



"Electrical and calcium signalling"

E&CS group



E&CS, a group formed on March 2009



← Dr. Claire Corratgé-Faillie (CDD Ing. Recherche)



← Dr. Tracey Ann Cuin (Post-doc Marie Curie)



← Dr. Erwan Michard (Post-doc Agropolis Fondation)



← Elsa Ronzier (PhD student)



← Frédéric Sanchez (TR)



← Dr. Jean-Baptiste Thibaud (DR)



← Dr. Tou Cheu Xiong (CR)





Disclosing the molecular basis of electrical and Ca^{2+} signalling in plants



Propagation of electrical signals in plants



Basipetal, then acropetal propagation of an electrical signal in Mimosa leaves

Electrical signalling and cytokinins mediate effects of light and root cutting on ion uptake in intact plants

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Plant, Cell and Environment (2007) 30, 249–257

doi: 10.1111/j.1365-3040.2006.01000.x

Electrical signals and their physiological significance in plants

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Planta (2009) 229:539–547
DOI 10.1007/s00425-008-0850-x

ORIGINAL ARTICLE

Dissection of heat-induced systemic signals: superiority of ion fluxes to voltage changes in substomatal cavities

Mathias R. Zimmermann · Hubert H. Felle

Plant, Cell and Environment (2009) 32, 319–326

doi: 10.1111/j.1365-3040.2008.01922

Heat-induced electrical signals affect cytoplasmic and apoplastic pH as well as photosynthesis during propagation through the maize leaf

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Voltage-dependent action potentials in *Arabidopsis thaliana*

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Planta (2007) 226:203–214

DOI 10.1007/s00425-006-0458-y

ORIGINAL ARTICLE

Systemic signalling in barley through action potentials

Hubert H. Felle · Matthias R. Zimmermann

Journal of Plant Physiology 168 (2011) 653–660

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Accession-dependent action potentials in *Arabidopsis*

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Role of electrical signalling in plants

WHAT DO PLANTS NEED ACTION POTENTIALS FOR?

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- trap closure and enzyme secretion (eg, Droseraceae): up to 250 mm/s !!
- fertilisation (pollen landing on the stigma): 10-30 mm/s
- defence (eg, *Mimosa pudica*): 4-40 mm/s
- defence/stress in non specialised plants/tissues
(cold, light/dark, heat/burning, etc.): 1-10 mm/s

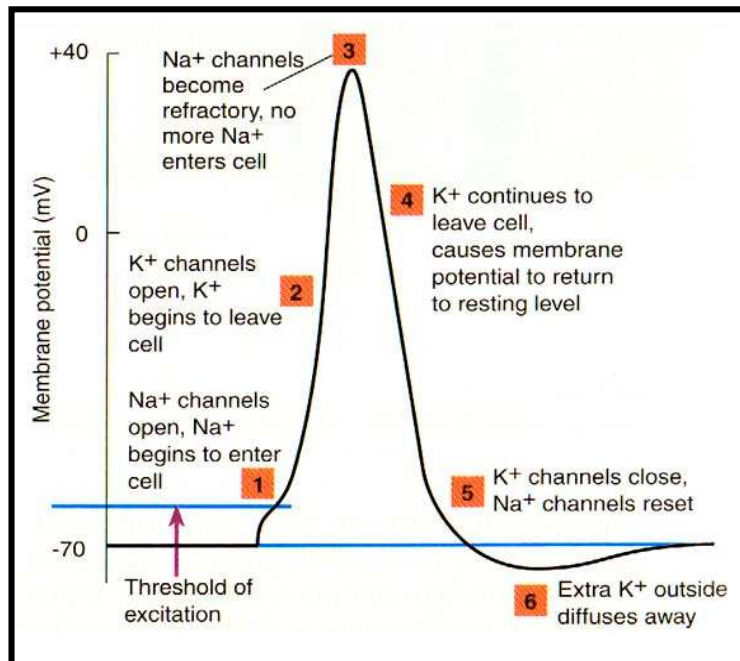
including *Arabidopsis*!



What is an action potential?

- Self-propagating, transient electrical signal
- Rapid depolarisation then repolarisation of membrane potential
- Travels from cell to cell with constant speed

