Bioelectricity of non-excitable cells: modeling of instructive multicellular patterns

*Javier Cervera, Michael Levin, Salvador Mafe**

Universitat de València, Spain, EU
Allen Discovery Center at Tufts University, MA, USA

Introduction

1) In *developmental processes*, a cell needs both individual *independence* to respond to external stimuli and multicellular *interdependence* with the rest of the multicellular aggregate. Crucial information concerning the body plan must reside within the *DNA*, a *single-cell language* for making proteins. However, a *multicellular language* is also needed to coordinate shape and function.

2) Here, we explore a *bioelectrical* multicellular language based on two experimental assumptions:

    2.1) *transcription* provides voltage-sensitive *ion channel* and intercellular *gap junction* proteins that regulate the *cell membrane potential*. In turn, the cell potential allows *feedback* mechanisms that modulate the *transcription* of these proteins (section 4b); and

    2.2) the spatio-temporal *pattern* of *membrane potentials* constitutes a *bioelectrical network* that contributes to the required *multicellular coordination* (section 5).

It is important to note that *point 2.1* allows a *full control* of the *bioelectrical hardware* (ion channel and gap junctions) which is crucial for the *bioelectrical software* of *point 2.2*.

Objectives

We describe a *qualitative model* of multicellular *bioelectrical networks* based on cell membrane potentials that are regulated by single-cell *ion channels* and intercellular *gap junctions.* In this model:

1) the bioelectrical concepts are complementary to traditional biochemical and biomechanical concepts and describe how small *bioelectrical asymmetries* or *disruptive events* can trigger downstream processes where *bioelectricity* and *transcription* are coupled. These events can occur at *random locations*, activating *programs* that *enhance* or *suppress* the *initial change* in the multicellular system;

2) we attempt to reduce biological complexity by identifying a small number of observable magnitudes that may control *crucial steps* in different processes, making emphasis on *bioelectrical mechanisms.* Subsequently, we suggest *operational actions* based on *average multicellular potentials*; and

3) we emphasize a *model limitation*: *operational actions* are not only *system-* but also *context-dependent* because of the *single-cell* and *multicellular* bioelectrical feedbacks of real systems.

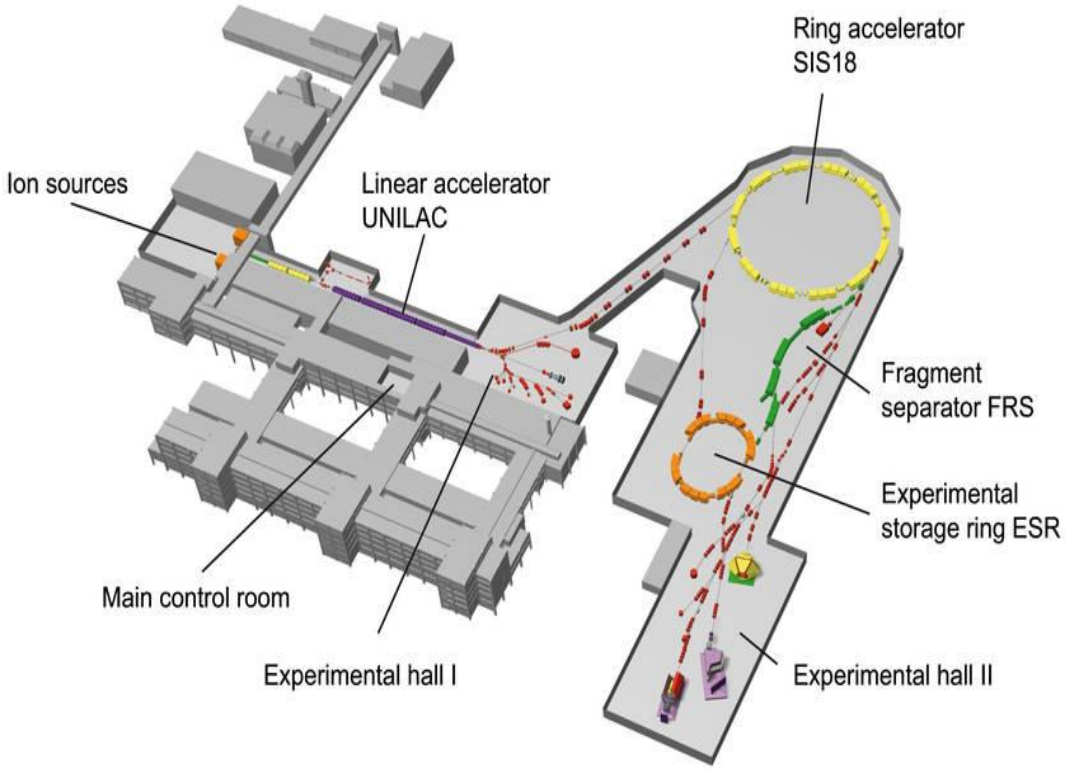
1. *Where we (Javier and Salvador) come from*
2. Multicellular organization: some bioengineering views
3. Some experimental facts of bioelectricity + molecular biology
4. Bioelectrical model assumptions and equations
5. Theoretical results of qualitative relevance
6. *Where we go*: identifying key bioelectrical steps in biological complexity

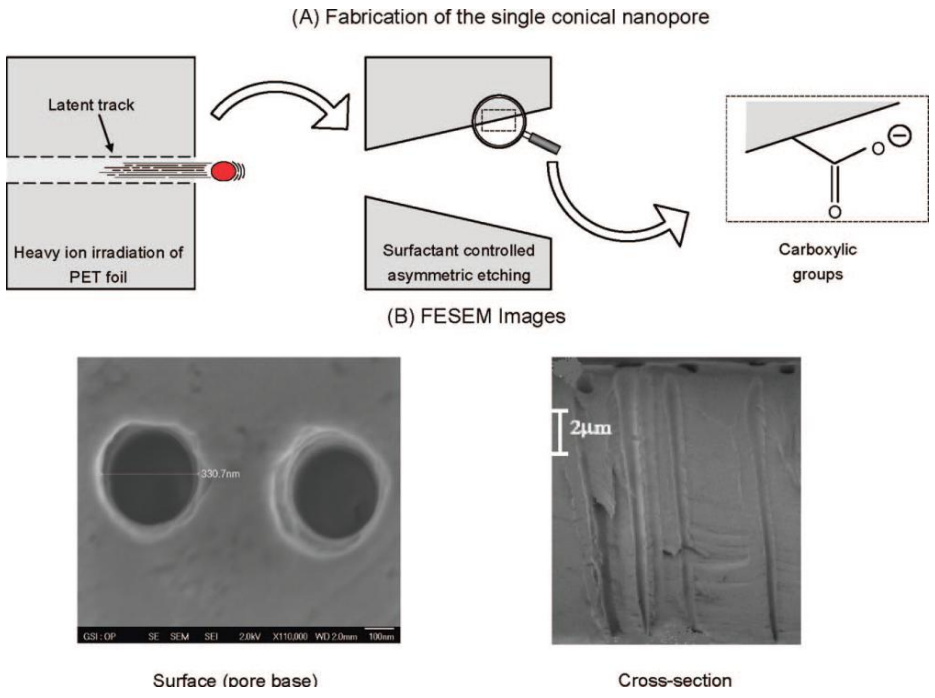
1. Where we (Javier and Salvador) come from

ACS Nano 2009
10.1021/nn900039f
Adv Funct Mat 2012
10.1002/adfm.201102146

*1a Applied Physics/Nanoscience/Biomimetic nanopores (with **Patricio Ramirez**)*

Biomimetic nanopores with voltage-gated conductances and memory effects provide *qualitative insights* on biological ionic currents. Single conical nanopore fabricated by irradiation of a polymer foil (polyethylene terephthalate or polyimide) at the linear accelerator *UNILAC* with single swift heavy ions (Pb, U, and Au) of energy 11.4 MeV per nucleon and chemical etching.



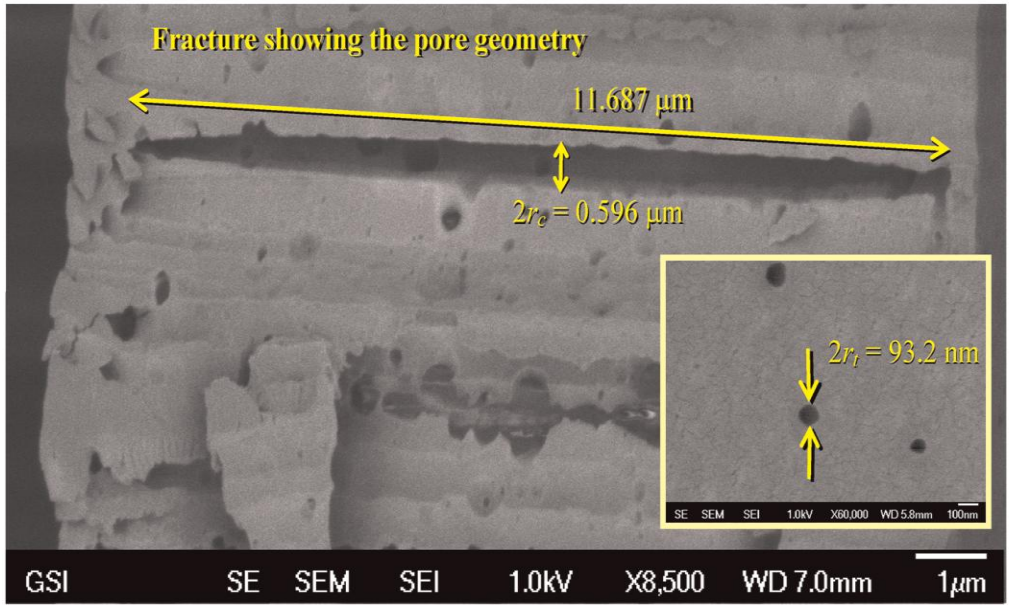


GSI Helmholtzzentrum für Schwerionenforschung GmbH

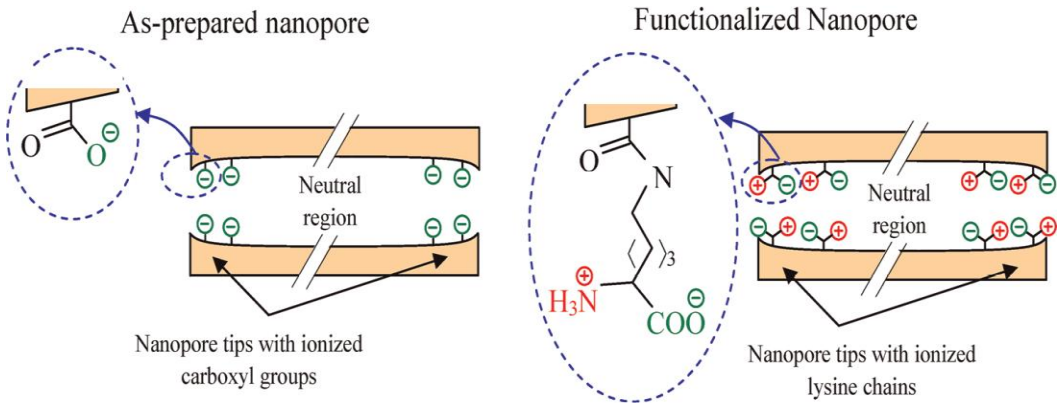
nanopores as chemically-gated transistors (with M. Ali and W. Ensinger)

ACS Nano 2012 10.1021/nn3010119
ACS Nano 2012 10.1021/nn303669g

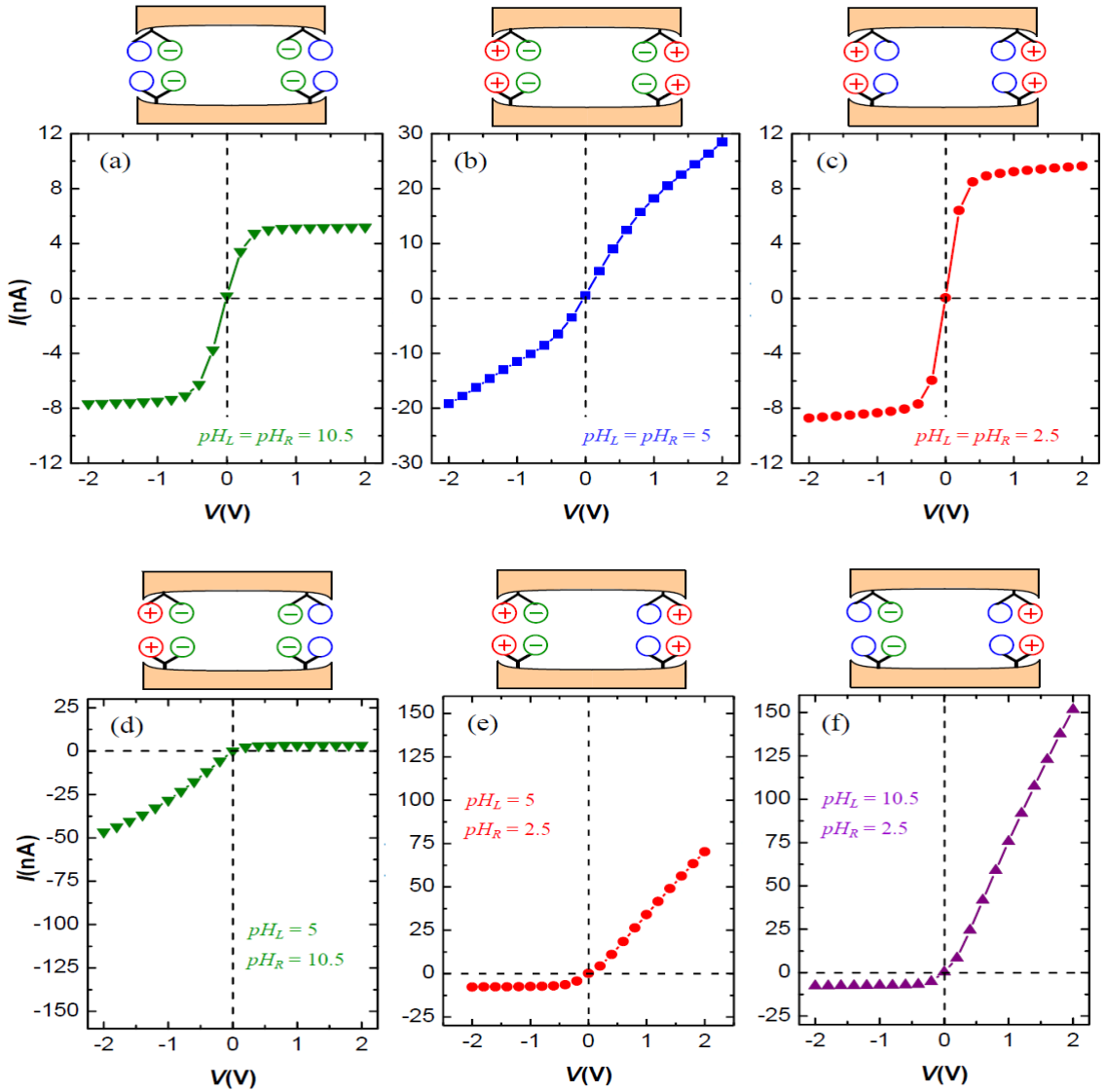
(a) Pore geometry



(b) Cigar-shaped pores after functionalization

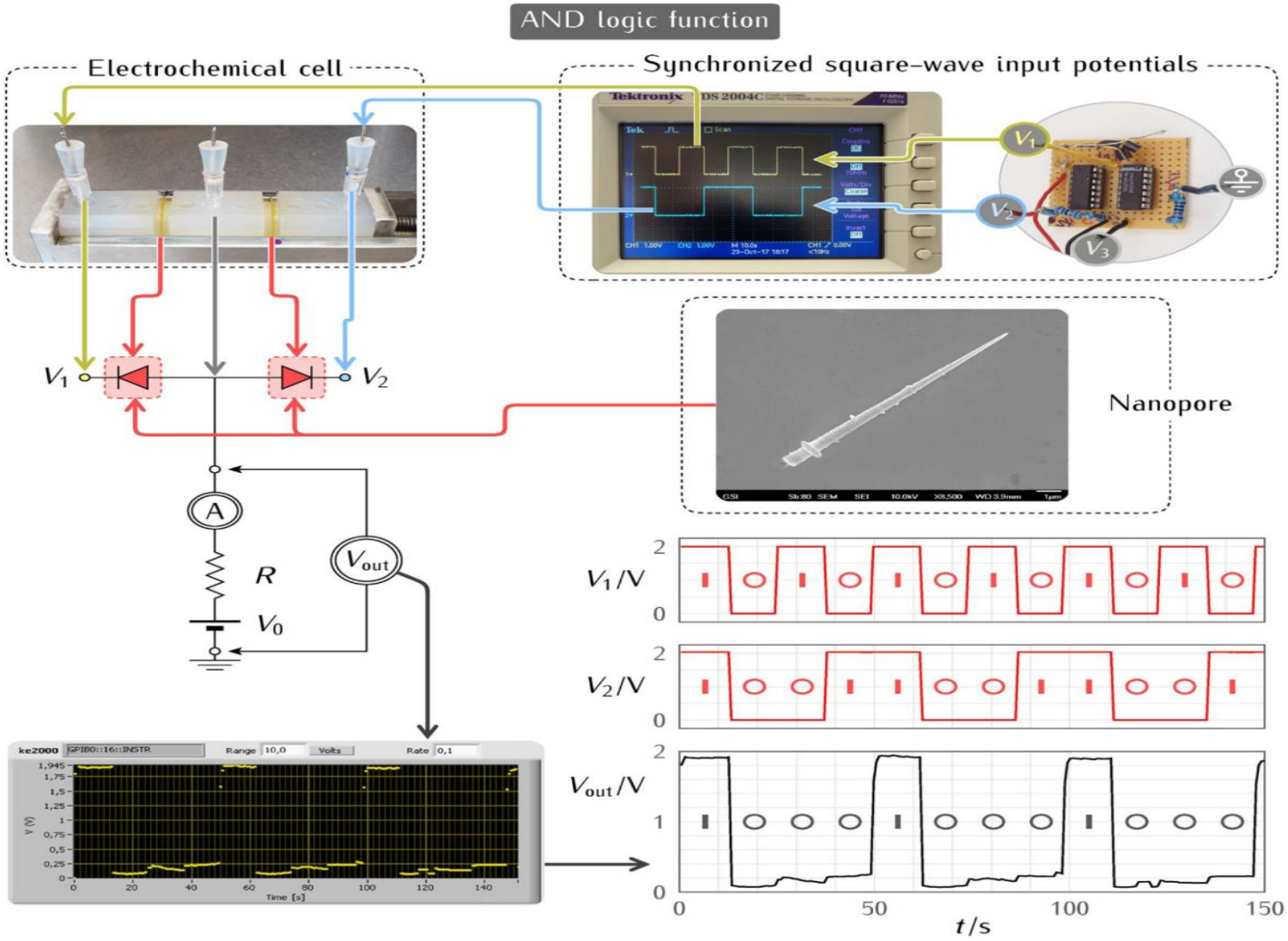


I-V curves of cigar-shaped nanopores functionalized with lysine chains



nanopore logical functions (with Vicente Gómez)

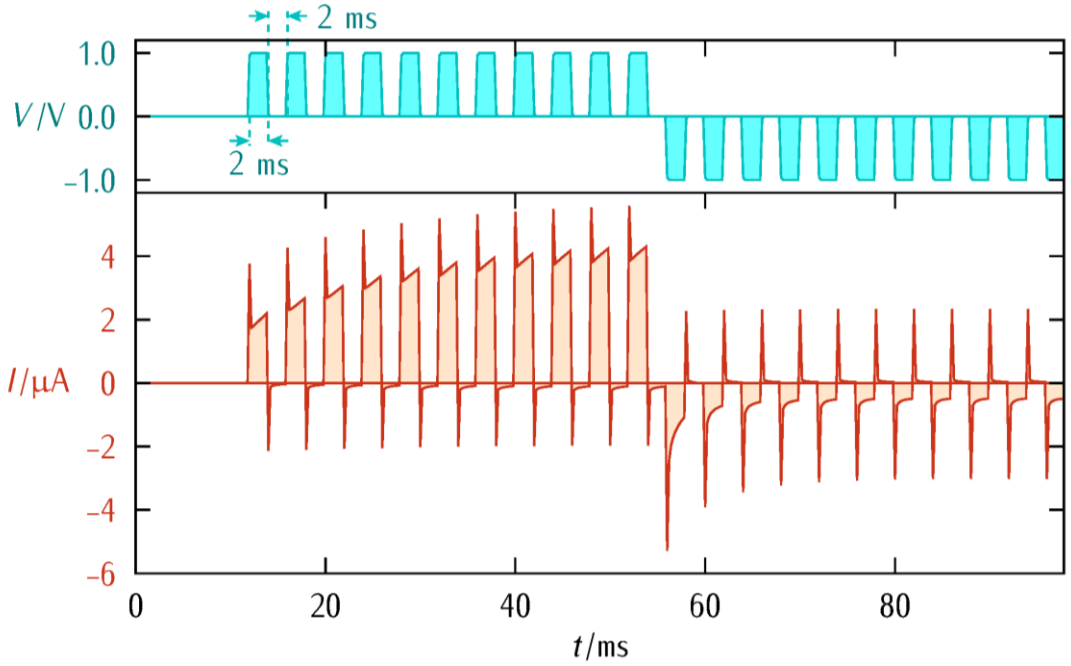
Phys. Rev. Applied 2017
10.1103/PhysRevApplied.7.064035
Appl. Phys. Lett. 2012, 2016
10.1063/1.4754845
10.1063/1.4954764
Electrochem. Commun. 2018
10.1016/j.elecom.2018.01.016

