhance guard cell responses to Abscisic Acid (ABA), but are not required for stomatal closure



https://en.wikipedia.org/wiki/Guard_cell

ABA-dependent activation of SLAC1 and SLAH3



Ende