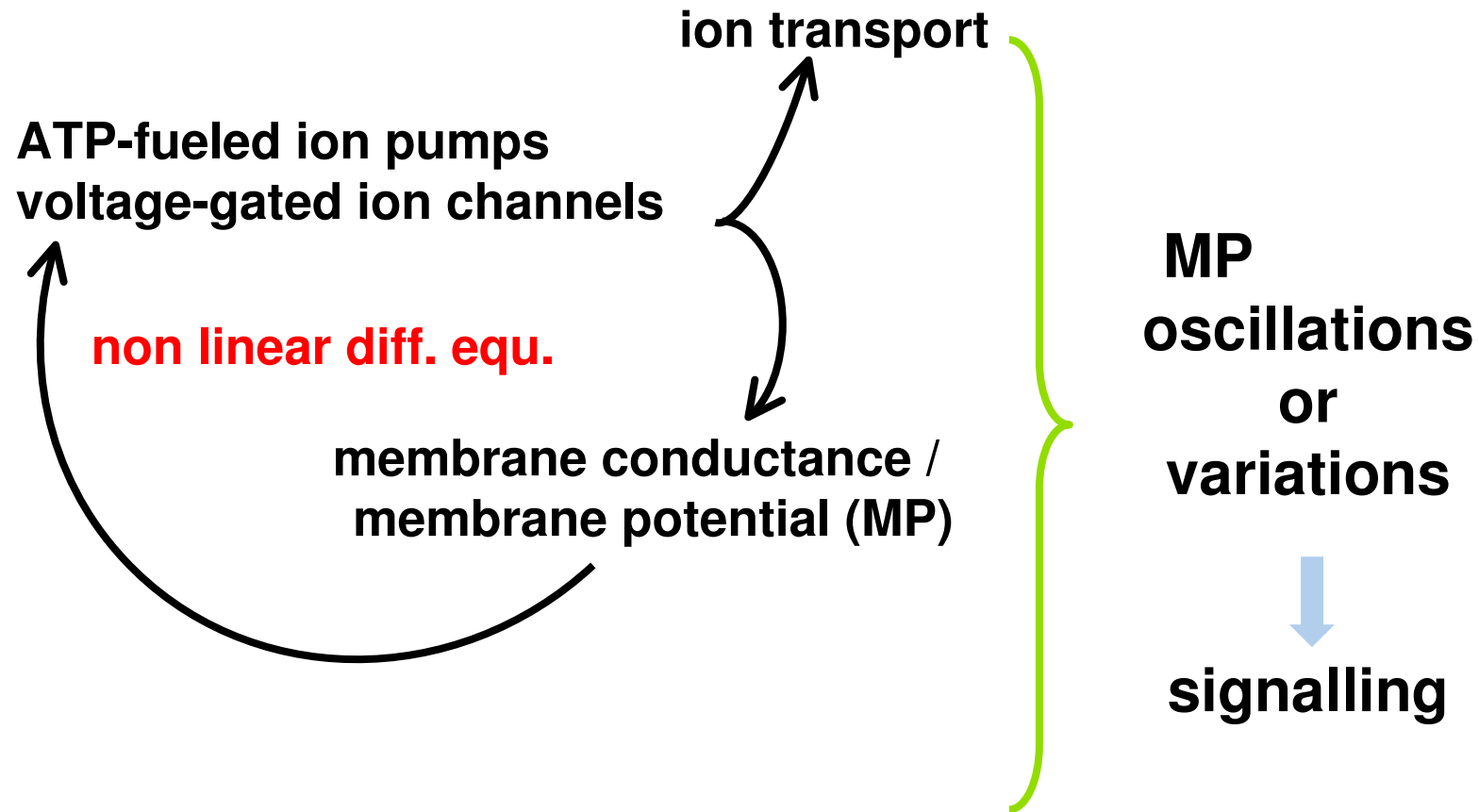
In animal cells..