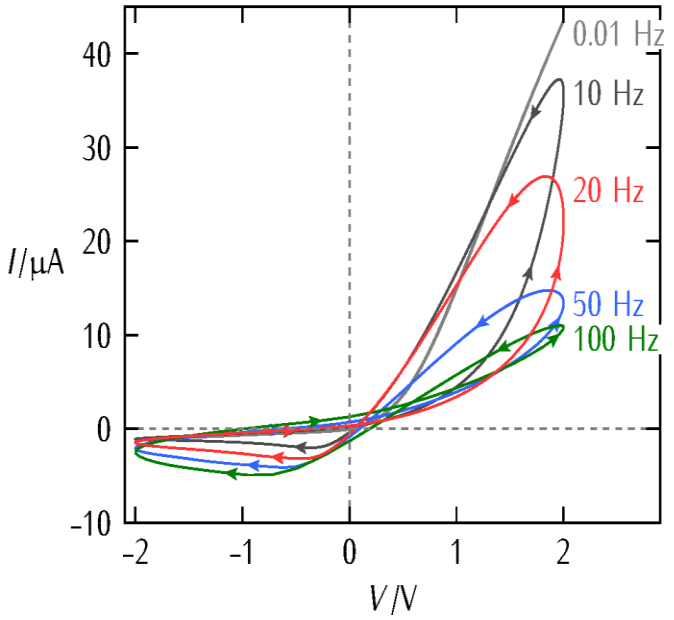


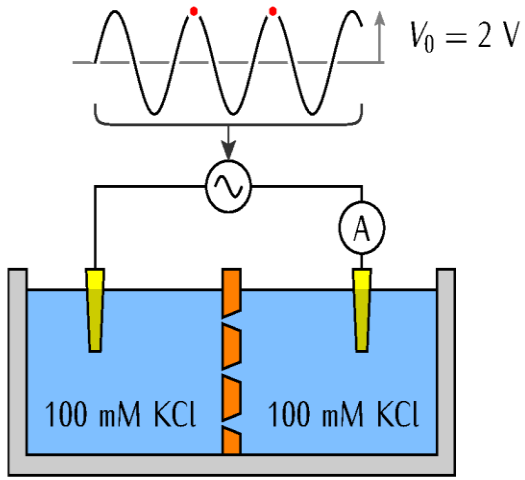
nanopores as rectifying neuromorphic memristors (with Juan Bisquert and Sergio Portillo)

J. Phys. Chem. Lett. 2023
10.1021/acs.jpclett.3c02796
Phys. Fluids 2024
10.1063/5.0204219

frequency-dependent ionic currents
and *voltage-gated* pore conductances





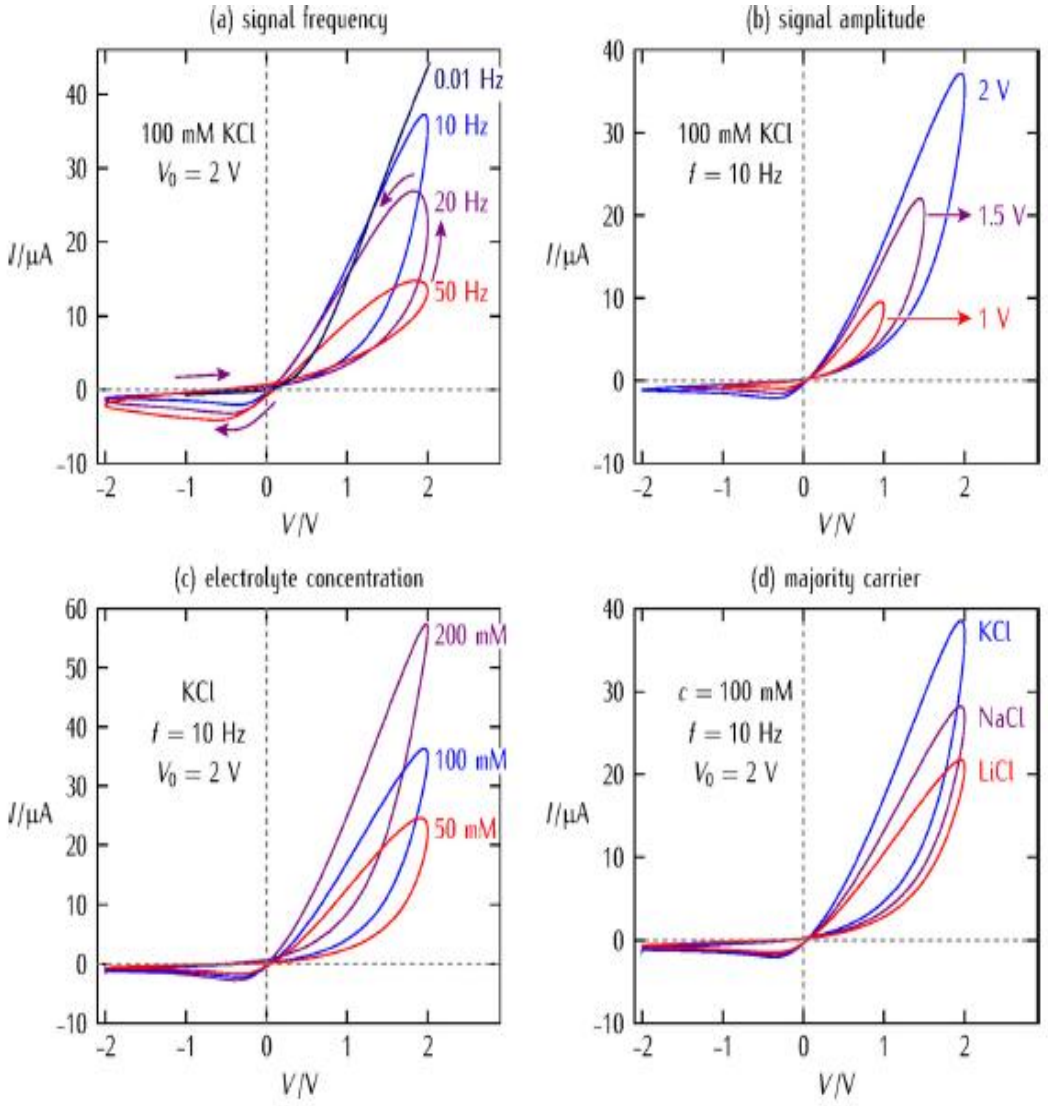


conductance *potentiation/depression*

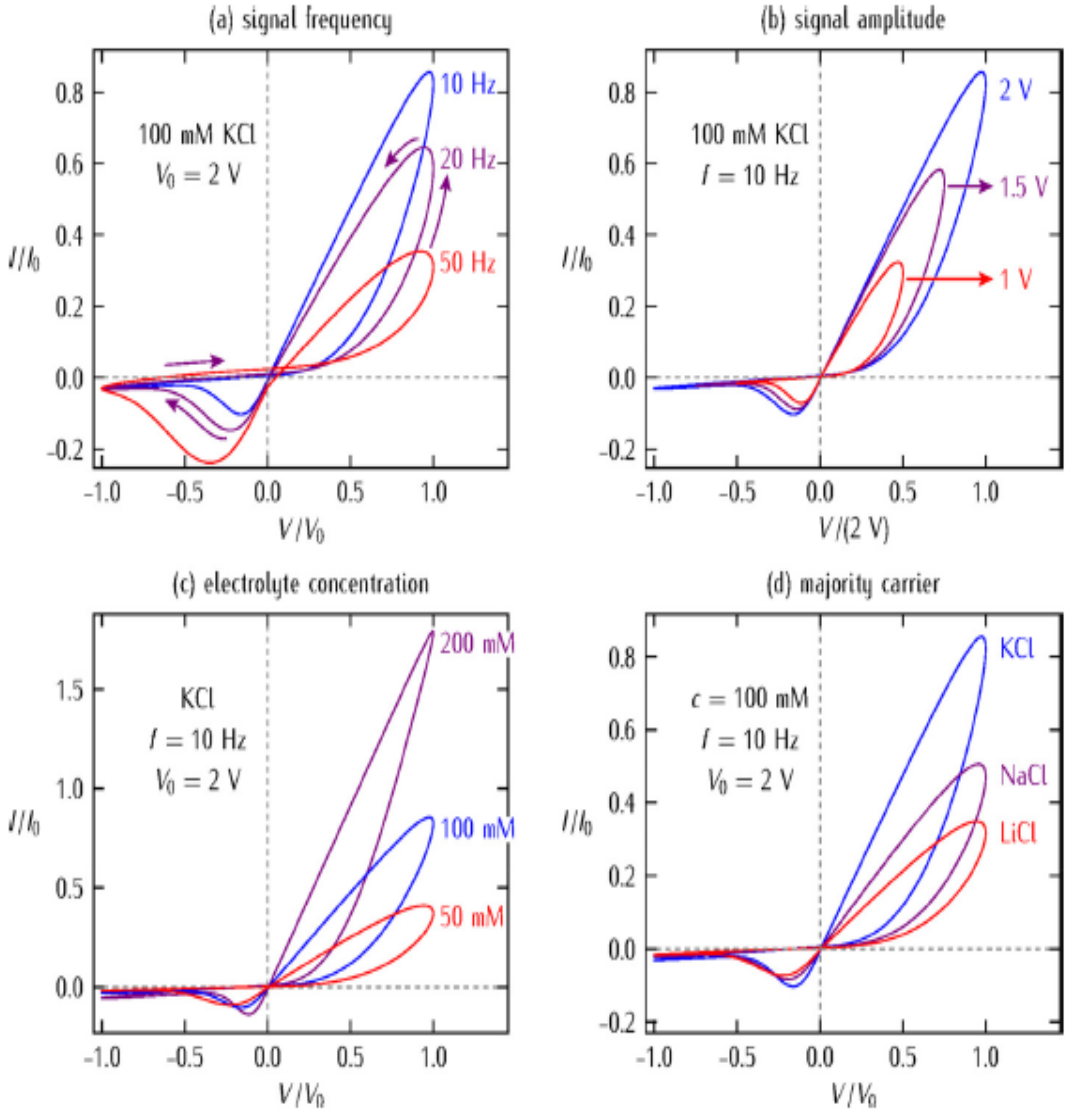
J. Phys. Chem. Lett. 2023
10.1021/acs.jpclett.3c02796

Phys.Fluids 2024
10.1063/5.0204219

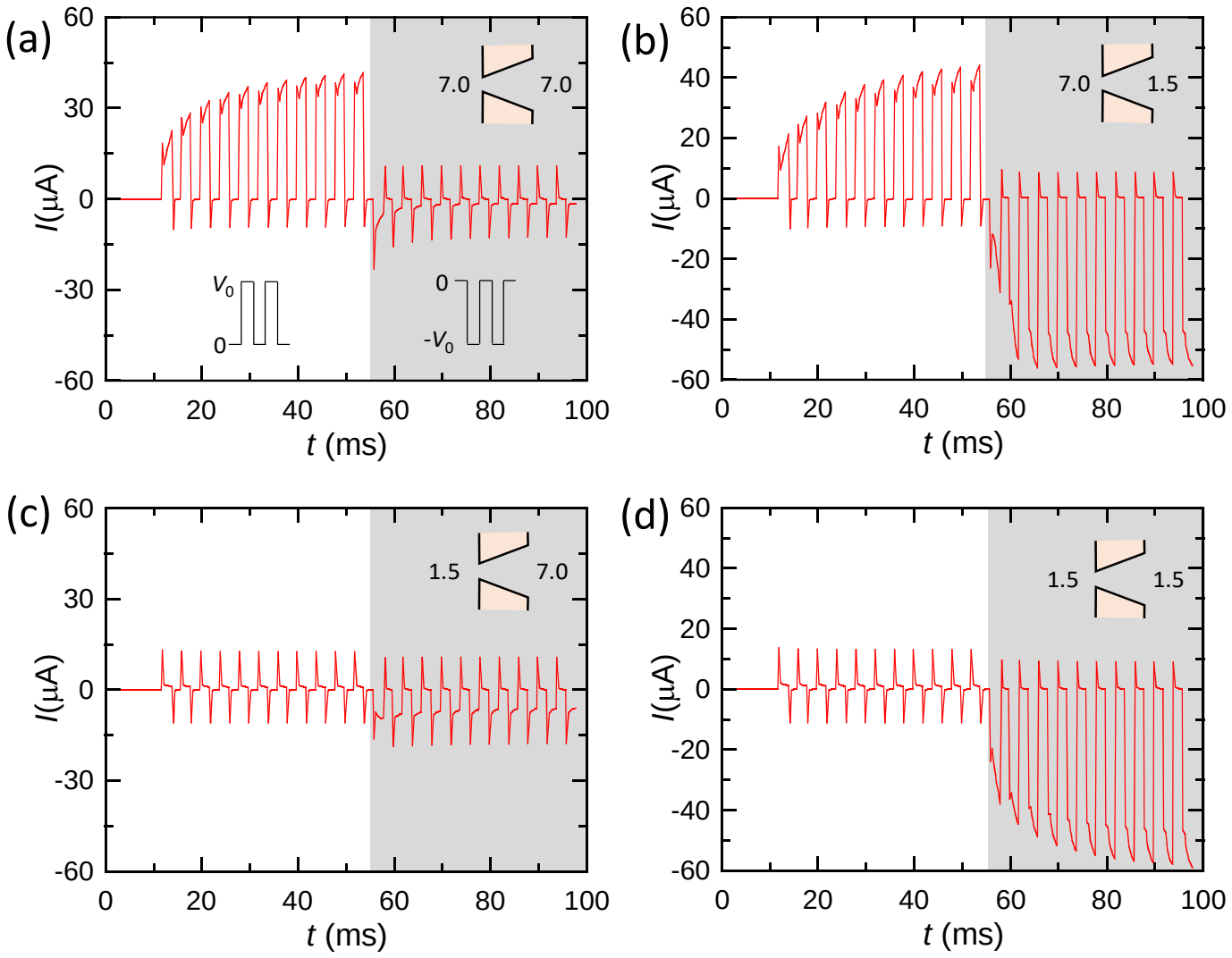
Experimental data



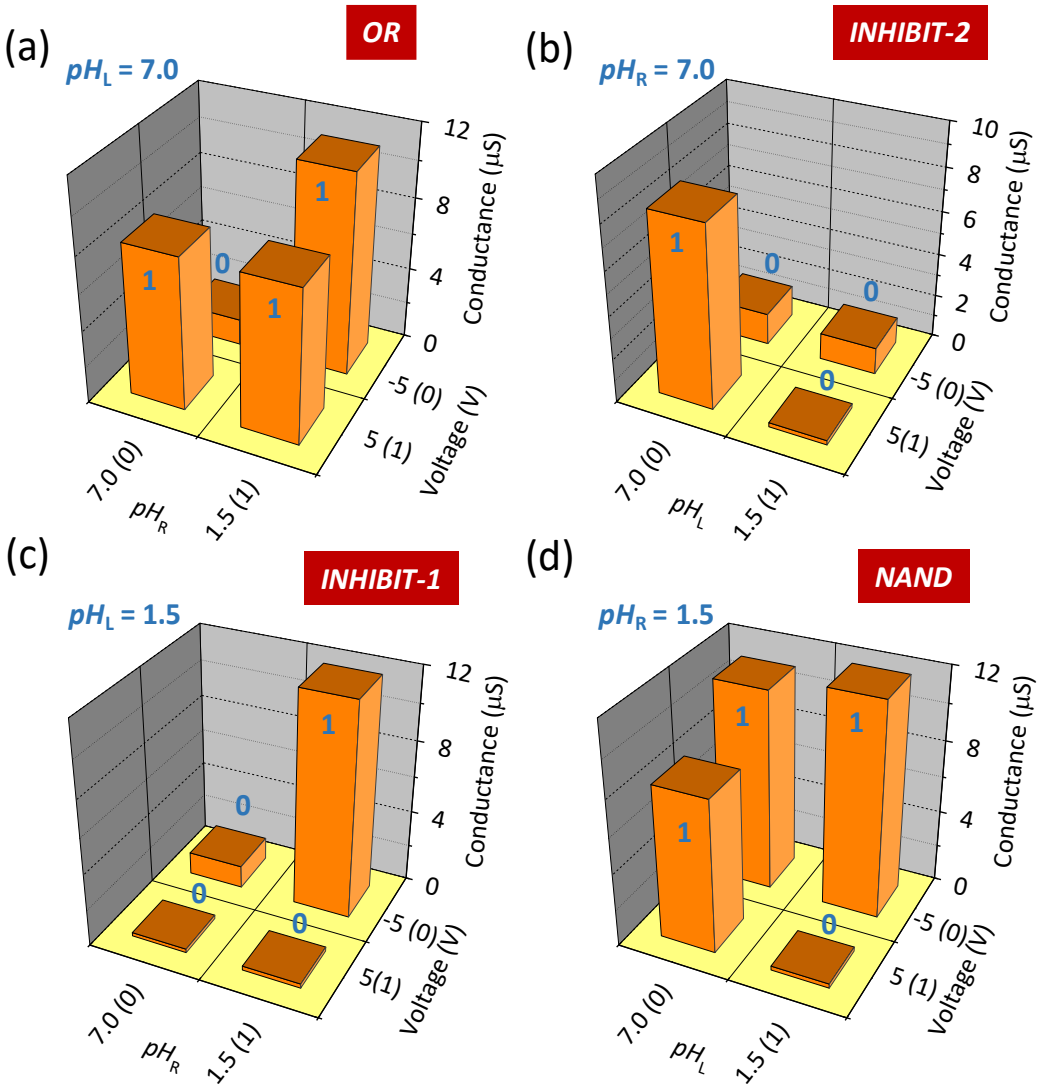
Model results



J. Phys. Chem. Lett. 2024
10.1021/acs.jpclett.4c01610

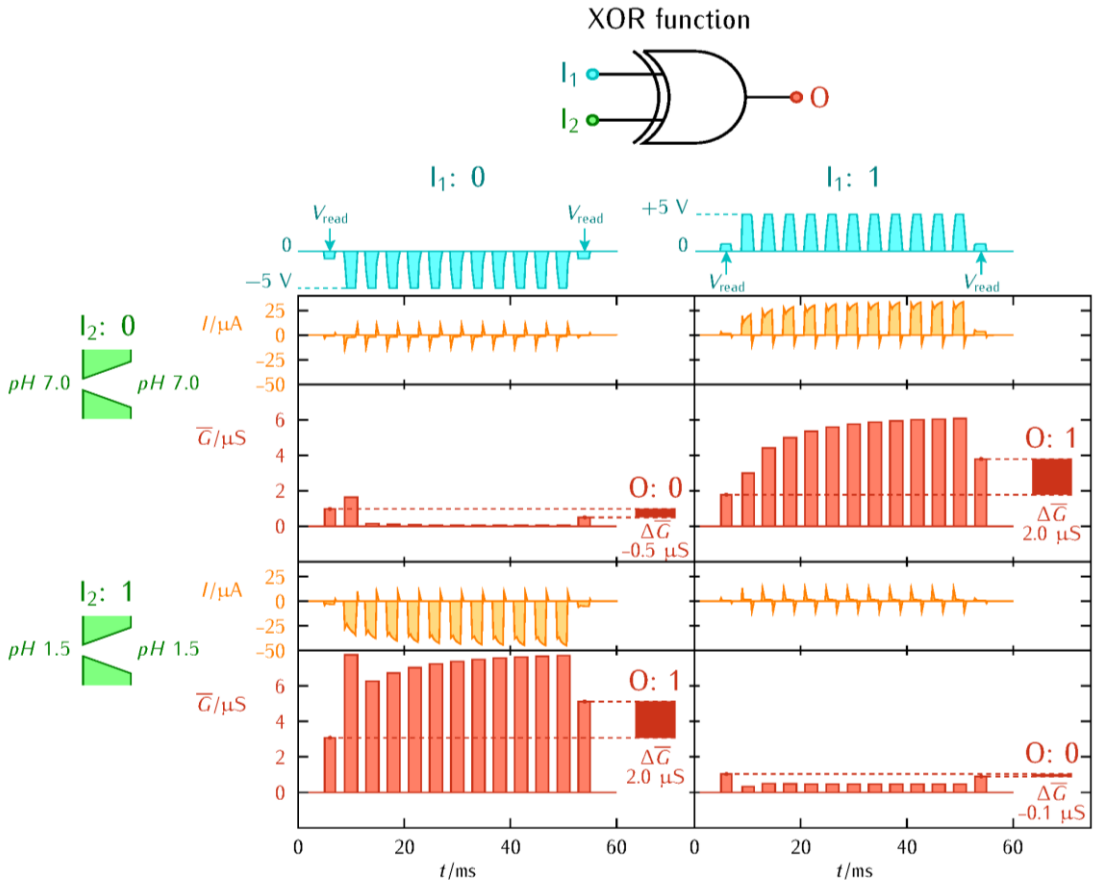


Current vs. time traces for different *pH tip/base membrane configurations* obtained with sequences of voltage pulses.