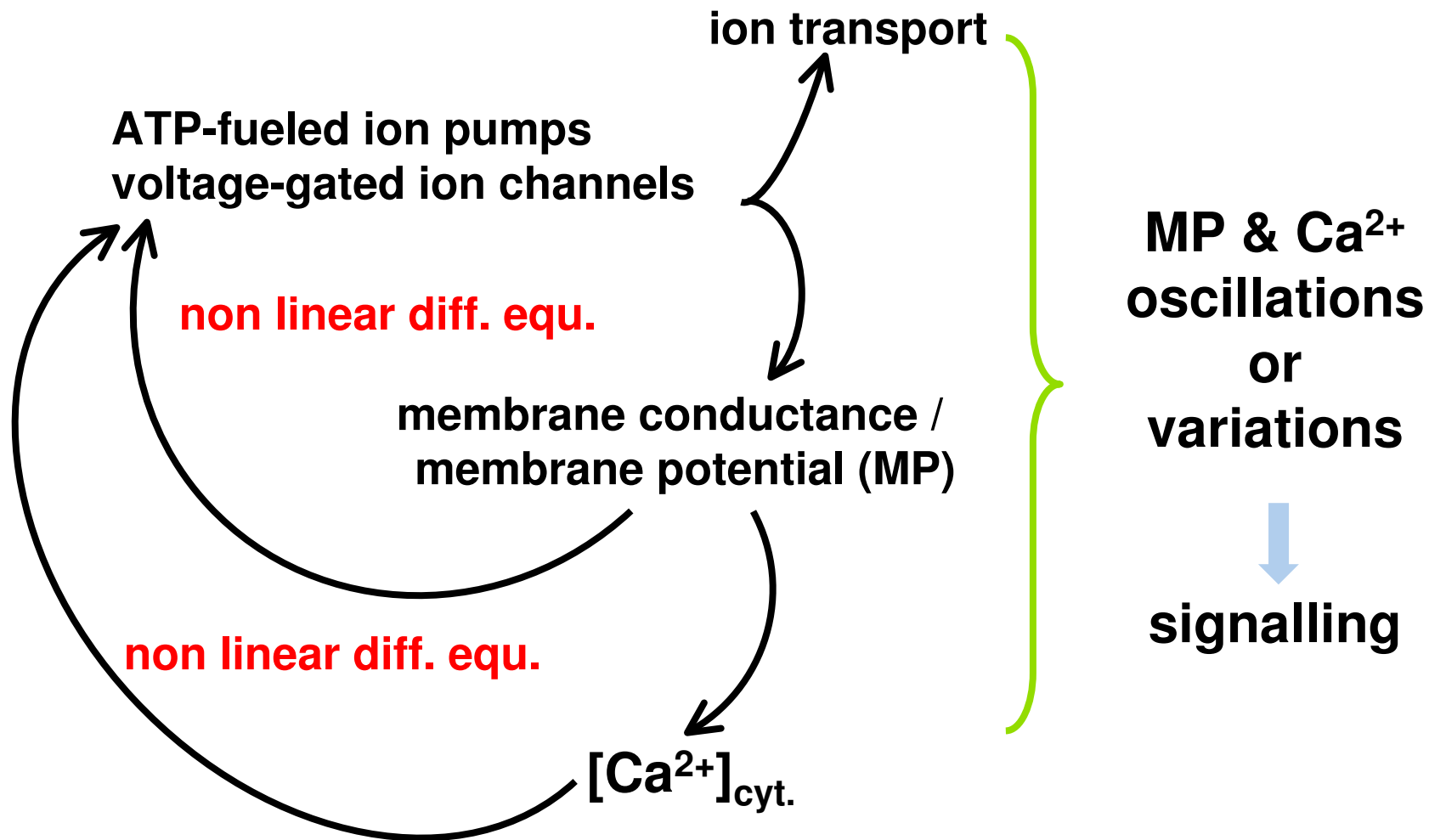
In plants...

- everything is much slower than in animals
- no Na⁺ involved in plant APs
- ion fluxes in plants during AP:
 - Ca²⁺ influx into the cell
 - then Cl⁻ & K⁺ efflux
- but we don't know which channels are involved

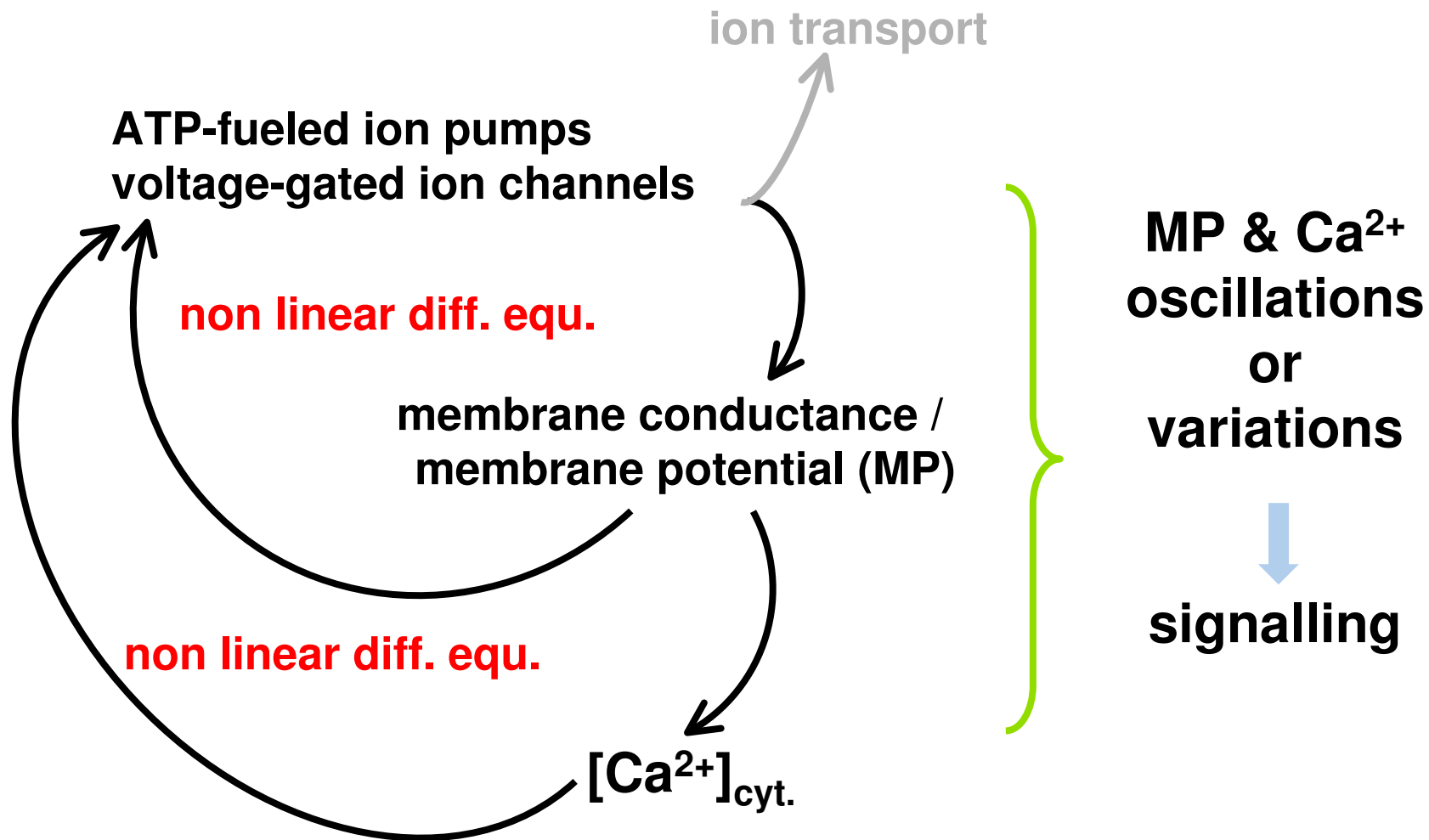
Need for mathematical modelling



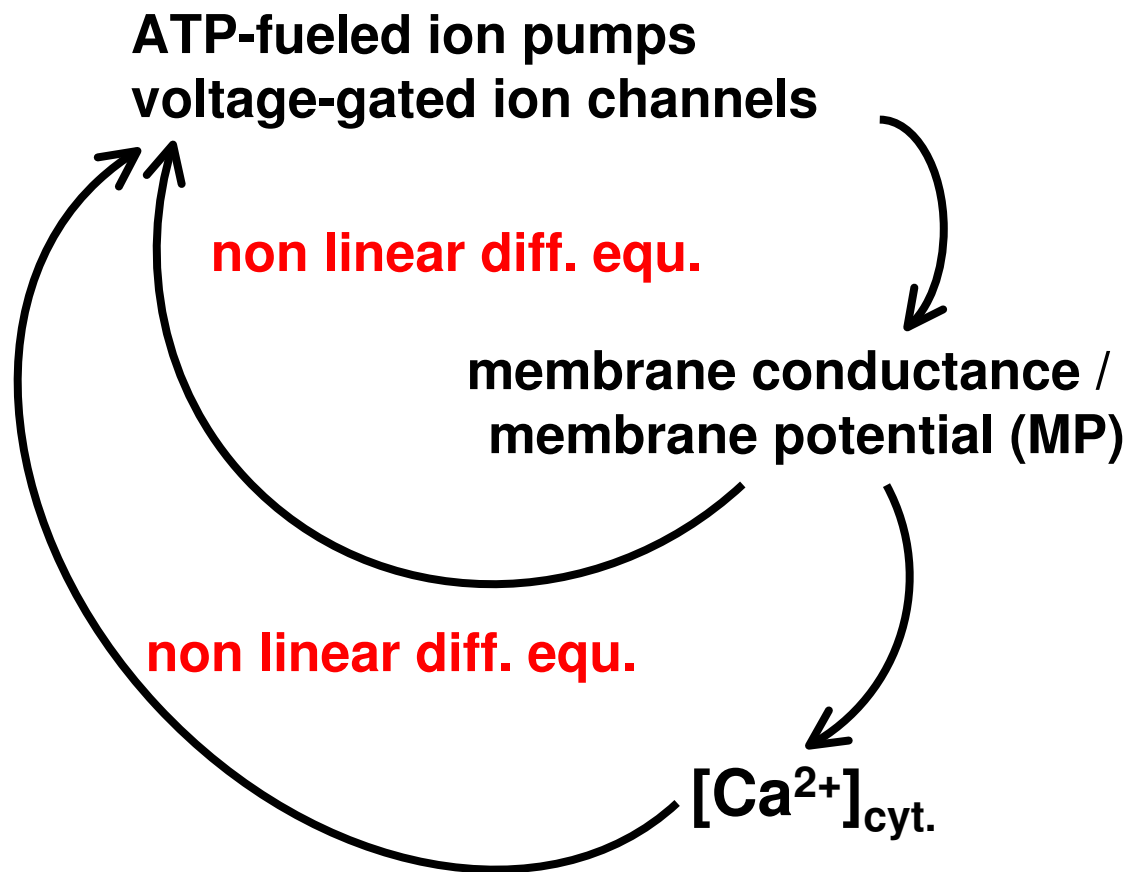
Need for mathematical modelling



We focus on voltage (and Ca^{2+}) waves



Our research aims



Addressed questions

- which ion transporters contribute to APs?
- how do they interplay to make an AP?
- how do APs travel?
- how is Ca^{2+} involved?
- do Ca^{2+} waves occur and travel?