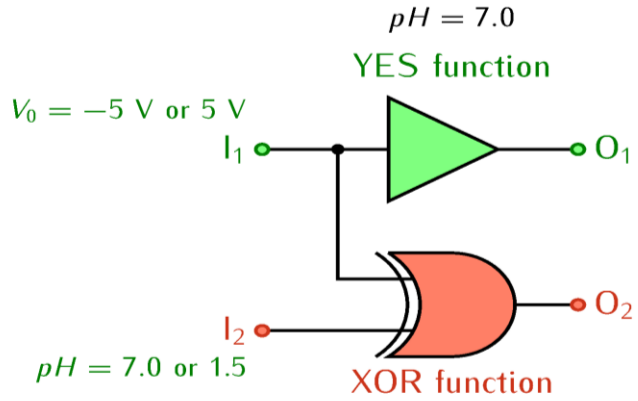


Logic functions: inputs *applied voltage V* and *pH*, output membrane *conductance G = I/V*.

Phys. Rev. E 2024
10.1103/PhysRevE.110.065101



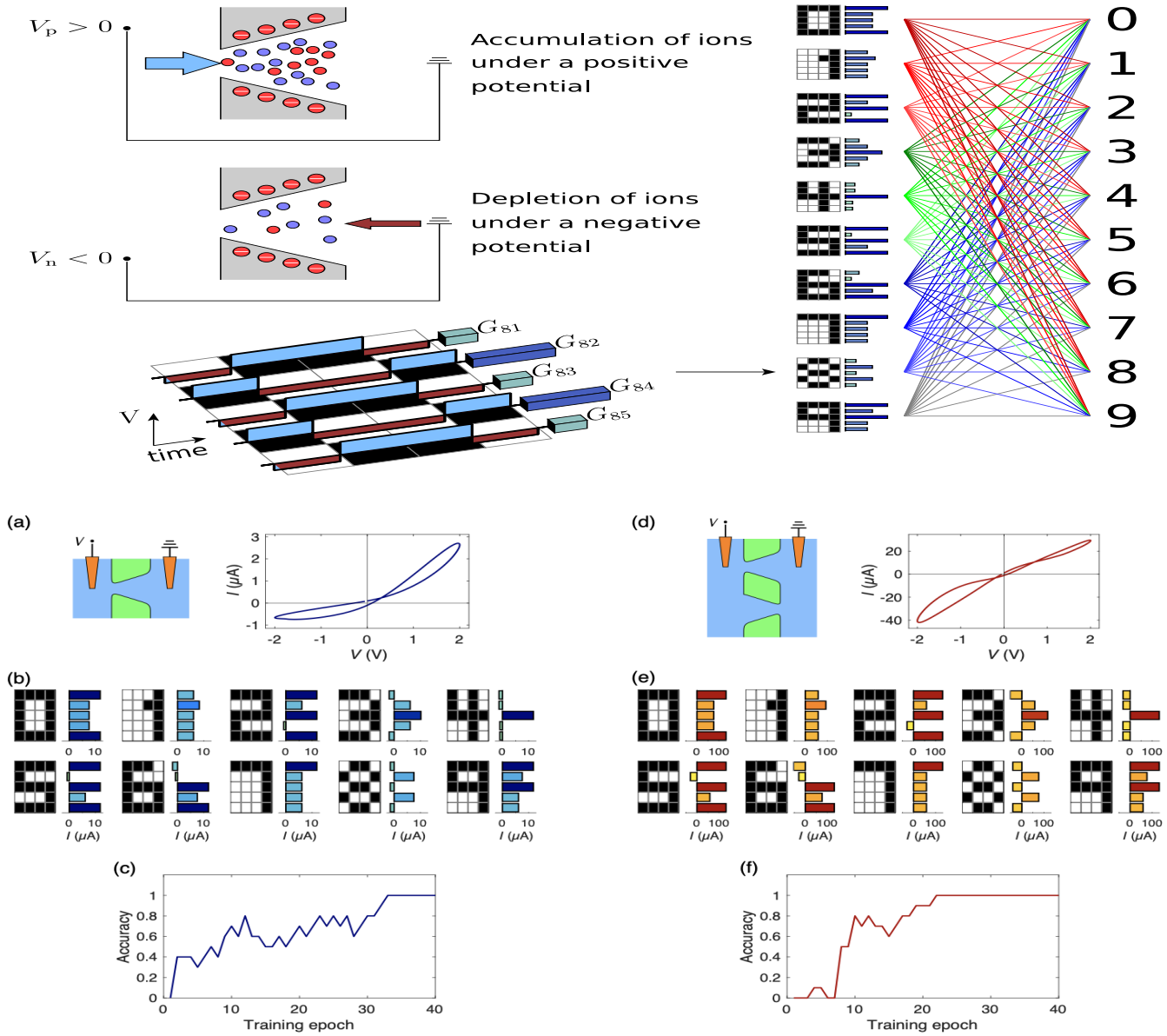
The *voltage sign* and the *pH solution* (*inputs*) give a *XOR* logics for the *membrane conductance* (*output*) in a 100 mM KCl solution. The sequences of negative and positive voltage pulses give the average conductance difference that characterizes the conductance potentiations.



Input		Output: $\lvert\Delta\overline{G}\rvert/\mu S$	
$I_1 \equiv V_0/V$	$I_2 \equiv pH$	O_1	O_2
−5 (**0**)	7.0 (**0**)	0.5 (**0**)	0.5 (**0**)
+5 (**1**)	7.0 (**0**)	2.0 (**1**)	2.0 (**1**)
−5 (**0**)	1.5 (**1**)	0.5 (**0**)	2.0 (**1**)
+5 (**1**)	1.5 (**1**)	2.0 (**1**)	0.1 (**0**)

Scheme of the *Feynman reversible logical function* implemented by the combination of the *YES* and the *XOR* functions.

Neuromorphic reservoir computing with memristive nanofluidic diodes

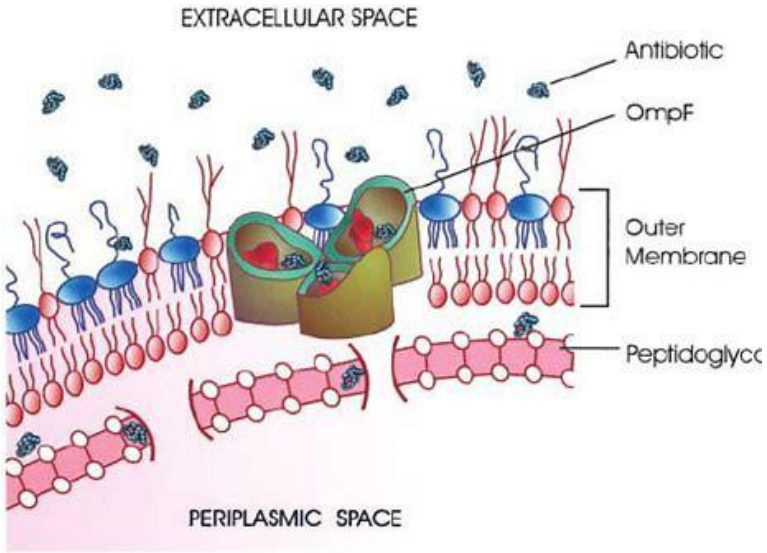


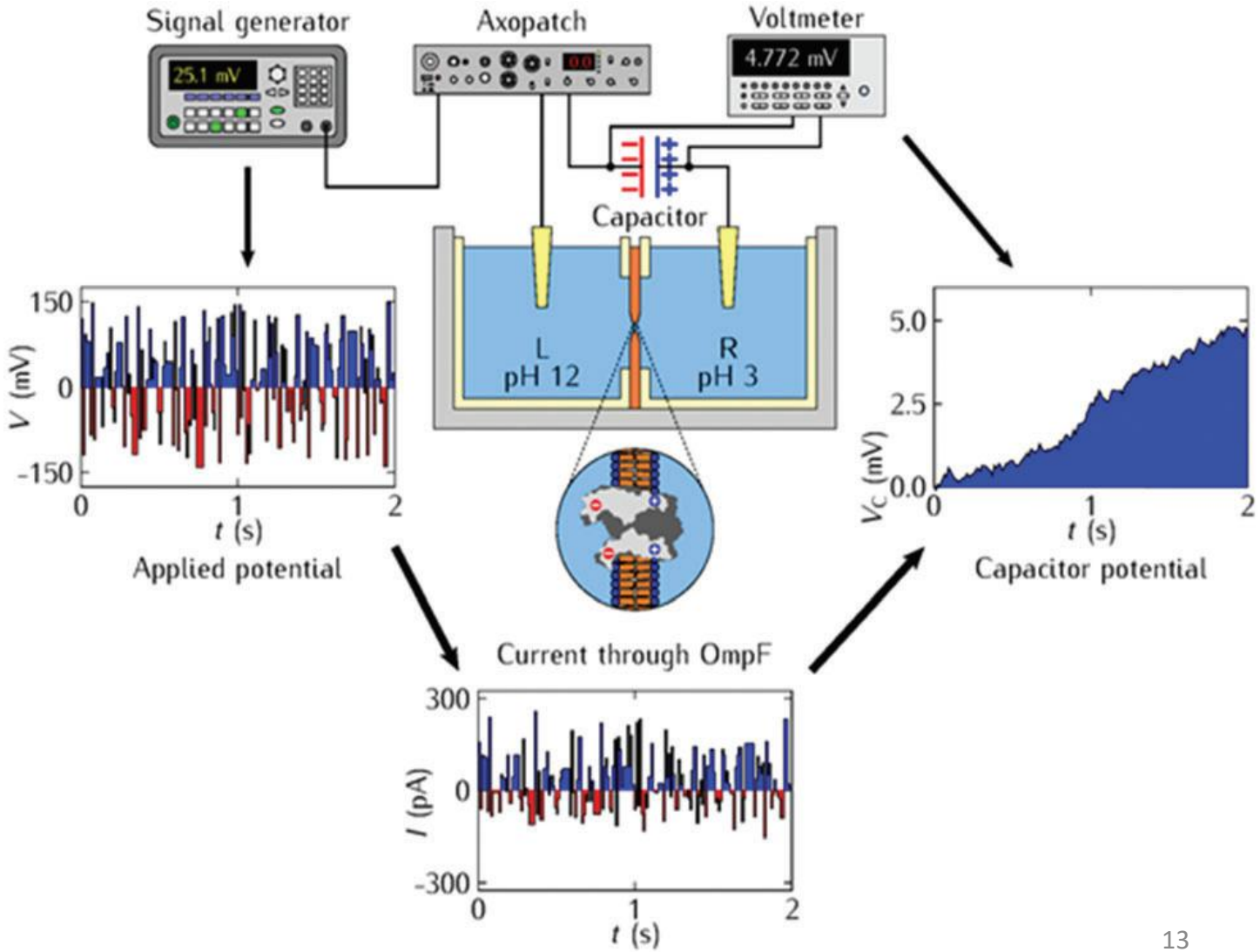
The mapping of the *positive and negative voltage pulses* (input) into the distinct reservoir states (read-out) permits to identify the *patterns of ten digits* (output). The ionic accumulation (voltage $V_p > 0$, high membrane conductance) and depletion (voltage $V_n < 0$, low membrane conductance) in the *nanofluidic conical pores* provides the *short-term plasticity* of the membrane conductance. The final conductance states corresponding to the digits $i = 0, 1, \ldots, 9$ and the assumed identical membranes $j = 1, 2, \ldots, 5$ are grouped in the *conductance matrix* G_{ij}. This reservoir states are then processed by the read-out layer, which associate the different pulse inputs patterns to the distinct digit outputs. The *accuracy vs. training epoch* an also use the current instead of the membrane conductance.

*1b Molecular biophysics: protein channels as nanofluidic diodes (with **Patricio Ramirez**)*

Small 2018
10.1002/smll.201702252
Phys. Chem. Chem. Phys. 2017
10.1039/c6cp06035h

Electrical rectification produce *cumulative effects* in the Outer Membrane Porin F of *E. Coli* inserted in an artificial lipid bilayer





2. Multicellular organization: some bioengineering views

2.a Distributed epigenetic shape formation and regeneration algorithm for a swarm of robots

Algorithm 2 Algorithm for the shape regeneration by the swarm of robots

```
1:  while TRUE do
2:    if State == Danger then
3:      LId == LeaderElection(); {the robot has the lowest Id
        among all the Queued robots}
4:      if Id == LId then
5:        State ← Leader;
6:      else
7:        State ← Queued;
8:        Algorithm-1();
9:      end if
10:   end if
11:   if State == Leader then
12:     CountPopulation(); {Count the remaining population}
13:     GenerateScaledShape(); {Scale down the input target
        shape}
14:     Share the new shape with the remaining robot;
15:     State ← Active;
16:     Form new seed robots;
17:     Algorithm-1();
18:   end if
19: end while
```

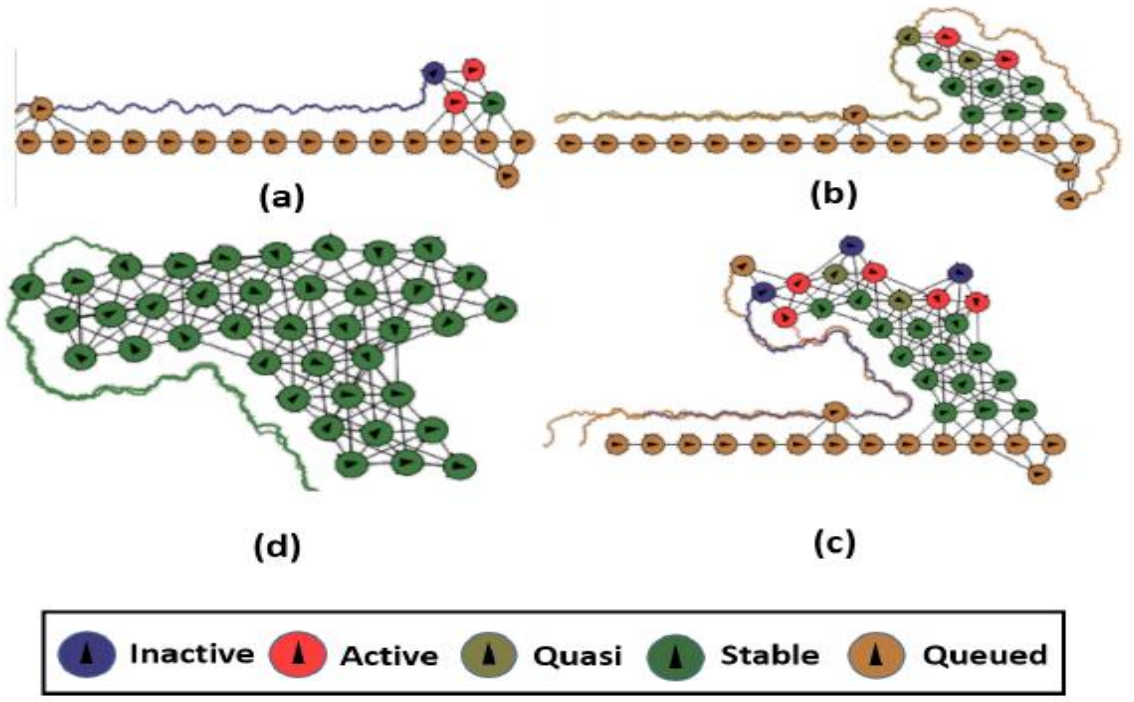


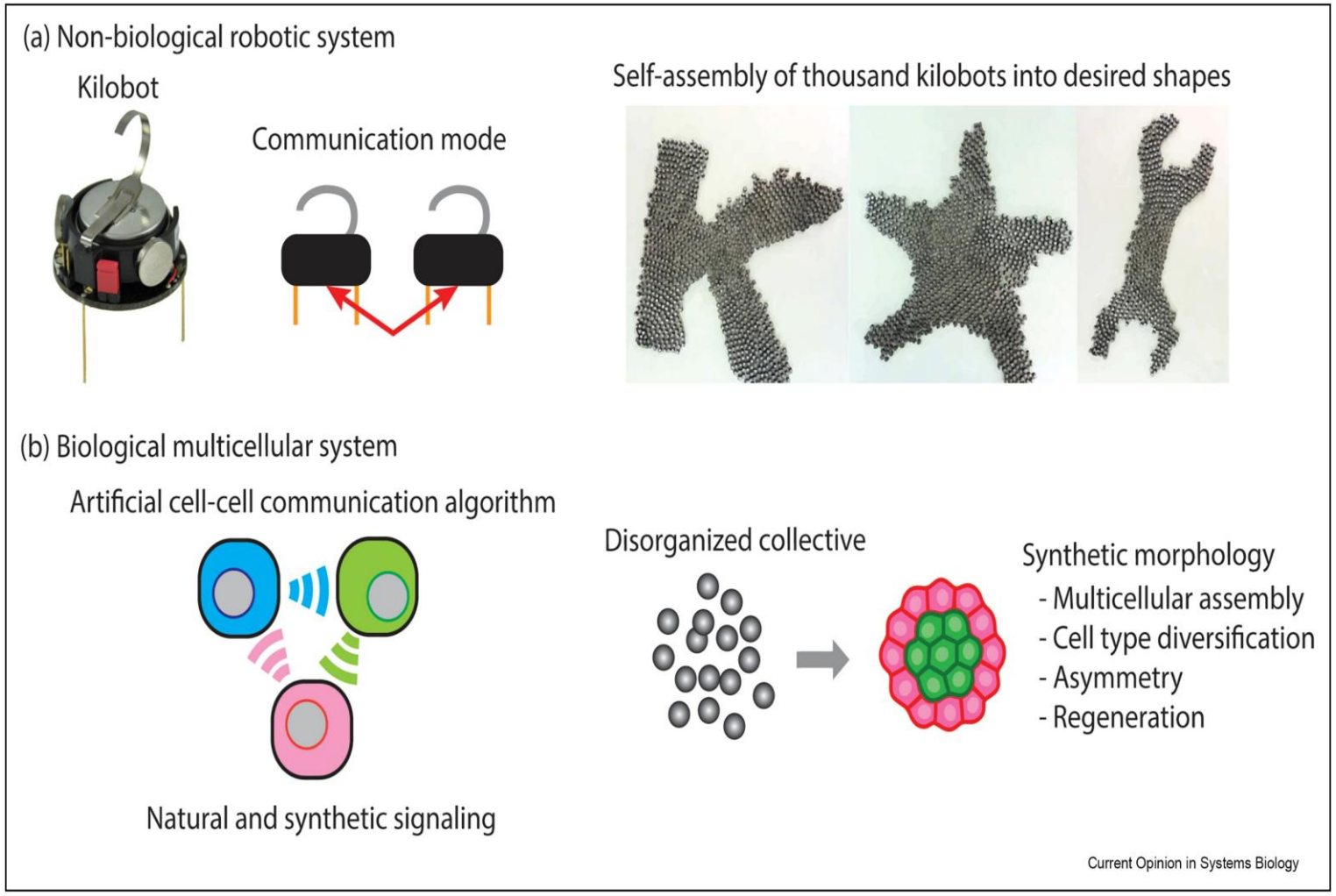
Figure 5: Generation of alphabet 'T' in 2 hours 50 minutes and 8 seconds

GECCO '18 Companion 2018 (ACM)
10.1145/3205651.3208300

Biologically inspired algorithm for shape formation and regeneration exhibited by a swarm of robots.. Here, a *gene* is similar to an *instruction manual* which aids cells to *reproduce and form* arbitrary shapes. Given an input binary image, the gene required for the shape formation is computed and transferred to *all the robots* constituting the swarm. The robots ("cells") then use this gene to form a shape in a distributed and decentralized manner.