E&CS group: the aims

- **to uncover the molecular mechanisms behind electrical signals in plants**
- **to disclose mechanisms of regulation by Ca^{2+} of K^{+} voltage-gated channels**
- **to establish *in planta* Ca^{2+} imaging at BPMP**
- **to evidence Ca^{2+} waves travelling through plant tissues**

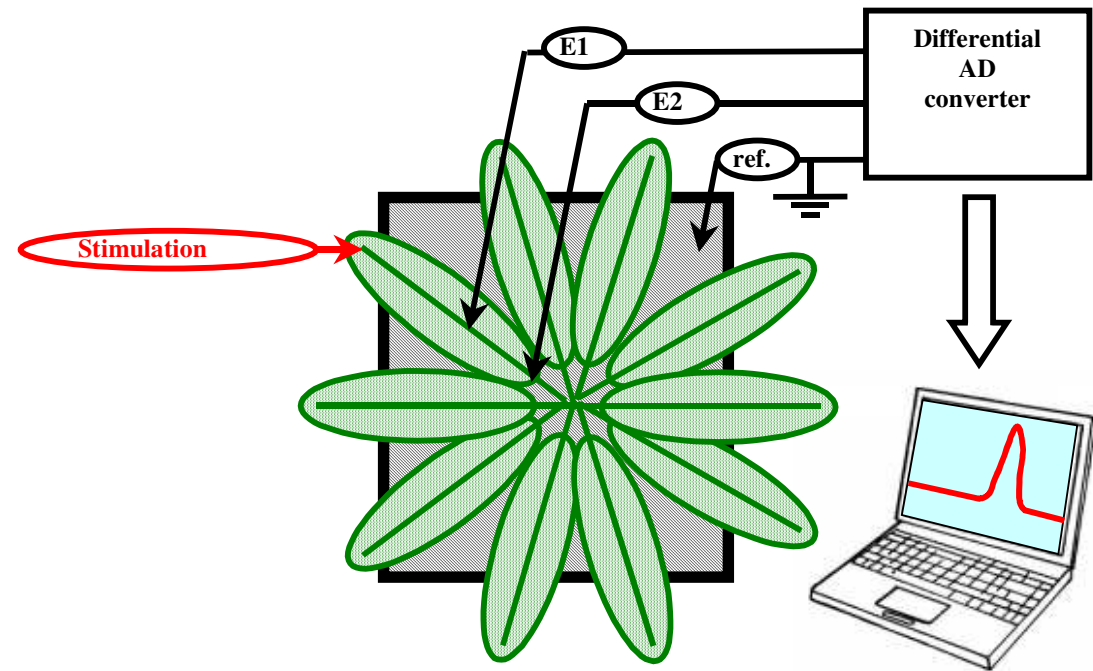
E&CS group: the tasks

- Install AP recording at BPMP
- Compare action potentials in WT and mutant lines (*AKT2* and *GORK* genes)
- Computer-assisted modelling:
a single-cell ("0D" model),
a file of cells ("1D" model)
a complex network of cells ("2D/3D" model)
- Regulation of Shaker channels by CPKs
- New tools for Ca^{2+} imaging
- Ca^{2+} waves in relation to APs?

Recording action potentials



- electrical stimulation



- mechanical stimulation



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The AKT2 channel controls "excitability"

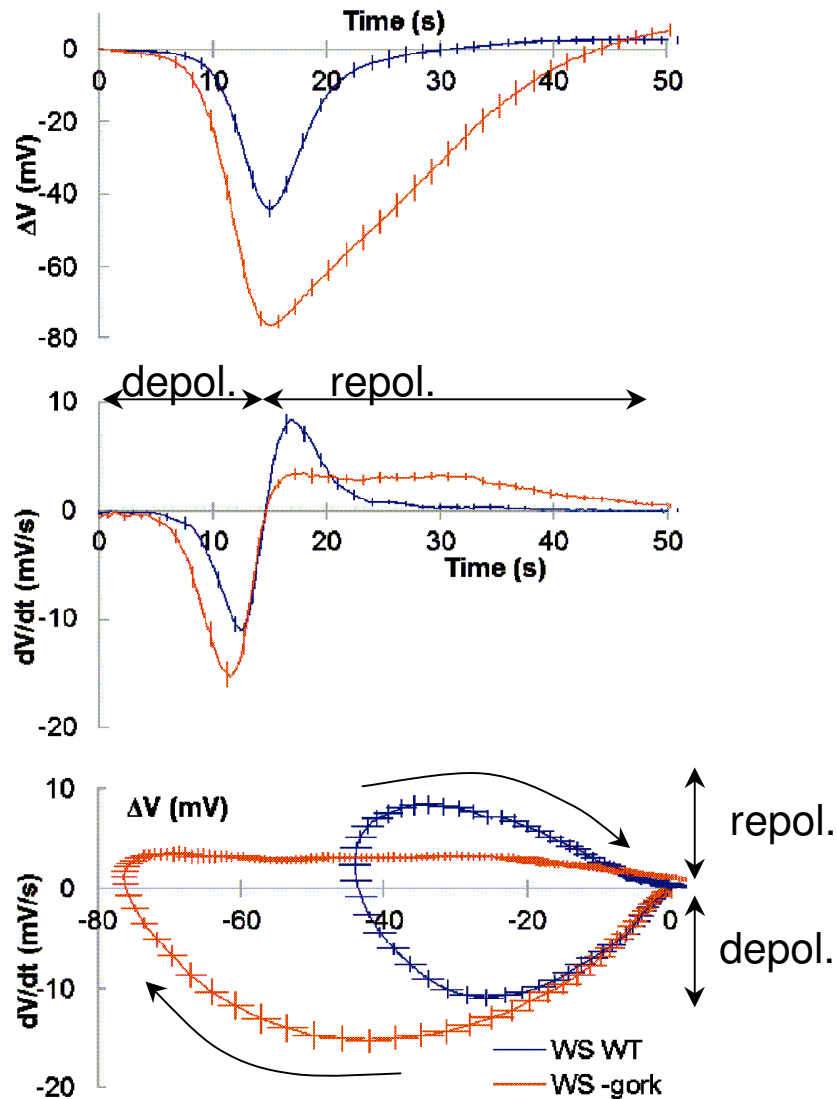
Line	WS ecotype			Col-0 ecotype	
	<i>akt2-1</i>	WT	AKT2-NN	<i>akt2-2</i>	WT
Excitability (%)	41%	51%	59%	63%	88%
Amplitude (mV)	38.1 ± 10.6	47.2 ± 11.2	53.0 ± 9.2	52.0 ± 12.3	62.2 ± 5.5
Width (sec)	6.6 ± 2.1	10.9 ± 4.2	6.7 ± 1.6	7.8 ± 2.5	7.8 ± 1.5
Speed (mm s ⁻¹)	1.2 ± 0.4	1.3 ± 0.3	1.1 ± 0.4	1.0 ± 0.2	1.2 ± 0.4

WS plants are less excitable than Col-0 plants

knocking-out the *AKT2* gene decreases excitability

AKT2 (phosphorylated) increases excitability

The GORK channel controls the shape of APs



knocking-out GORK lengthens and amplifies the AP

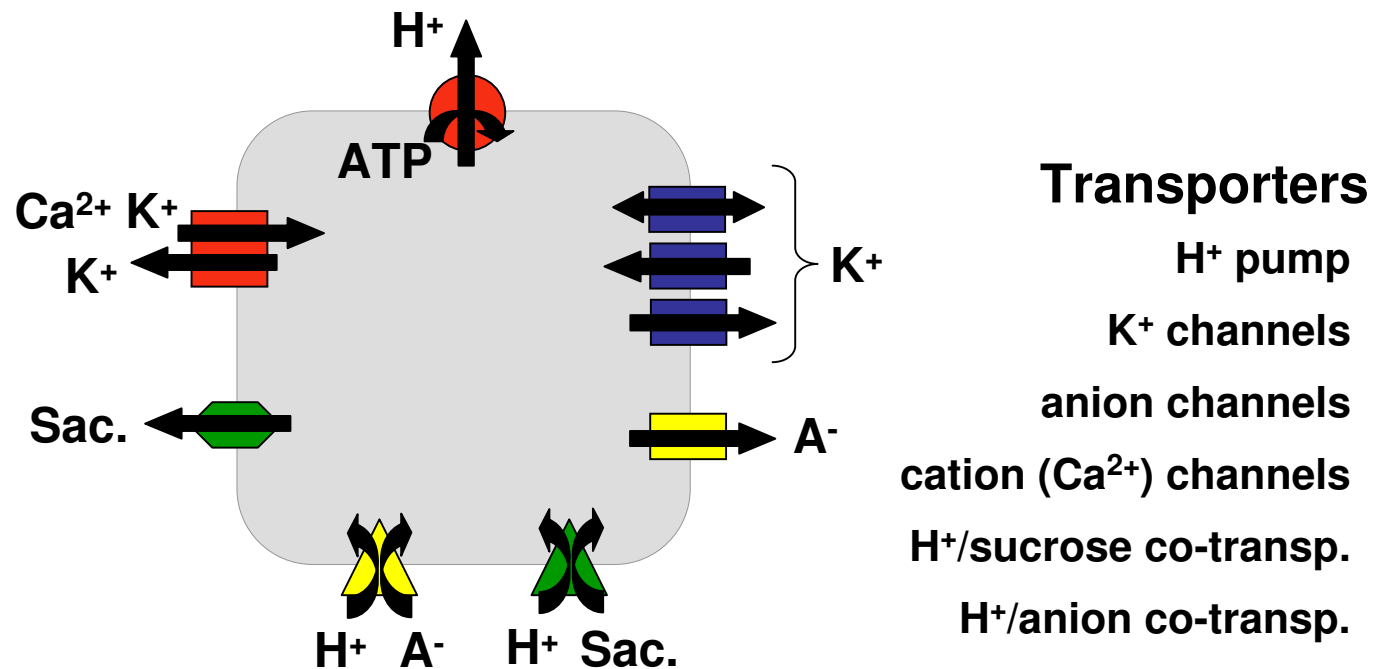
knocking-out GORK has little effect on depolarising currents but flattens time-course of repolarising currents