2.b Forward engineering of collective systems

Curr. Opin. Syst. Biol. 2019
10.1016/j.coisb.2019.02.008

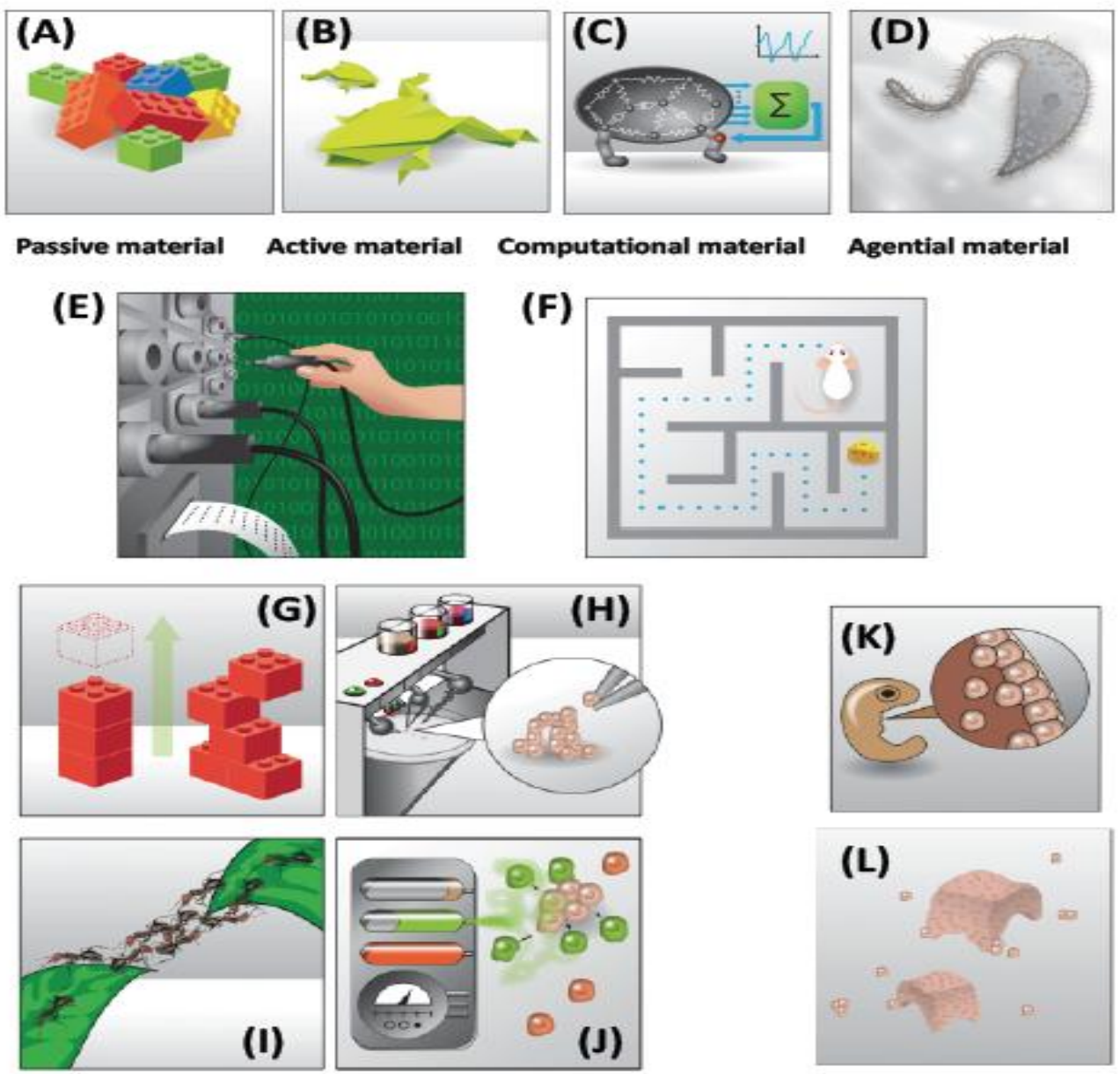


(a) *Robotic swarms of Kilobots* can communicate with neighbors by reflecting infrared light off the table below to decide how they move according to a *user-designed communication algorithm*. Examples of collective emergent patterns.

(b) *Engineering of cell–cell interactions to drive multicellular self-organization*. Artificial algorithms can program disorganized cells to behave collectively and self-organize into particular structures. Features can mimic processes in natural developmental systems (self-assembly, cell type diversification, symmetry breaking, and regeneration).

2.c Engineering with agential materials

Soft Robot. 2023
10.1089/soro.2022.0142



Engineering with passive materials (A) managed for desired functionality has moved toward active matter (B) and computational media (C). Biorobotics considers material as *agential*, composed of subunits (D, living cells) that include *problem-solving* in *problem spaces*. They are not only subjected to rewiring (E) but also to behavior-shaping (F). In complement to 3D-printing designed for building with passive matter (G), which also works with cells (H), collective intelligence of living systems, e.g. ant swarm (I), allows manipulating the collective behavior of cells (J) in *anatomical morphospace*, as instructive signals from other cells cause frog ectodermal cells to be a 2-dimensional barrier in embryos (K) and stimuli can achieve guided self-assembly toward novel form and function (L).

2.d Biochemical multicellular patterns

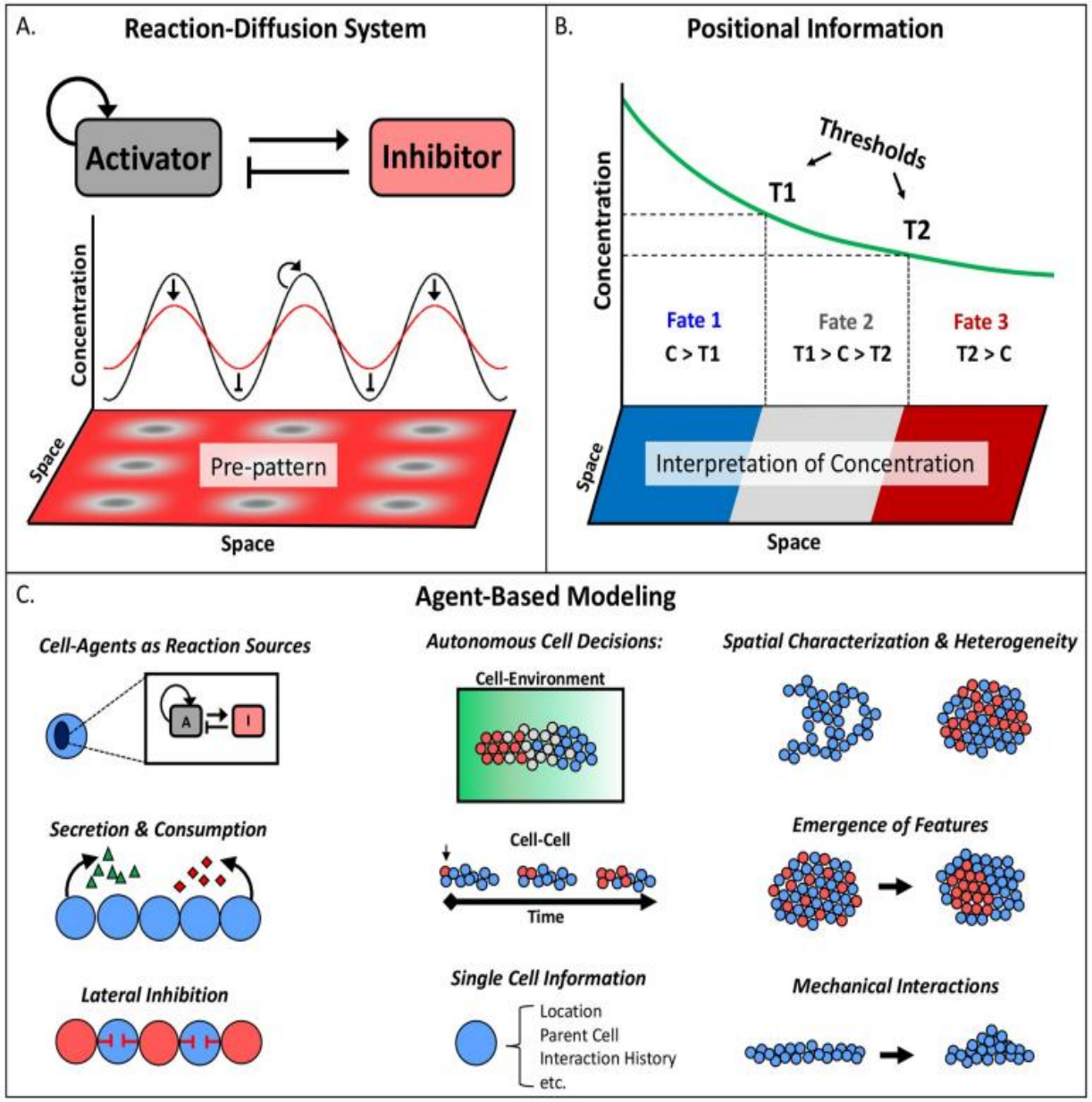
In *Biology*:

- individual cells can make decisions with *local info,* usually without having a highly precise knowledge of its particular position in a multicellular ensemble;

- *environmental changes* and *individual variability* are crucial and may help *system exploration*. The result is a continuous interplay between *short-term* individual changes and *long-term* collective persistence; and

- traditional attempts to understand *instructive patterns*, which are relatively *simple* with respect to the *high complexity* of *single-cell mechanisms,* have a solid experimental basis and have favored spatio-temporal distributions of *biochemical* signals. We explore here a *complementary* bioelectrical view.

Plos Biol. Comput. 2019
10.1371/journal.pcbi.1006577



pattern formation during morphogenesis

(A) *Turing reaction–diffusion system* with an activator and an inhibitor: a chemical prepattern is established in advance of cell fate decision.

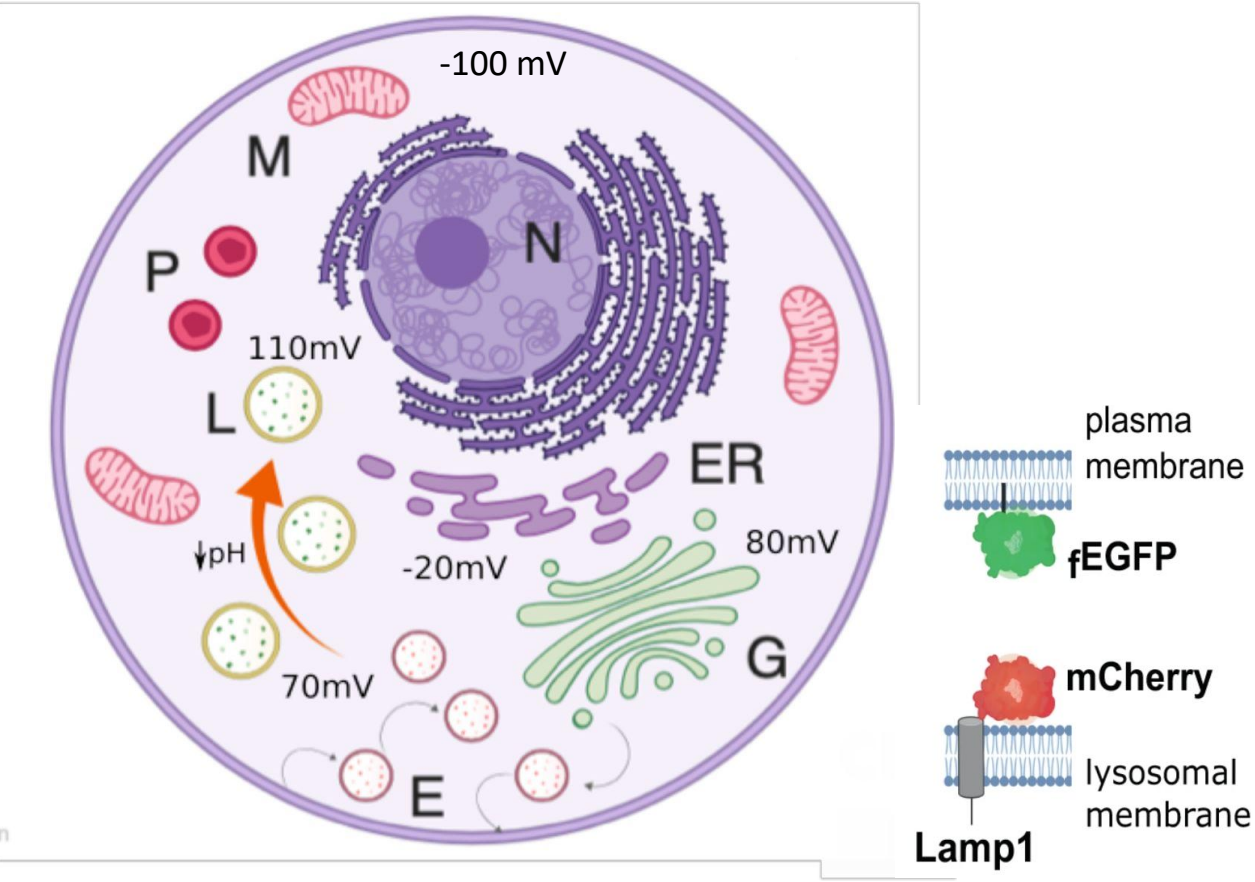
(B) *Wolpert's positional information:* concentration thresholds allow cell multiple fate decisions from a single molecular gradient.

(C) *Agent-based models* incorporate features from both theories. Cell agents are sources of activators and inhibitors, permit localized reactions, and make autonomous decisions in response to local environment. Static and dynamic spatio-temporal patterns can reproduce complex emergent behaviors.

3. Some experimental facts of bioelectricity + molecular biology

bioRxiv 2019
10.1101/578765
Comm. Biol. 2021
10.1038/s42003-021-01916-6

Multiscale bioelectricity: single-cell *regionalization* of organelle *membrane potentials* as low scale spatio-temporal integration: can *higher scales* be defined?

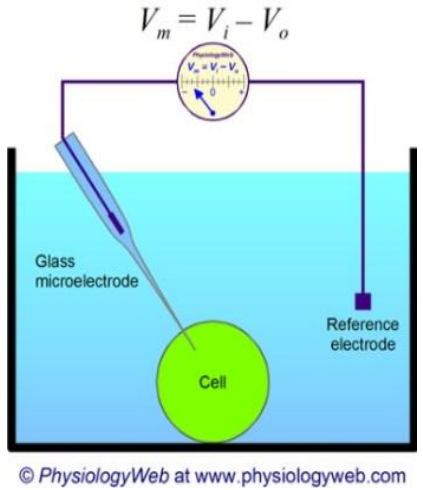


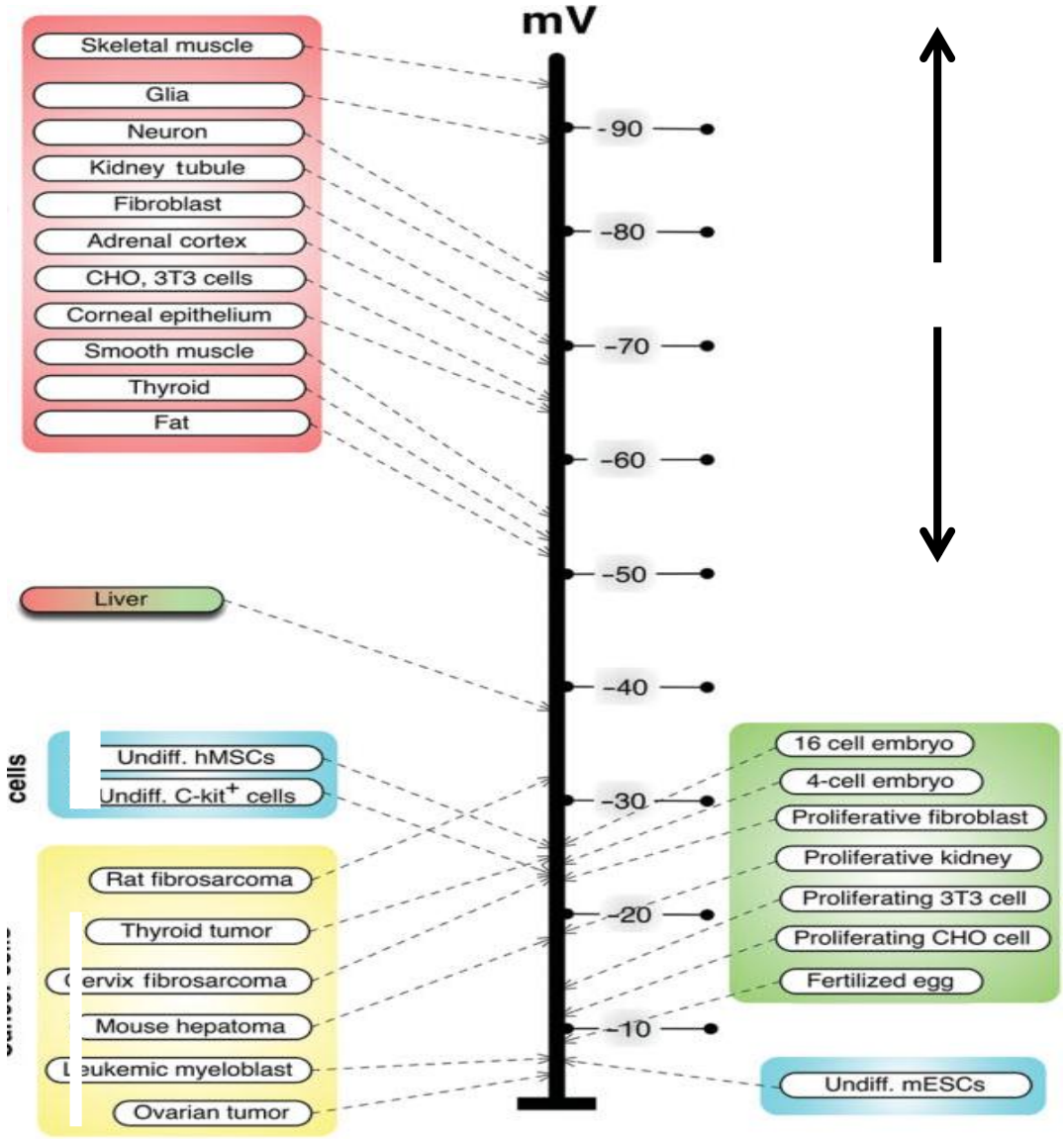
Organellar membrane potential compartmentalization. *Lysosomes* (L, yellow) and *golgi* (G, green) compartments have a relatively large and positive resting potential in contrast with the modest and negative inside potential of *ER* (purple). Pumping of protons into the lysosomal lumen by the V-ATPase leads to acidification and a more hyperpolarized membrane potential in the mature lysosome (orange arrow). *Mitochondria* (M, red) have a relatively large and negative potential.

Optical multiplexing of organellar activity and topology of the probes.

3.a Single-cell potentials

Wires. Sys. Biol. Med. 2013
10.1002/wsbm.1236



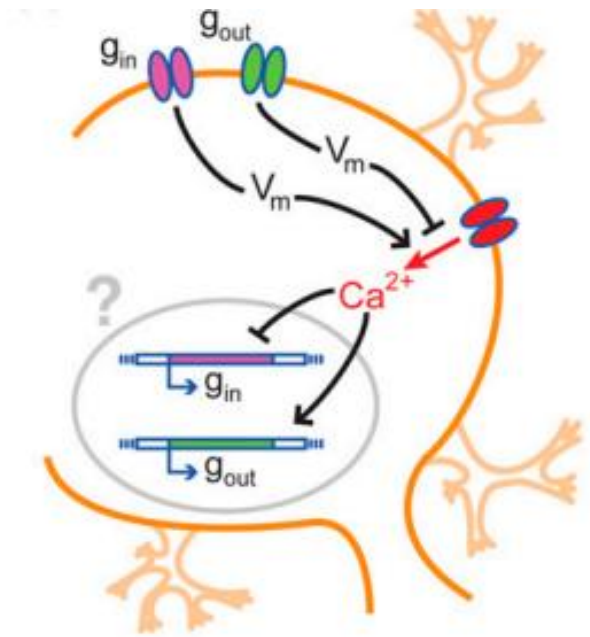


Mature cells are *polarized* (High $|V_m|$)

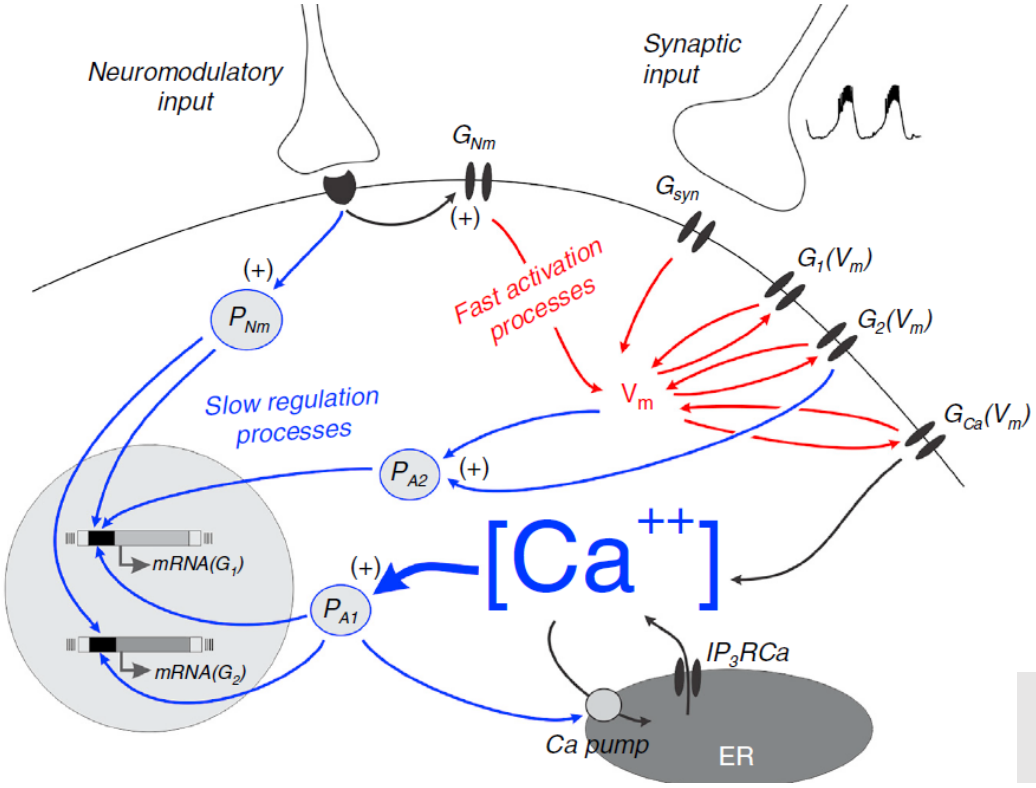
Proliferative cells are *depolarized* (low $|V_m|$)

Experimental results suggest *polarizing* and *depolarizing* potential *windows* at the *cell scale*. But we are interested in *multicelular patterns* here.

3.b A reduced number of counteracting voltage-gated channels, which influence protein transcription, regulate single-cell and intercellular potentials



PNAS 2013
10.1073/pnas.1309966110



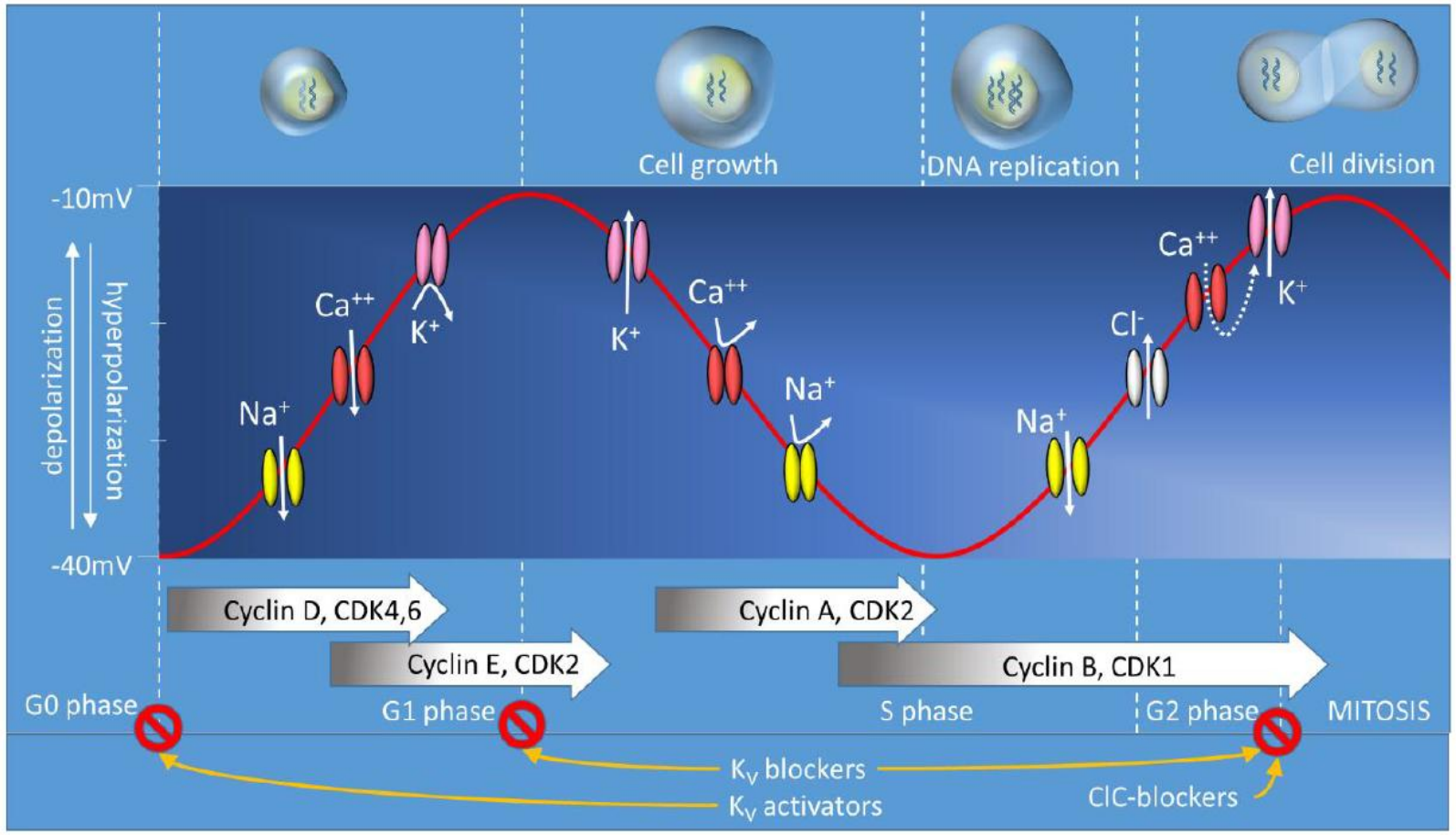
homeostatic channel regulation in neurons

Current Biol. 2019
10.1016/j.cub.2019.05.029

The possible *interplay* between *bioelectricity* and *transcription*: voltage-gated channel proteins have mRNA levels modulated by a *calcium-dependent transcription*. A neuron regulated by inward (g_{in}) and outward (g_{out}) conductances where the Ca^{2+} influx through voltage-gated calcium channels depends on the membrane potential, which is regulated in turn by these channel conductances.

cell cycle

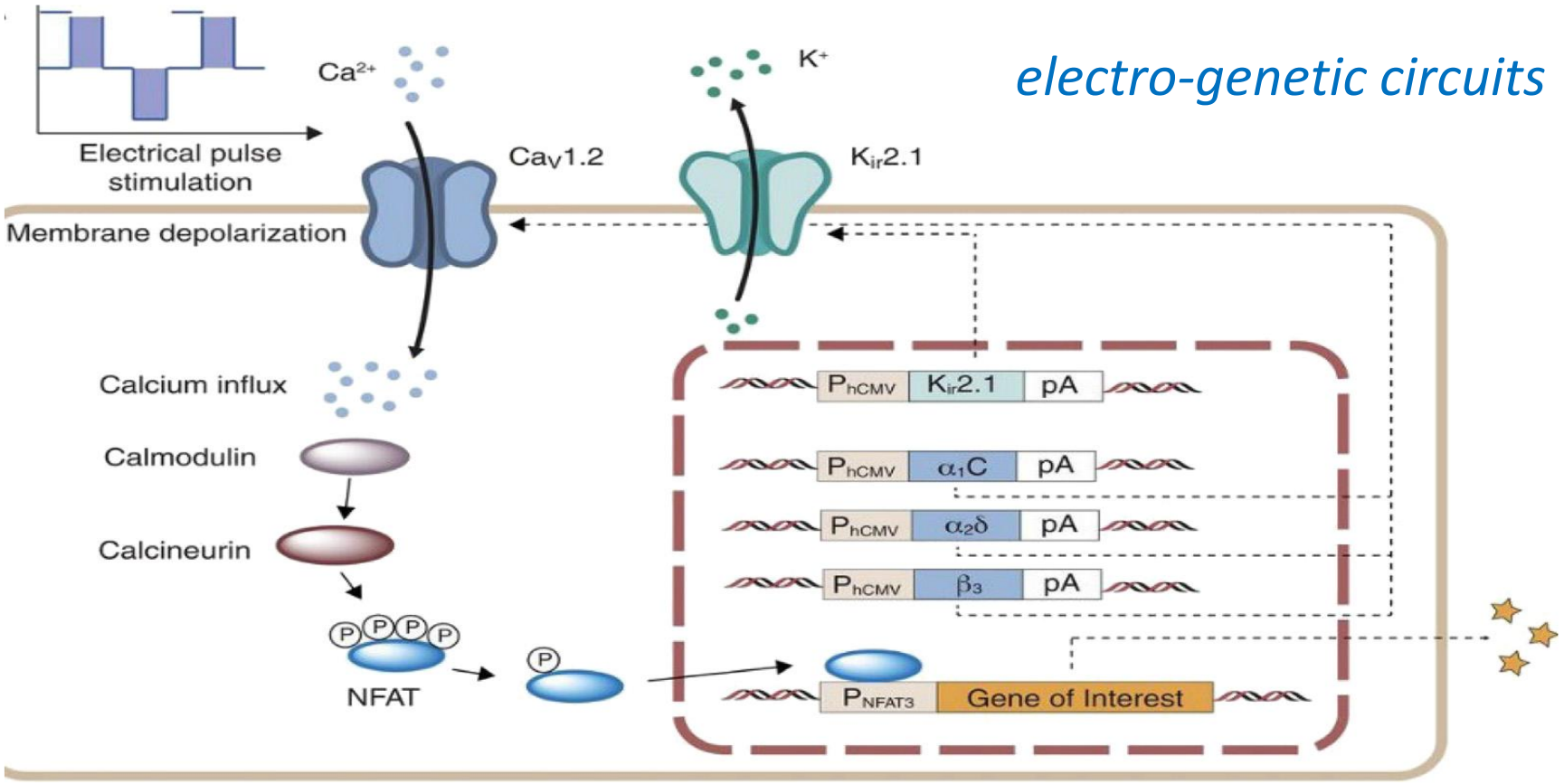
Cancers 2015
10.3390/cancers7020813



Proposed model of voltage-gated ion channel activity during the *cell cycle*. Note the *counteracting channel actions* based on their different expression levels. The oscillation of the membrane potential appears to follow the progression of the cell cycle. In principle, this *bioelectrical process* could be arrested at particular *polarized* or *depolarized* cell states by acting on specific channels.

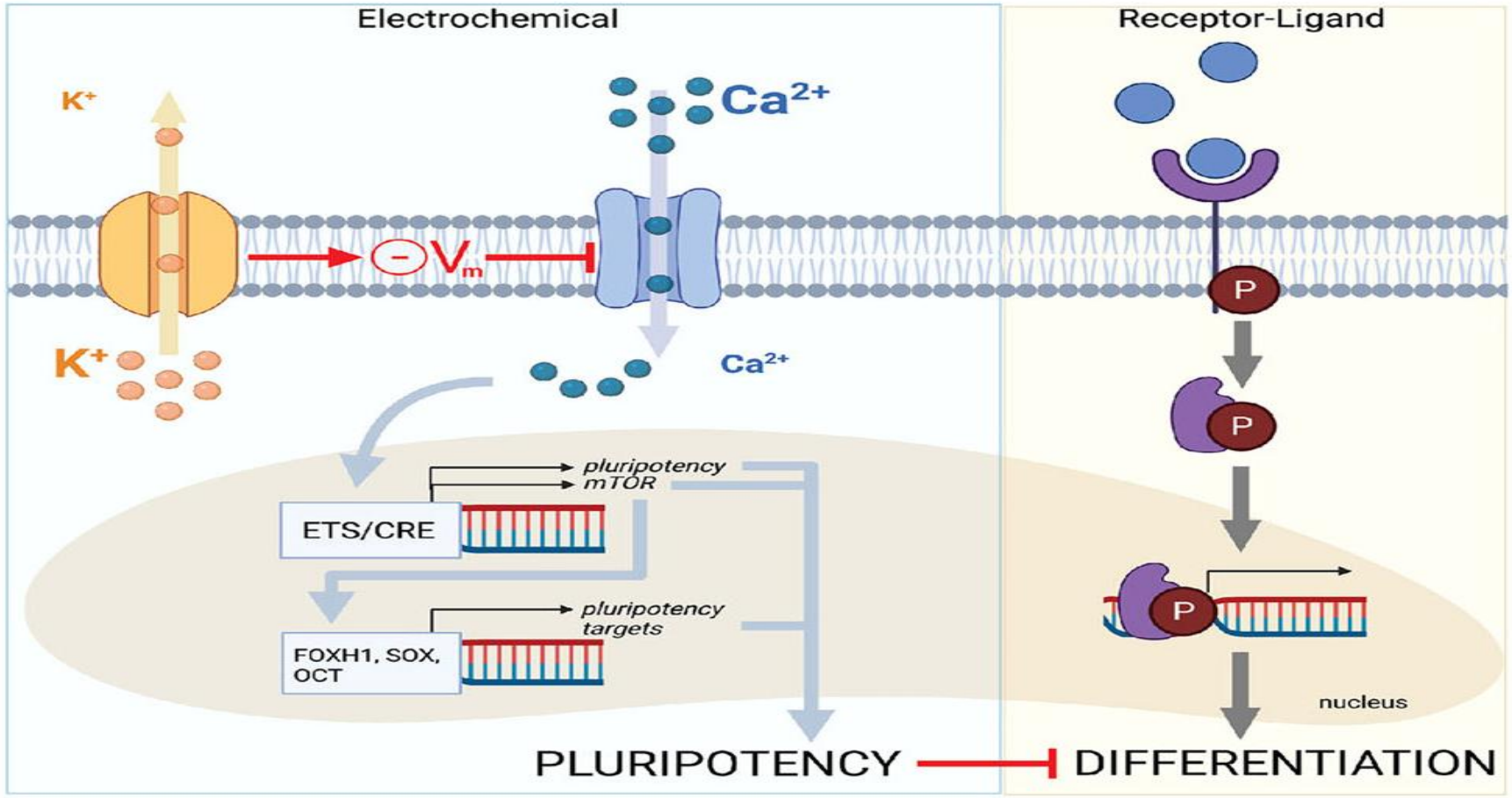
Science 2020
10.1126/science.aau7187

electro-genetic circuits



Transcriptional control of mammalian cells by membrane depolarization shows the *coupling* between *bioelectrical* and *biochemical signals*. In this electrogenetic circuit, an inward-rectifying potassium (*Kir*) channel sets initially the potential of human embryonic kidney (*HEK-293T*) cells. External pulses provoke the depolarization that opens the voltage-gated calcium channel (*Cav*). The increase in the calcium level yields *downstream processes* (activation of the calmodulin/calcineurin pathway, dephosphorylation of the nuclear factor of activated T cells (NFAT), and the NFAT-sensitive promoter) which give the *target transgene expression*.

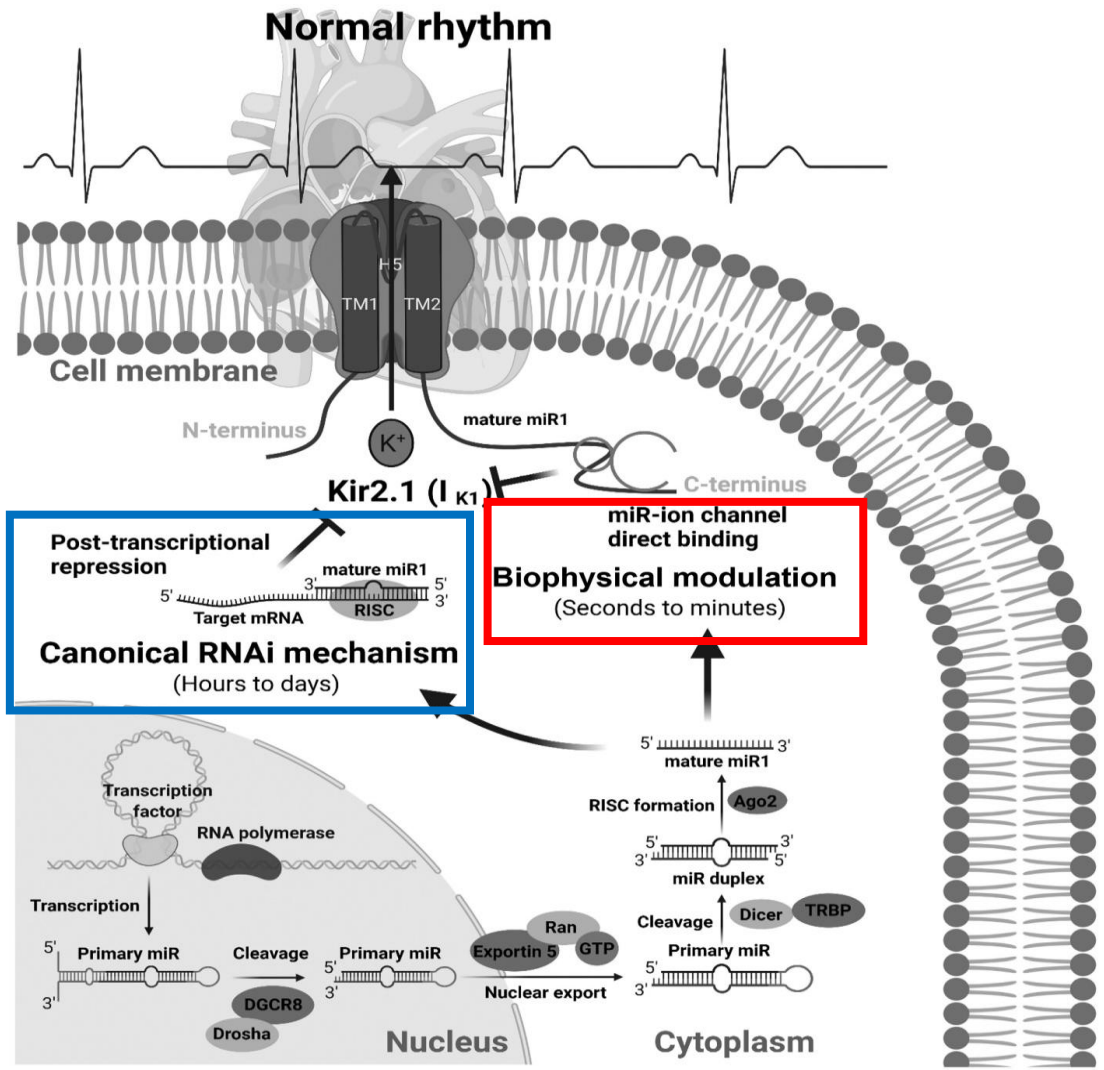
Nat. Commun. 2022
10.1038/s41467-022-34363-w



embryonic differentiation

Membrane potential (*left*) and *receptor-ligand* (*right*) *signaling* in the onset of *embryonic differentiation*. Potassium channels set the polarized cell potential, which keeps voltage-gated calcium channels inactivated, thus avoiding the high calcium levels required for downstream processes to pluripotency. While gene expression is also mediated by intracellular signal transducers and biochemical pathways essential to the expression of differentiation factors, *bioelectricity* may influence also the *timing of pluripotency genes*.

J. Mol. Cell Cardiol. 2022
10.1016/j.yjmcc.2022.02.007



cardiac electrophysiology

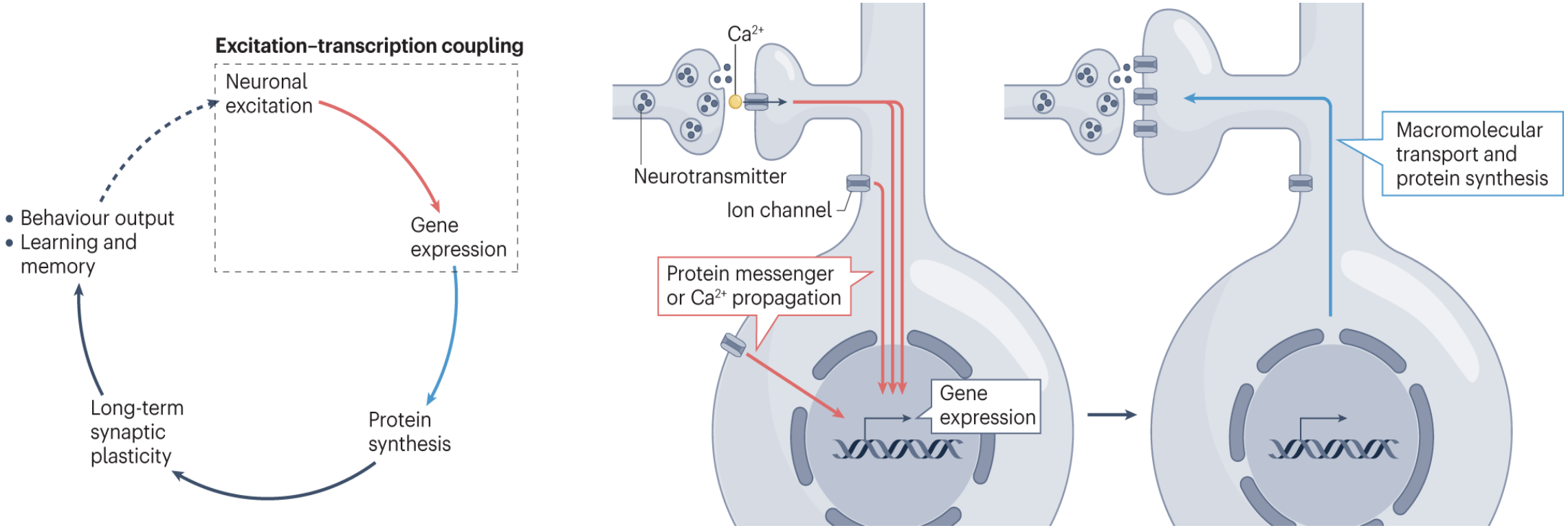
The *different bioelectrical* and *transcriptional time scales*.