knocking-out GORK suppresses the voltage-dependence of repolarising currents

GORK is the main V-gated repolarising transporter

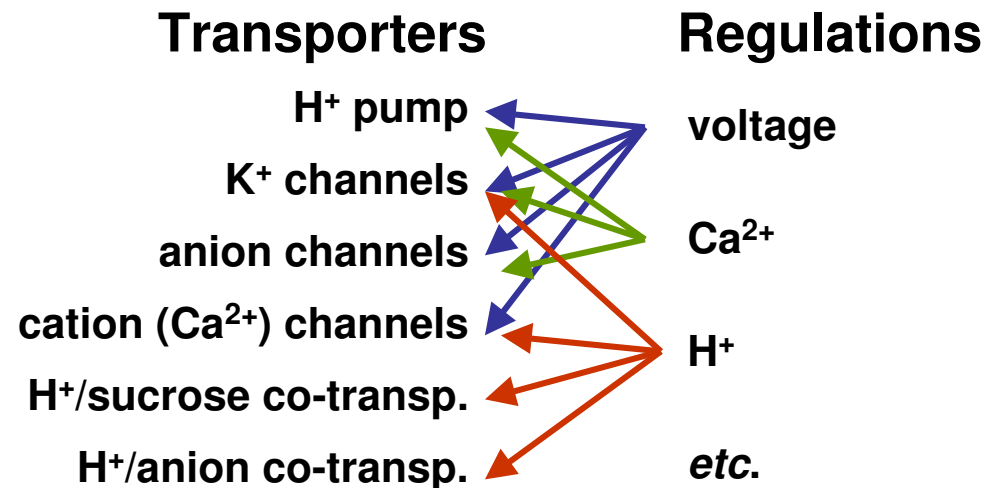
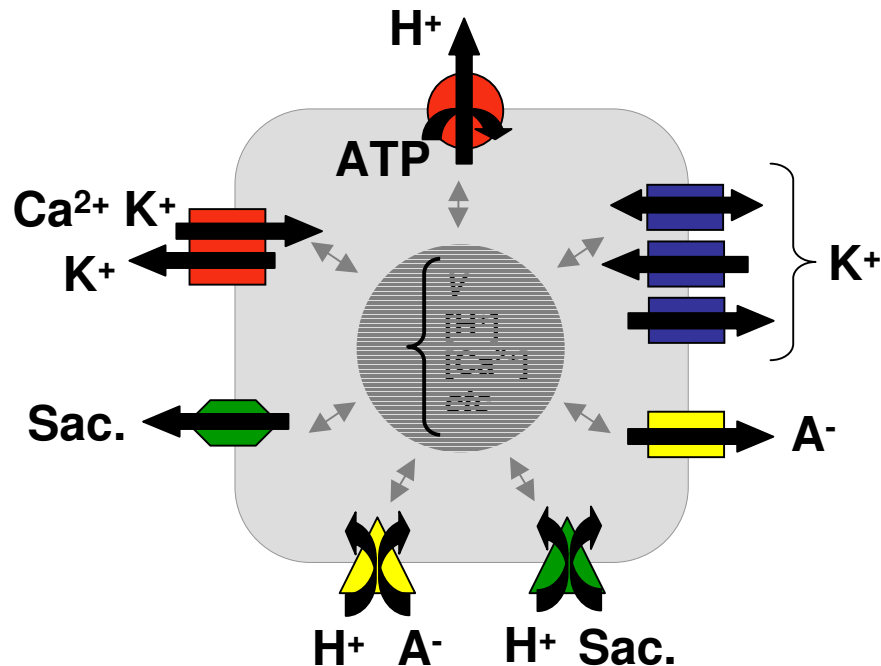
What about the model?

functional model of a cell membrane



What about the model?

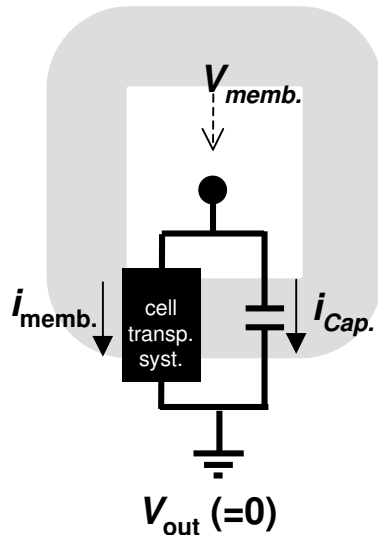
functional model of a cell membrane
+ transporter regulations



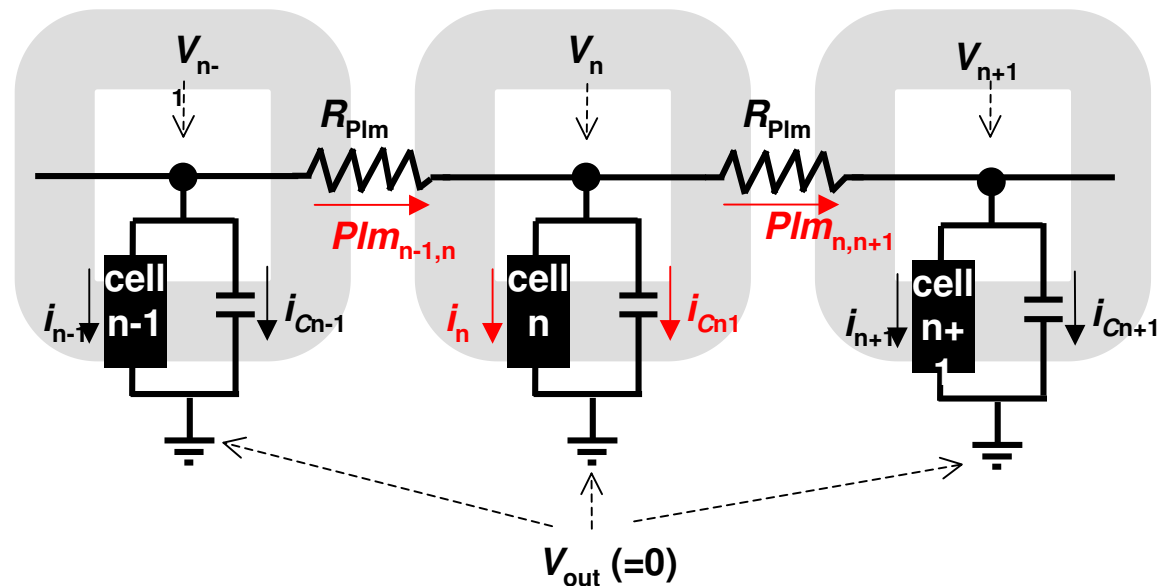
application of laws of biophysics and biochemistry
(Nernst, Goldman-Hodgkin-Katz, mass action, Michaelis...)

What about the model?

electrical model of a cell membrane...



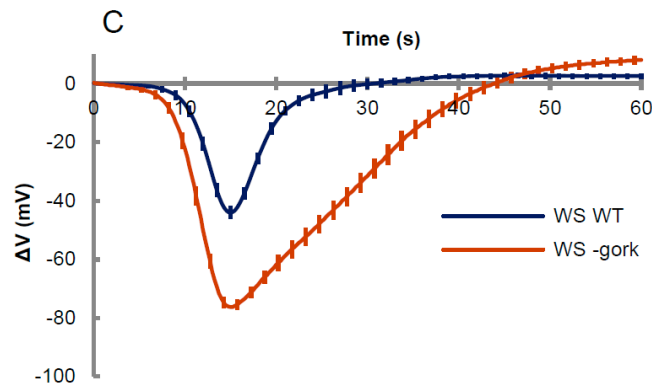
... and of a file of cells



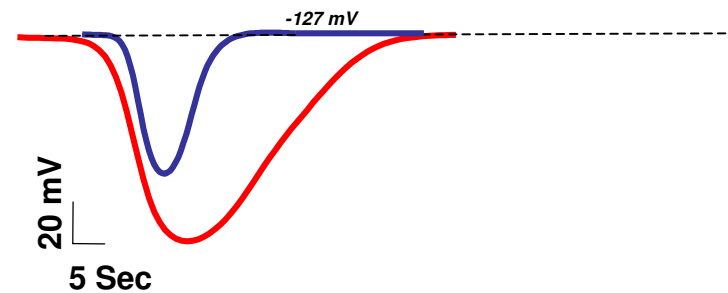
application of laws of electricity (Kirchhoff, Ohm...)

The model confirms experimental results

- exp. WT/*gork*-



- modelled exp. WT/*gork*-



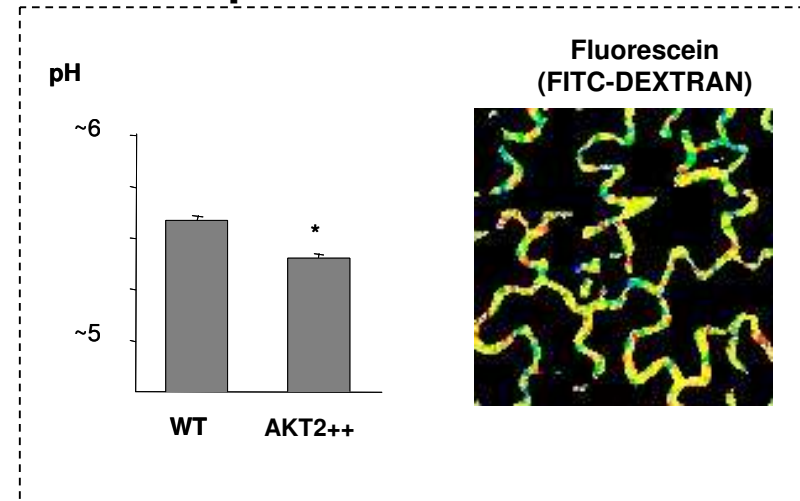
knocking out the *GORK* gene

- increases the AP amplitude
- lengthens de AP (slows the repolarisation phase)
- lets the propagation speed unchanged

Model predictions

- overexpressing AKT2 should decrease the leaf apoplastic pH

Experimentation



- ABA application should trigger APs in leaves
- propagating APs make "Ca²⁺ waves"

soon tested!

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