The miRs regulate cardiac electrophysiology and heart rhythm through *two different mechanisms*:

- canonical post-transcriptional RNAi, which regulates the expression of ion channels, takes *hours to days*;
- bioelectrical modulation, however, quickly changes the dynamics of ion channels (*seconds to minutes*).

Nature Rev. Neurosci. 2023
10.1038/s41583-023-00742-5

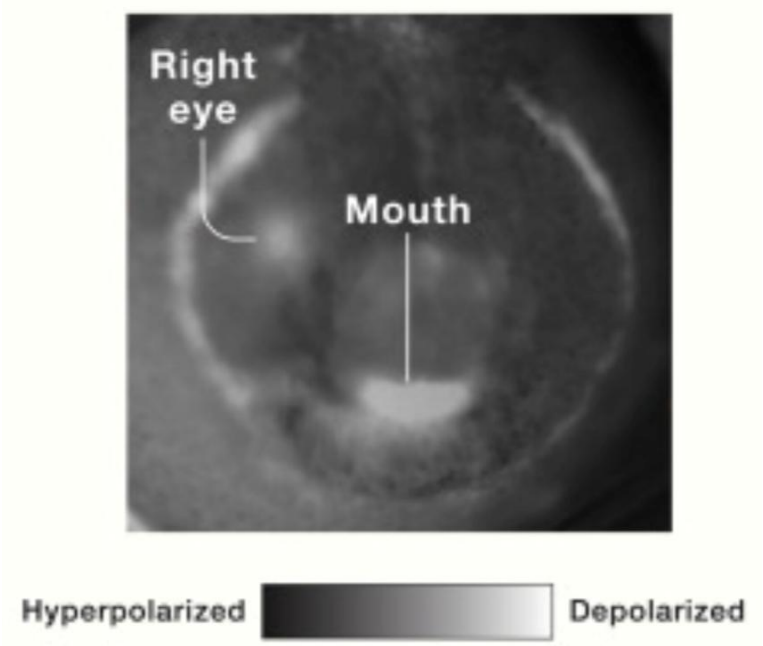
excitation–transcription coupling in synaptic plasticity



A model of *lasting synaptic plasticity* relies on neuronal activity-induced transcription in the nucleus and transport of translated proteins back to the synapse. The conversion of neuronal excitation to gene expression involves an *excitation–transcription coupling* initiated by membrane depolarization at the synapse and the soma, which drives Ca^{2+} entry via ion channels and activates Ca^{2+}-sensitive transcriptional signaling cascades (*red arrows, left*). The gene expression yields macromolecules from the nucleus back to the synapse (*blue arrow, right*) to sustain long-term potentiation.

*3.c Multiscale bioelectricity: patterns of cell potentials as a **high** scale spatio-temporal integration (**Michael Levin**)*

Wires. Sys. Biol. Med. 2013
10.1002/wsbm.1236

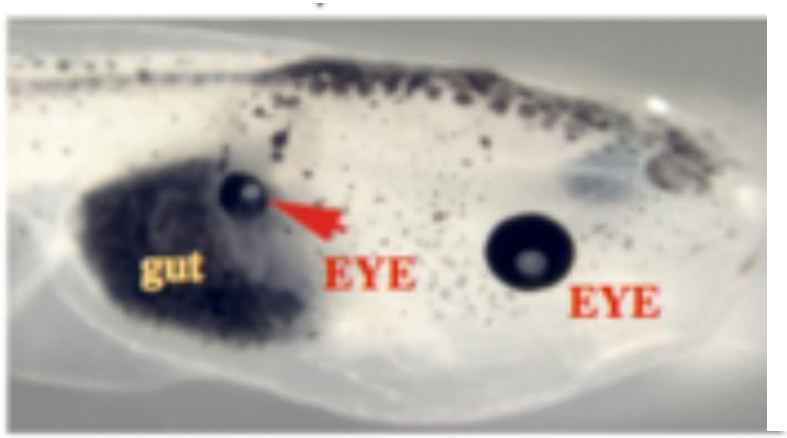


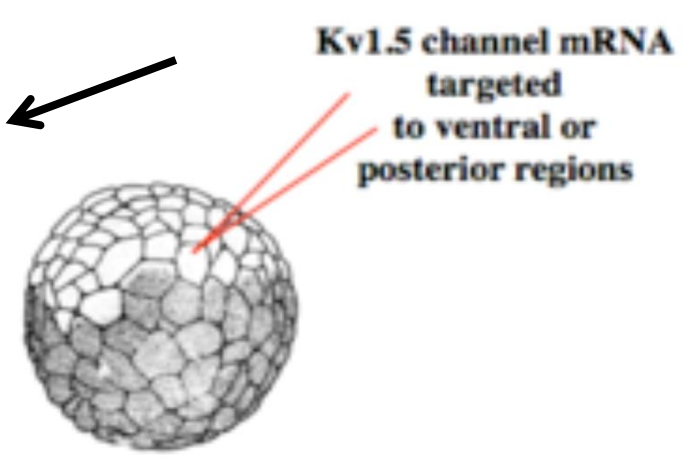
Voltage-sensitive fluorescent dyes non-invasively reveal bioelectric patterns.

Multicellular potentials act as functionally *instructive patterns*.

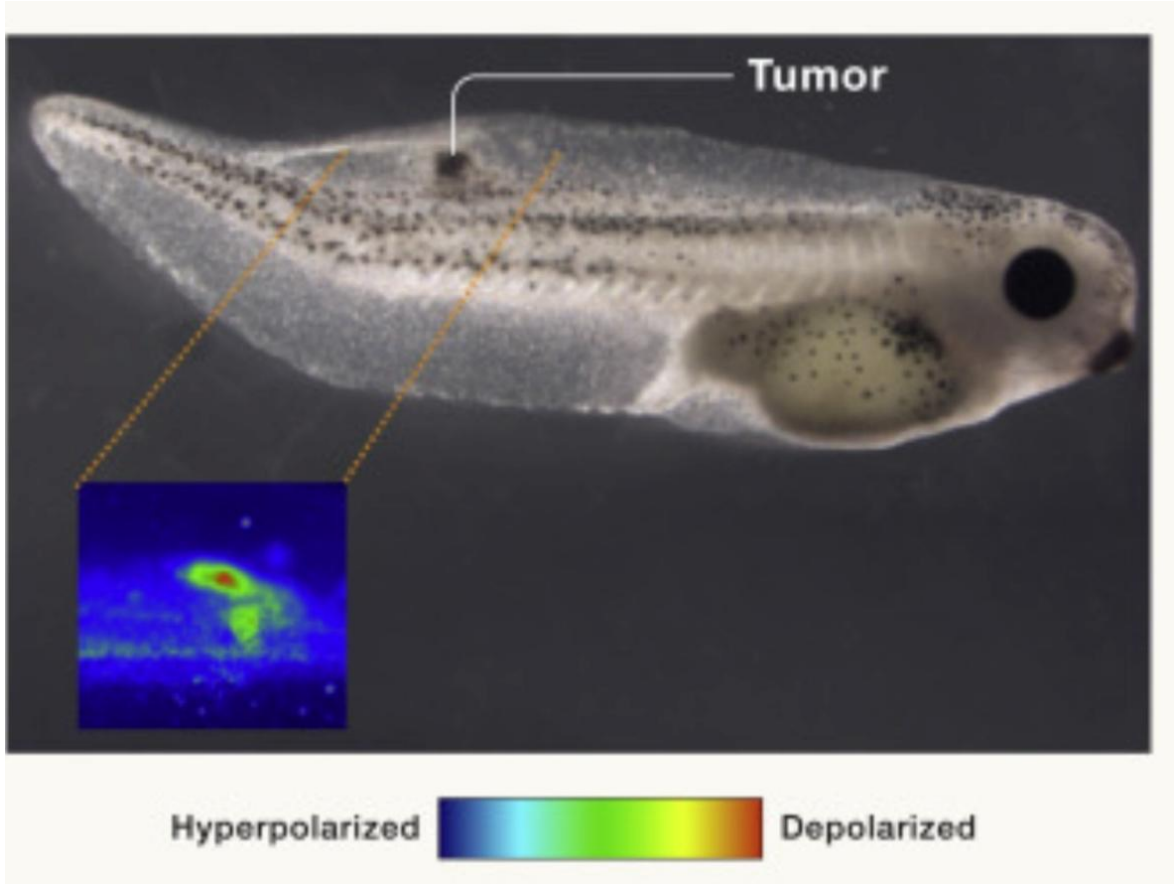
The *bioelectrical face*: prior to organ generation, fluorescence dyes show hyerpolarized regions where the face organs (eye, mouth) will develop.

Local *mRNA transfer* experiments may generate *ectopic eyes* by changing the multicellular potential of a localized region.





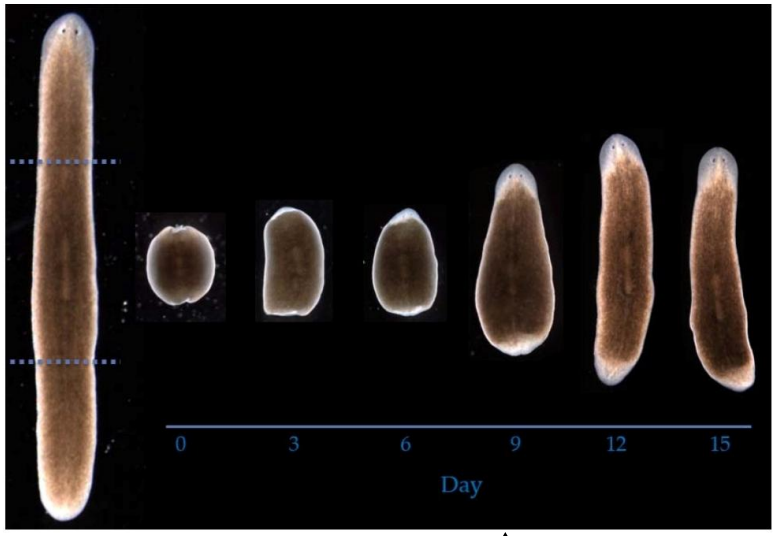
Cell 2021
10.1016/j.cell.2021.02.034

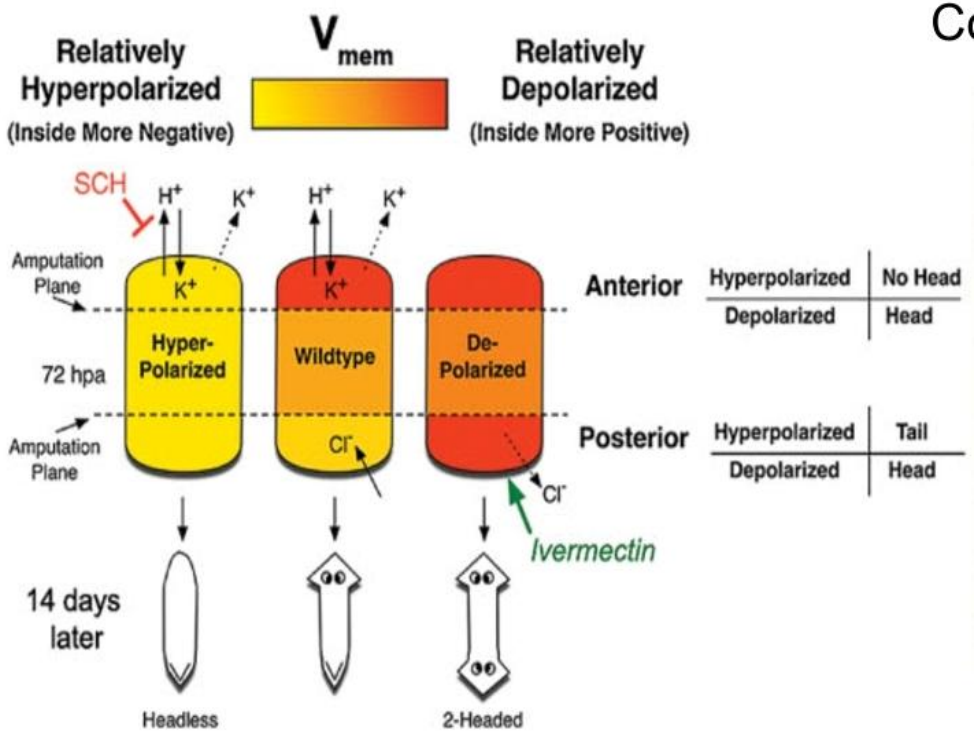


Experimental tools can *read* and *write* the *bioelectric patterns* of non-neural tissues using molecular-genetics, pharmacological, and optogenetical techniques to open and close ion channels and gap junctions.

A *tumor induced* on a *zebrafish embryo* shows a *locally depolarized* region suggesting a possible role of multicellular *bioelectricity*.

Mol. Biol. Cell 2017
10.1091/mbc.e13-12-0708

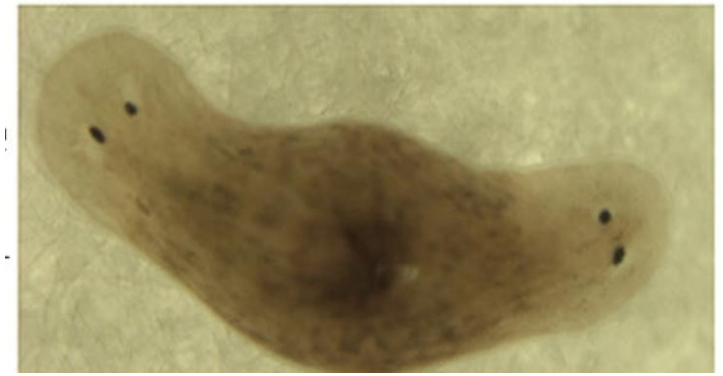




Pattern completion by *bioelectrical modules* may allow generating the entire anatomy from a small piece. The bioelectric network stores a pattern memory that controls individual cells to restore the whole organism.

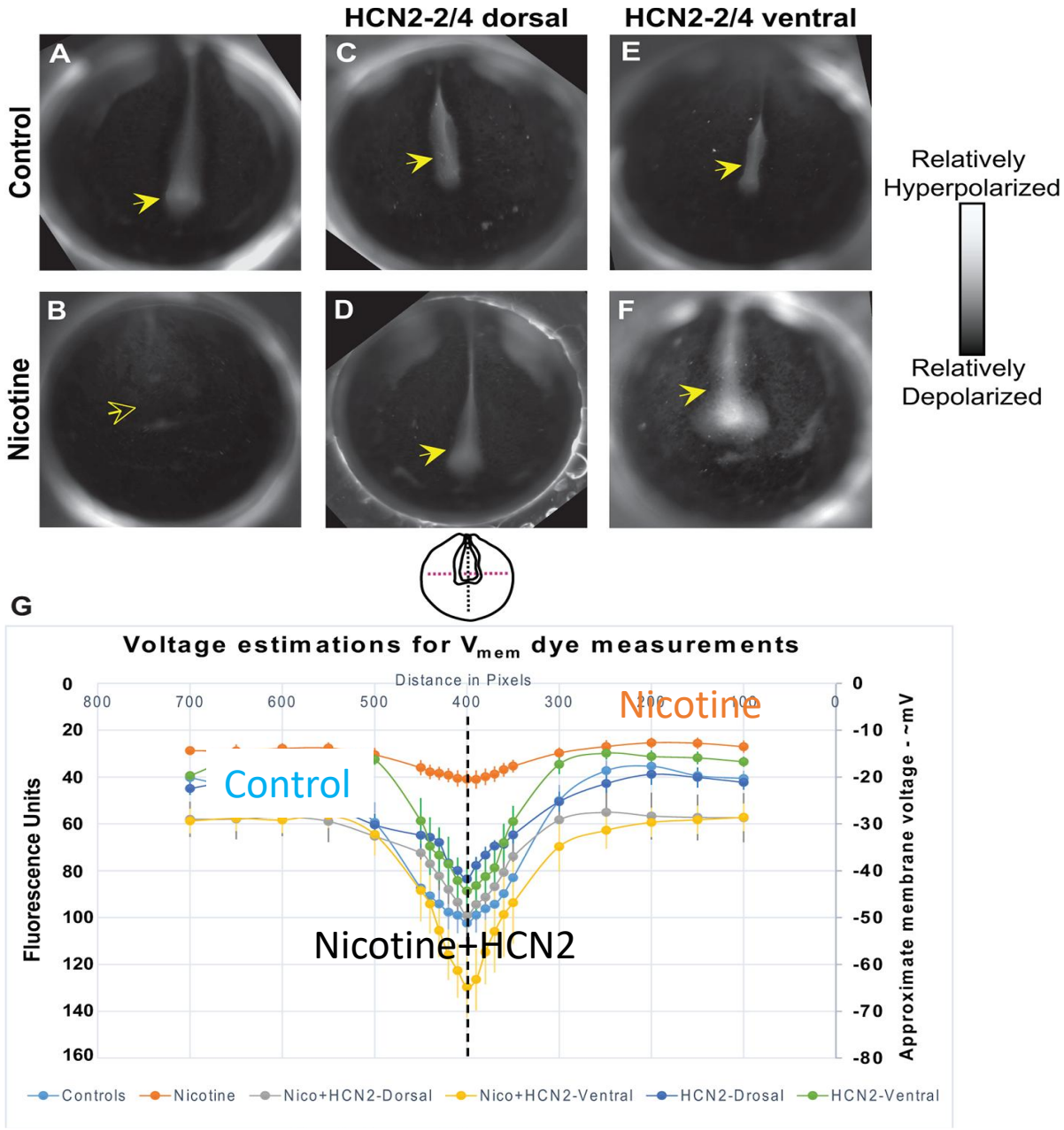
Planaria can regenerate their body upon amputation.

By controlling the *multicellular potential map*, normal, headless, and double-head planaria can be obtained.



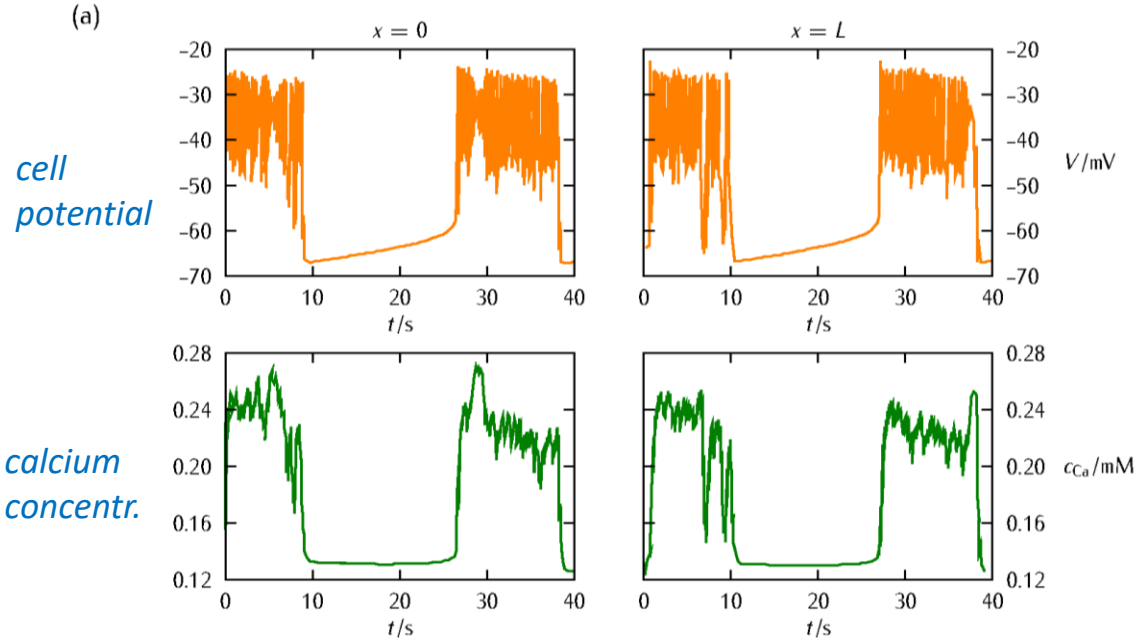
Embryonic exposure to teratogen *nicotine* results in *brain defects,* apparently by *disrupting* the *multicellular potential* endogenous pre-pattern.

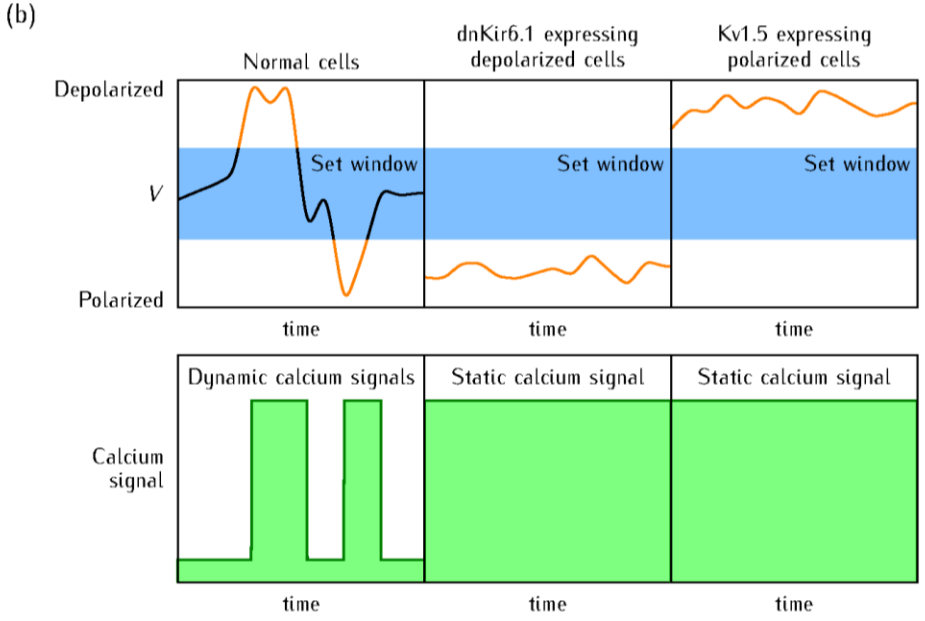
Upregulation of an *ion channel* (*HCN2*) can recover the potential pre-pattern *at distance*, acting to reverse profound defects and *rescue* brain anatomy and learning.



Nat. Commun. 2018 10.1038/s41467-018-03334-5
Front. in Cell. Neurosci. 2020 10.3389/fncel.2020.00136
iScience 2023 10.1016/j.isci.2023.108398

Phys. Rep. 2023
10.1016/j.physrep.2022.12.0030370-1573





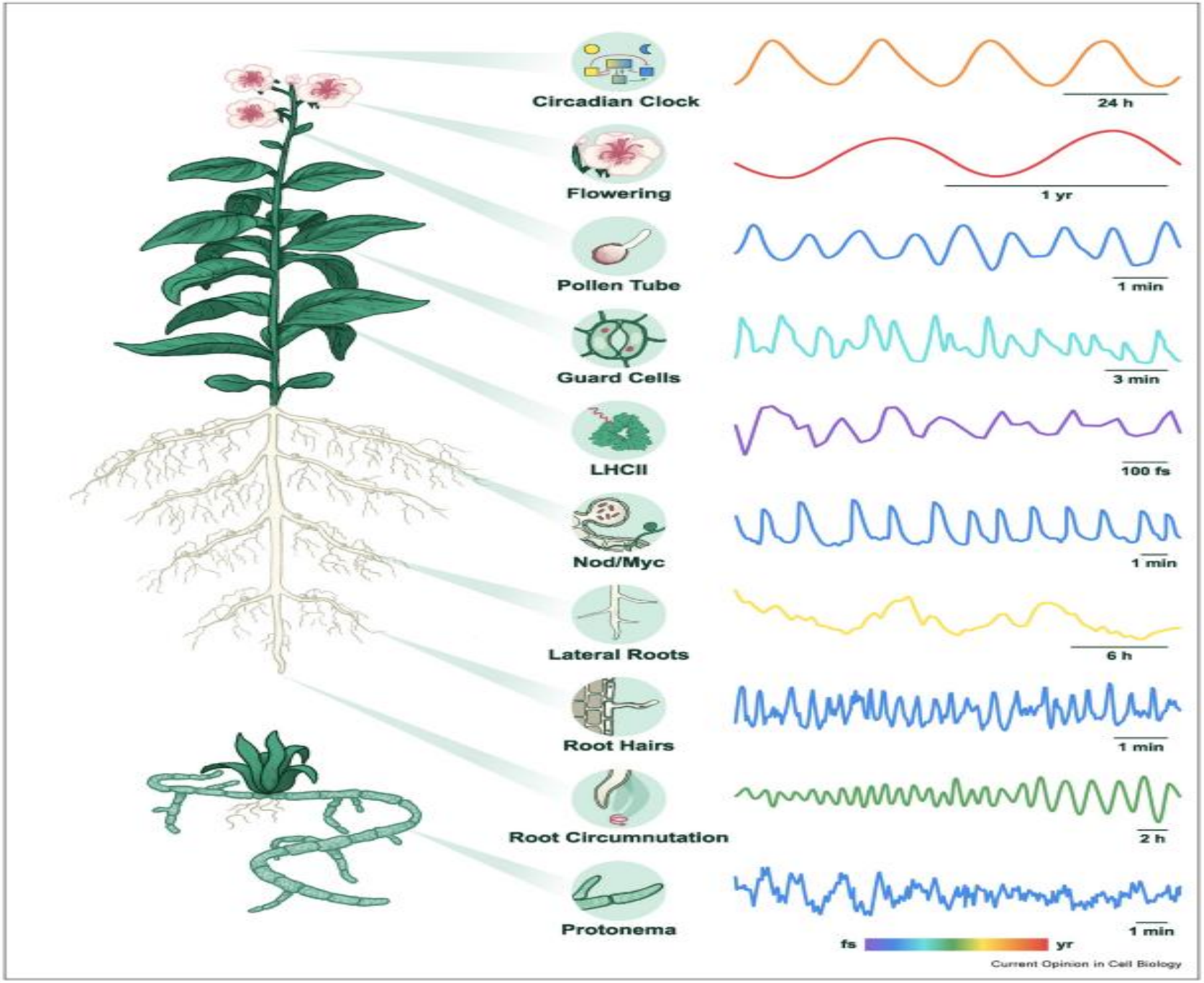
Experimental *coupling* between *bioelectrical* and *biochemical (Ca^{2+})* signals.

(a) The propagation of a bioelectrical excitation wave in multicellular *pancreatic islets*. The activity time delay for the first ($x = 0$) and last ($x = L$) cells of a linear chain is shown. Note *correlation* between the time traces of the *cell potential V* and the intracellular *calcium concentration* c_{Ca}.

(b) *Cell potentials* and *cytoplasmic calcium levels* are interrelated in *Xenopus embryogenesis*. Normally developing cells show a *potential window*. Polarization and depolarization phenomena associated with the expression of two potassium channels (inward-rectifying pore-mutant dnKir6.1 and Kv1.5) cause elevations of calcium levels that fade over developmental time.

The examples suggest that *ion channels, cell potentials,* and *signaling ions levels* can be *coupled* in *oscillatory* and *bi-stable memories*, as assumed in the model.

Curr. Opin. Cell Biol. 2022
10.1016/j.ceb.2022.102113



Plants also generate electric potential and Ca^{2+} level *oscillations* in many spatio-temporal scales of biological organization. Also, *calcium-binding proteins* influence key *transcriptional processes* here.

Ion channels are involved in most *oscillatory regulations*, as shown by optogenetic techniques, which allow direct manipulation of membrane potential and hypothesis testing.

3.d Experimental systems and facts

- *Homeostatic channel regulation in neurons*
- *Cell cycle*
- *Electro-genetic circuits*
- *Embryonic differentiation*
- *Cardiac electrophysiology*
- *Excitation-transcription coupling in synaptic plasticity*
- *Developmental processes*
- *Calcium levels*

suggest that *bioelectricity* can influence *protein expression* through a *small number* of *counteracting voltage-gated channels*. In particular, *single-cell* and *multicellular potentials* can participate in transcription, together with other transporters such as *ligand-gated channels,* through complex downstream processes.

4. Bioelectrical model assumptions and equations

J. Phys. Chem. Lett. 2020
10.1021/acs.jpclett.0c00641
Cell 2021
10.1016/j.cell.2021.02.03

General ideas to be incorporated in a *biophysical* model:

- *complex patterns* can result from relatively *simple local rules* of interaction between individual units. The final pattern can emerge with *no central coordination* because of the multiplicity of *multiscale competencies* provided by single-cell characteristics and *multicellular fields*;

- *a cell* has *limited local information*; however, the whole *multicellular system* of locally interacting cells may have a *complex representation* of *itself* and the *external environment*. Here, we consider that multicellular bioelectricity contributes to this abstraction; and

- biophysical units in different ensembles, e.g. neurons, non-excitable cells, and bacteria, may show *distinct individual characteristics* but spatio-temporal patterns characterized by *polarized/depolarized multicellular regions* can be identified experimentally.

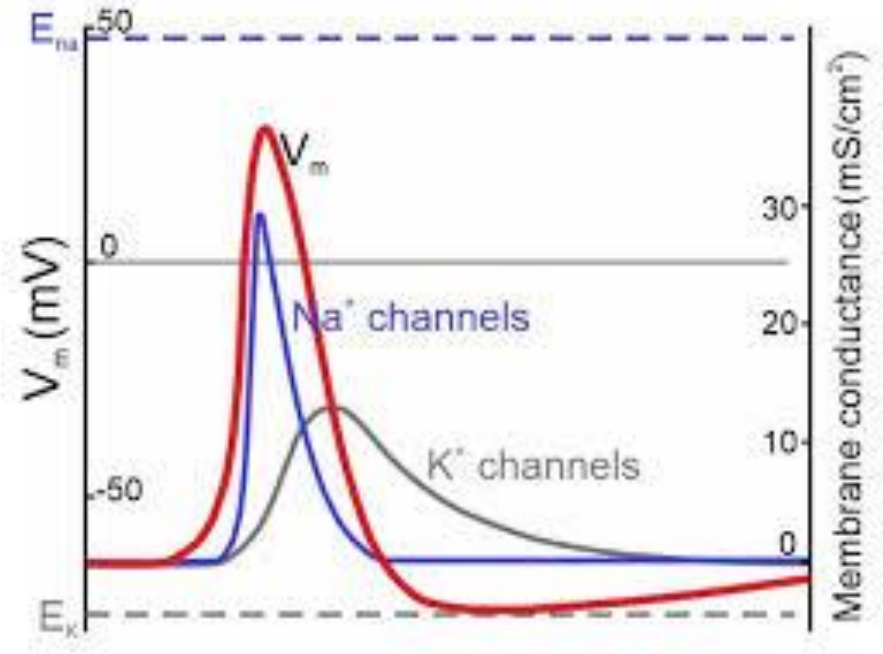
The above facts suggest that *slow bioelectrical mechanisms* in ensembles of non-excitable cells, reminiscent of the relatively fast processes found in neural networks, should be explored.

Sci. Rep. 2016
10.1038/srep35201
J. Phys. Chem. Lett. 2020
10.1021/acs.jpclett.0c00641

Specific facts to be taken into account in a simple *bioelectrical* model:

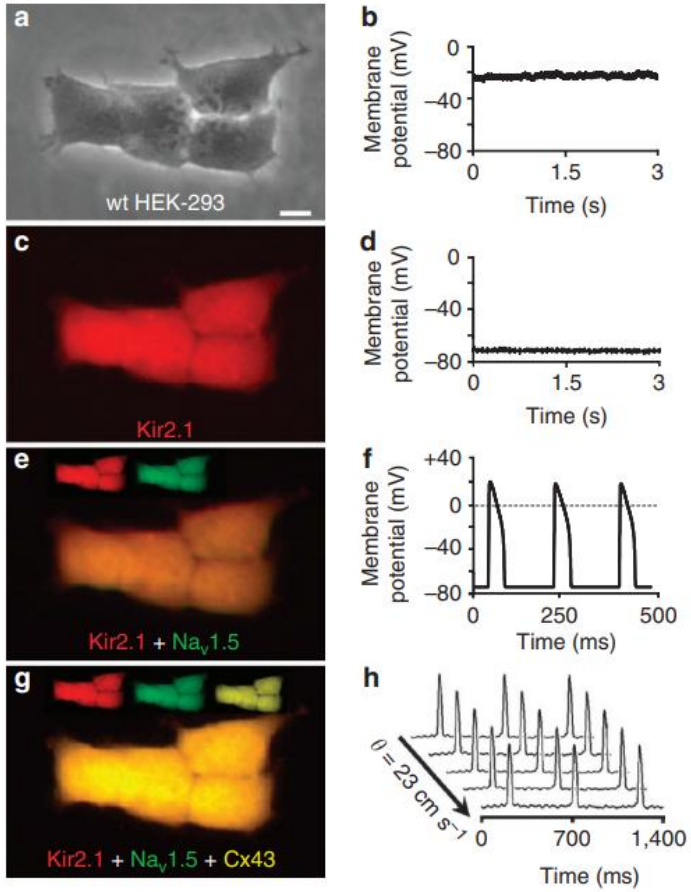
1) many *ion channels* are *voltage-sensitive* and operate over a *wide voltage window*: channels can *polarize* or *depolarize* the cell depending on the particular cell state and the environmental conditions;

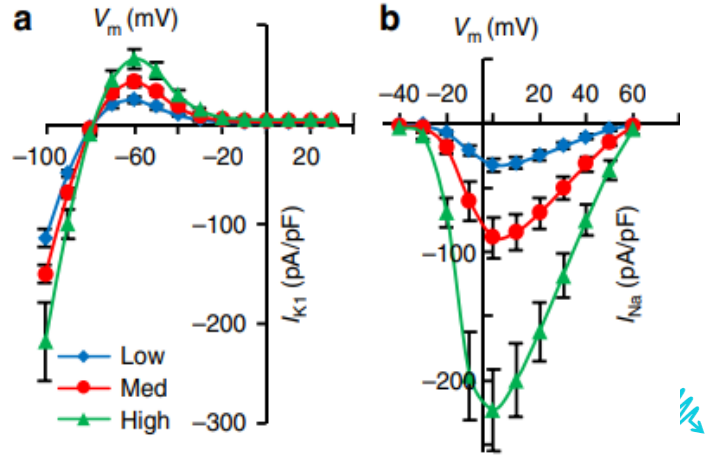
2) in most cases, the voltage-gated conductances of a *small number* of *counteracting channels* are central to the regulation of polarized (*pol*) and depolarized (*dep*) cell states. A paradigm here may be the action potential, where the combined action of *sodium* and *potassium* channels contribute to the observed *depolarization* and *polarization* phenomena;

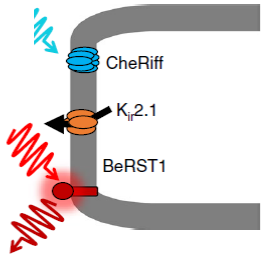


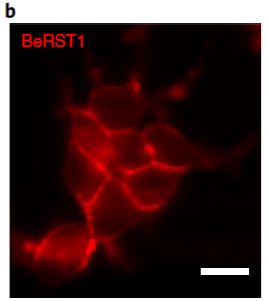
3) biosynthetic *excitable tissues* can be designed from non-excitable cells using a *reduced number* of channels and intercellular junctions. Also, nearly homogeneous tissues show spatial *symmetry breaking*, with the formation of *domains* of *bi-stable cell potentials,* in cell lines engineered with a small number of channels;

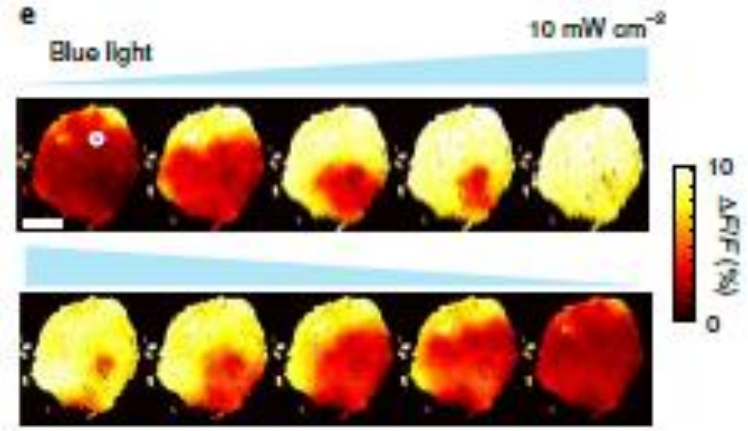
Nat. Commun. 2011, 2016
10.1038/ncomms1302
10.1038/ncomms13132
Nat. Phys. 2020
10.1038/s41567-019-0765-4

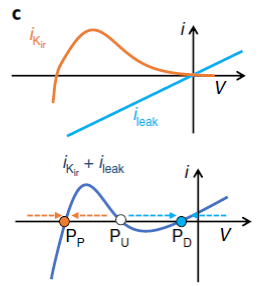


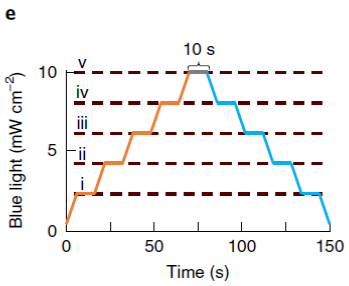


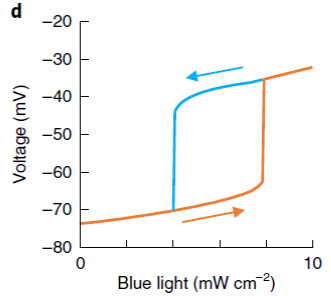


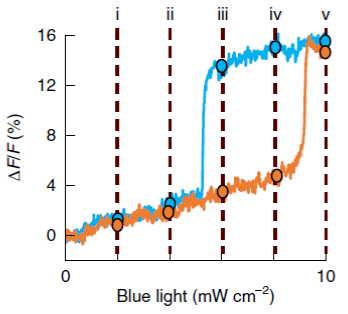








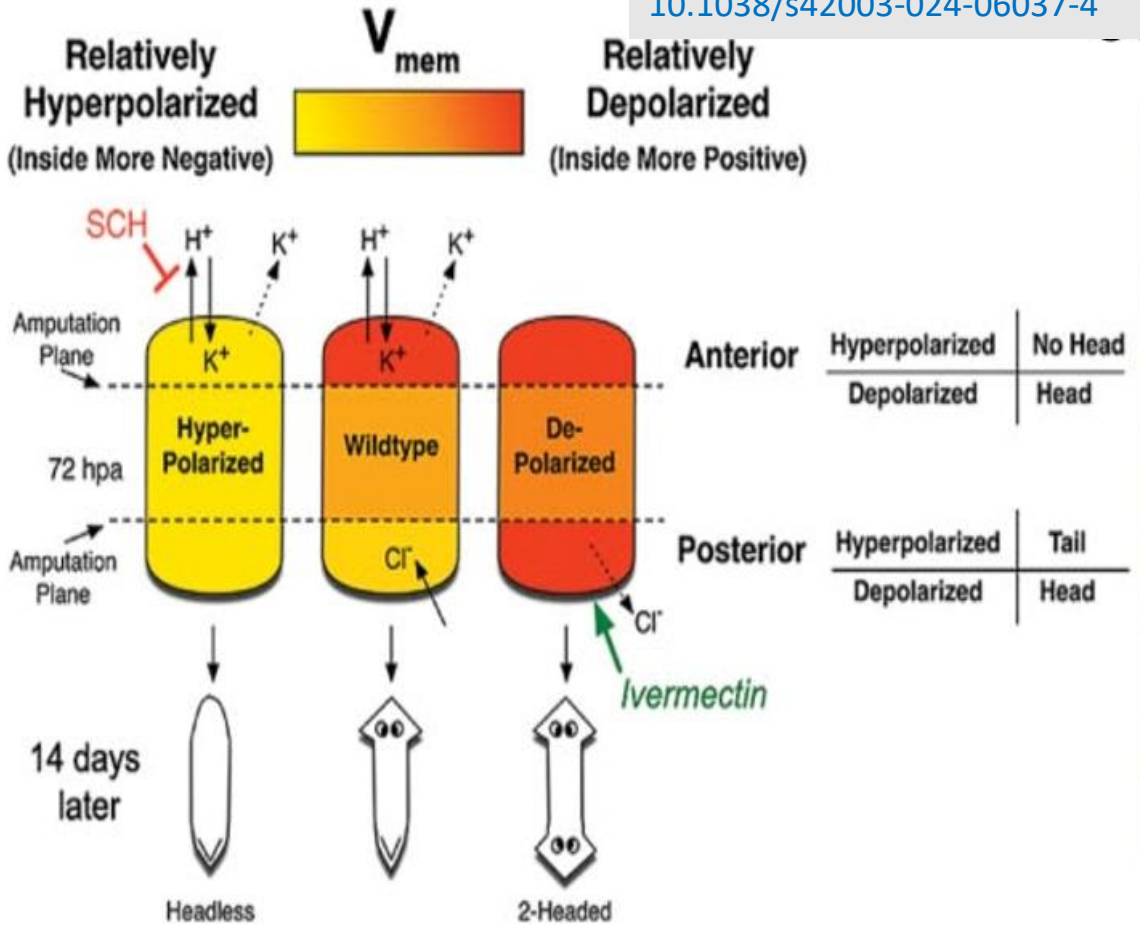




4) channel proteins and cell *membrane potential* influence *transcription via second-messengers* such as calcium, serotonin or butyrate that can regulate downstream processes. The *coupling* between *bioelectricity* and *transcription* can encode *spatio-temporal information* through the response of *multicellular potentials* to *external inputs*, e.g. different symmetry-breaking phenomena; and

5) the cell state is *updated* in response to *environmental changes*. Because of the intercellular connectivity, average *multicellular potentials* can *suppress perturbations* in local cell polarization but may also *permit morphologically instructive* transitions induced by environmental changes. Thus, *bioelectricity* can contribute to the *adaptive* phenomena observed in complex *cognitive systems* that show abilities to *solve problems* and deal with *novel challenges*.

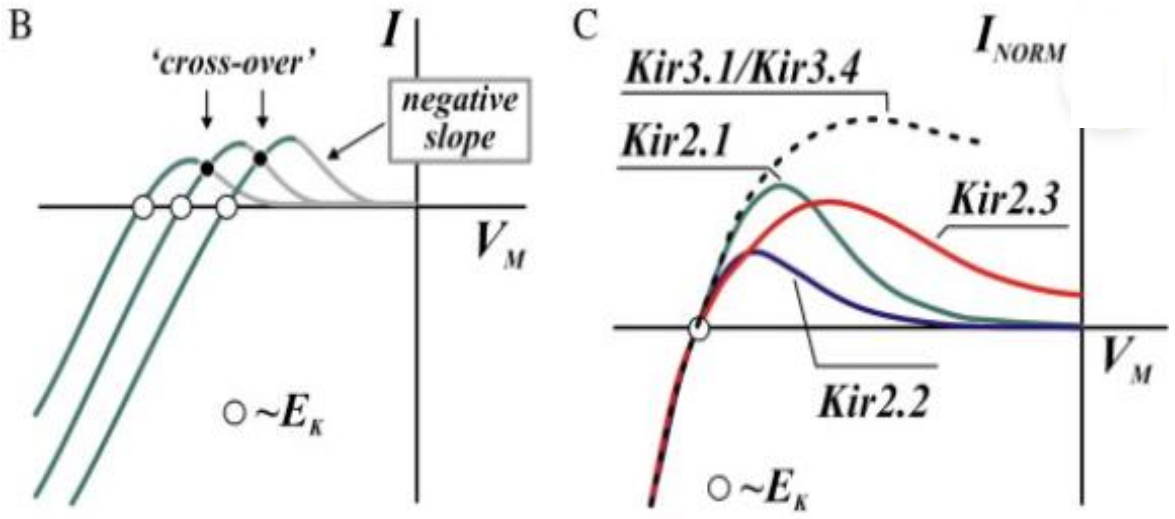
J. Phys. Chem. Lett. 2020
10.1021/acs.jpclett.0c00641
Cell 2021
10.1016/j.cell.2021.02.03
Commun. Biol. 2024
10.1038/s42003-024-06037-4

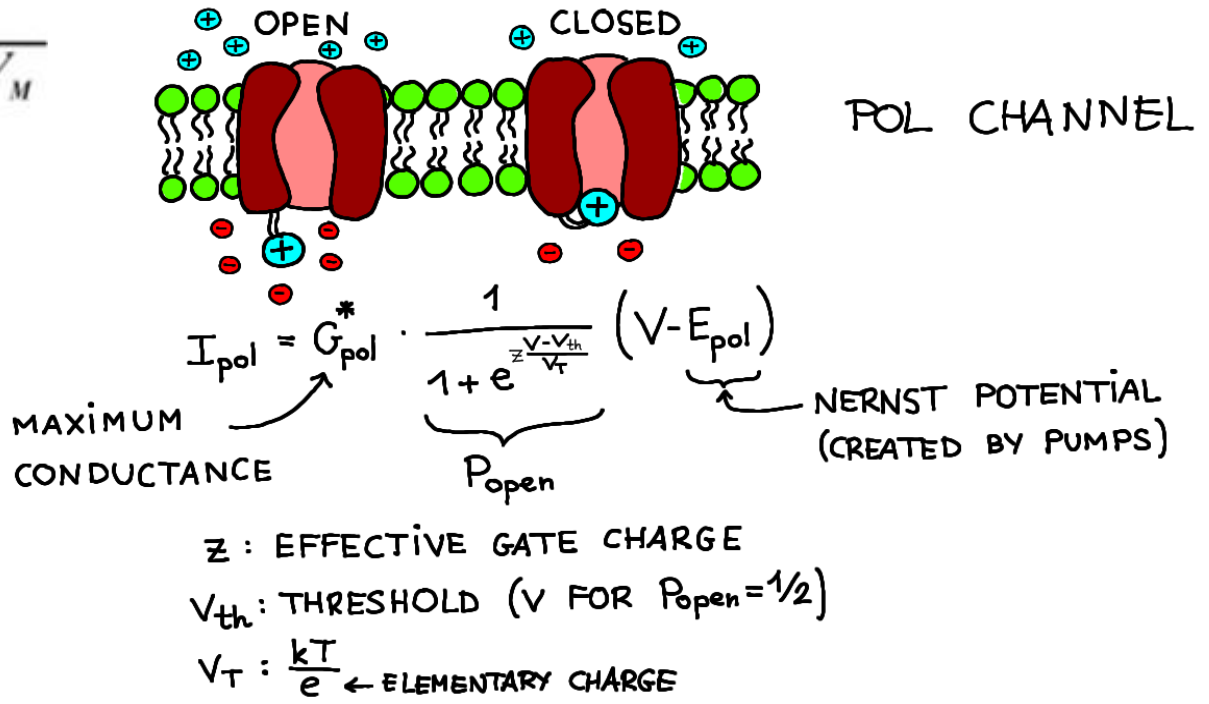


4a Single-cell bioelectrical model

J. Mol. Cell. Cardiol. 2009
10.1016/j.yjmcc.2009.08.013
J. Phys. Chem. B 2014
10.1021/jp508304h

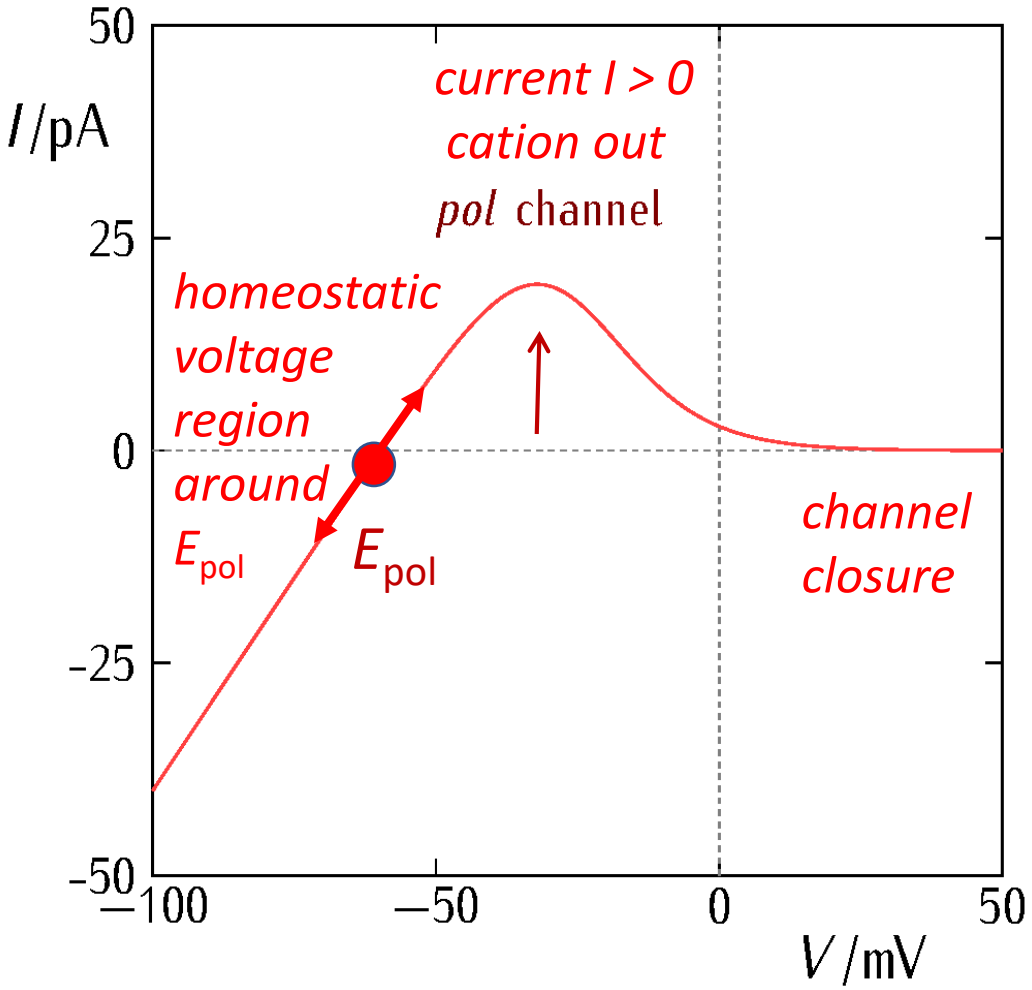
K^+ channel
I-V curves





In the model, a *generic pol* conductance simulates a *diverse family* of channels acting to *polarize* the cell.

J. Phys. Chem. B 2014
10.1021/jp508304h



context-sensitive:

channel can repolarize *slight* depolarization (*homeostatic* region, disruptive event *suppresed*)

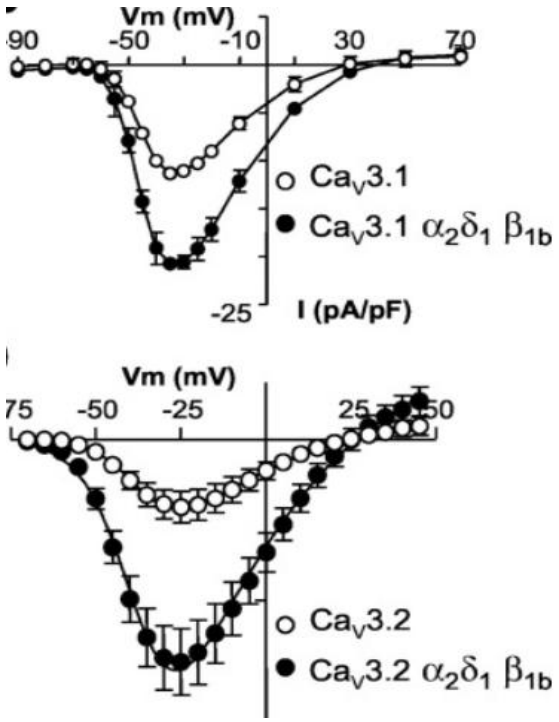
but

may permit *strong* depolarization (*closure*), thus allowing a

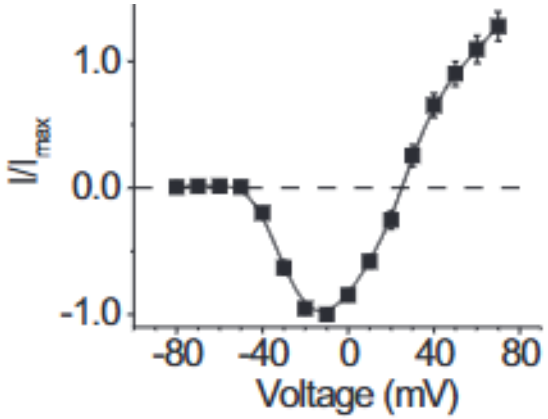
bioelectrical regionalization

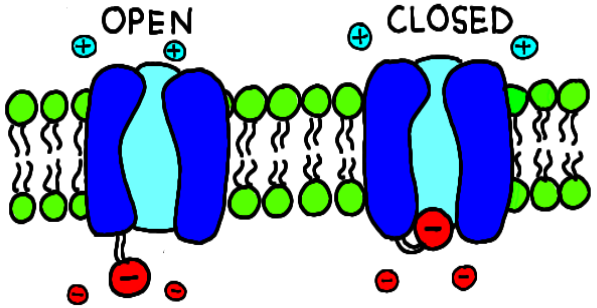
PNAS 2012
10.1073/pnas.1115575109
J. Biol. Chem. 2004
10.1074/jbc.M313450200
J. Phys. Chem. B 2014
10.1021/jp508304h

Ca^{2+} channel
I-V curves



Na^+ channel
I-V curve





DEP CHANNEL

$$I_{dep} = G^*_{dep} \cdot \frac{1}{1+e^{z\frac{V-V_{th}}{V_T}}} (V-E_{dep})$$

MAXIMUM CONDUCTANCE

P_{open}

NERNST POTENTIAL (CREATED BY PUMPS)

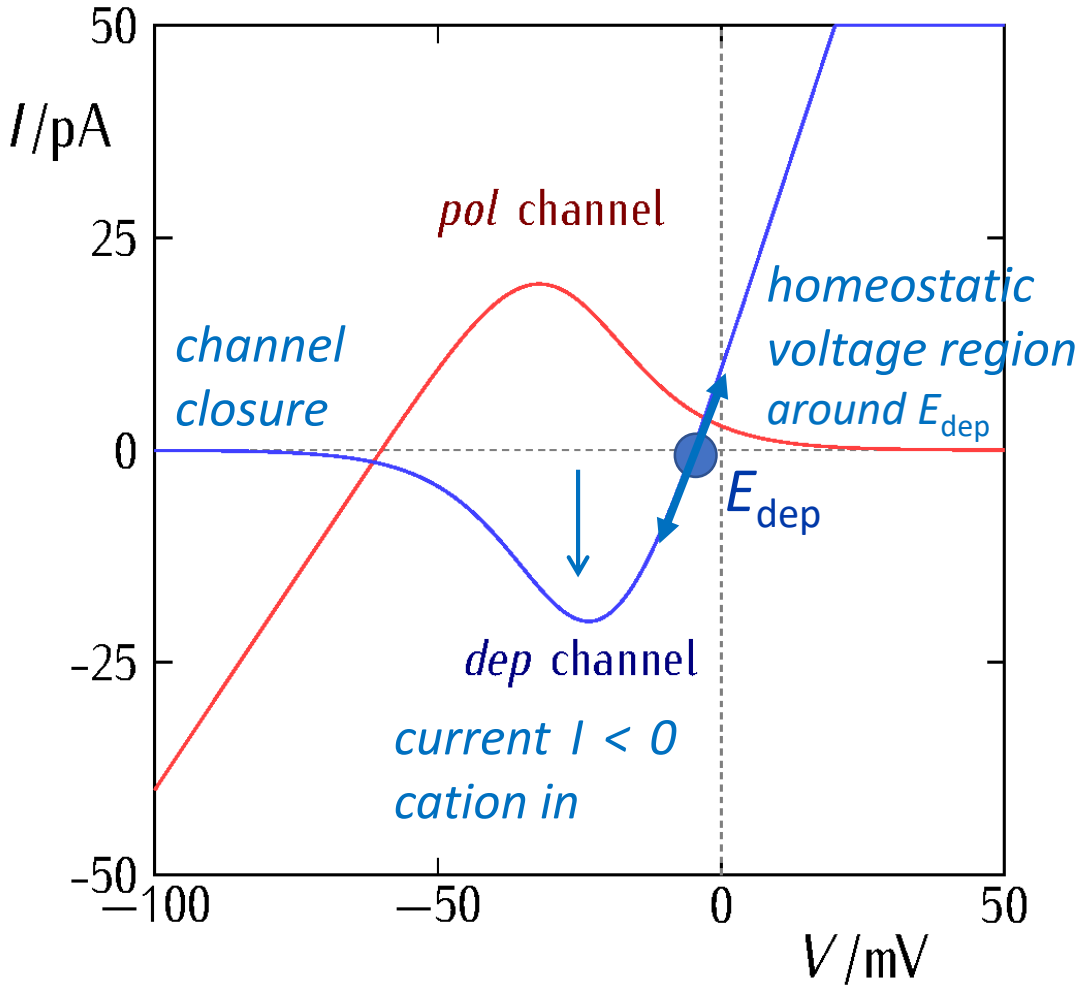
z : EFFECTIVE GATE CHARGE ($z<0$)

V_{th} : THRESHOLD (V FOR $P_{open}=1/2$)

V_T : $\frac{kT}{e}$ ← ELEMENTARY CHARGE

In the model, a *generic dep* conductance simulates a *diverse family* of channels acting to *depolarize* the cell.

J. Phys. Chem. B 2014
10.1021/jp508304h
Sci. Rep. 2016
10.1038/srep20403



context-sensitive:

channel can depolarize *slight* repolarization (*homeostatic* region, disruptive event *suppresed*)

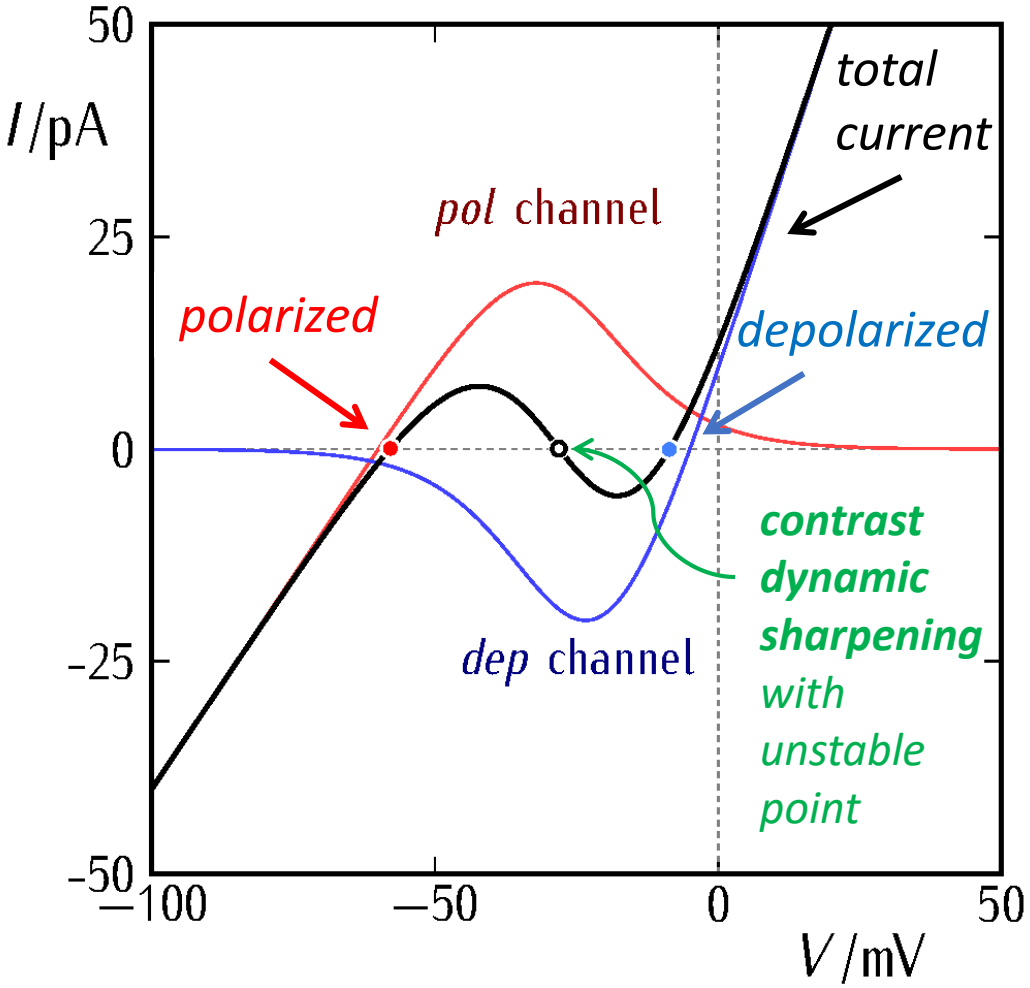
but

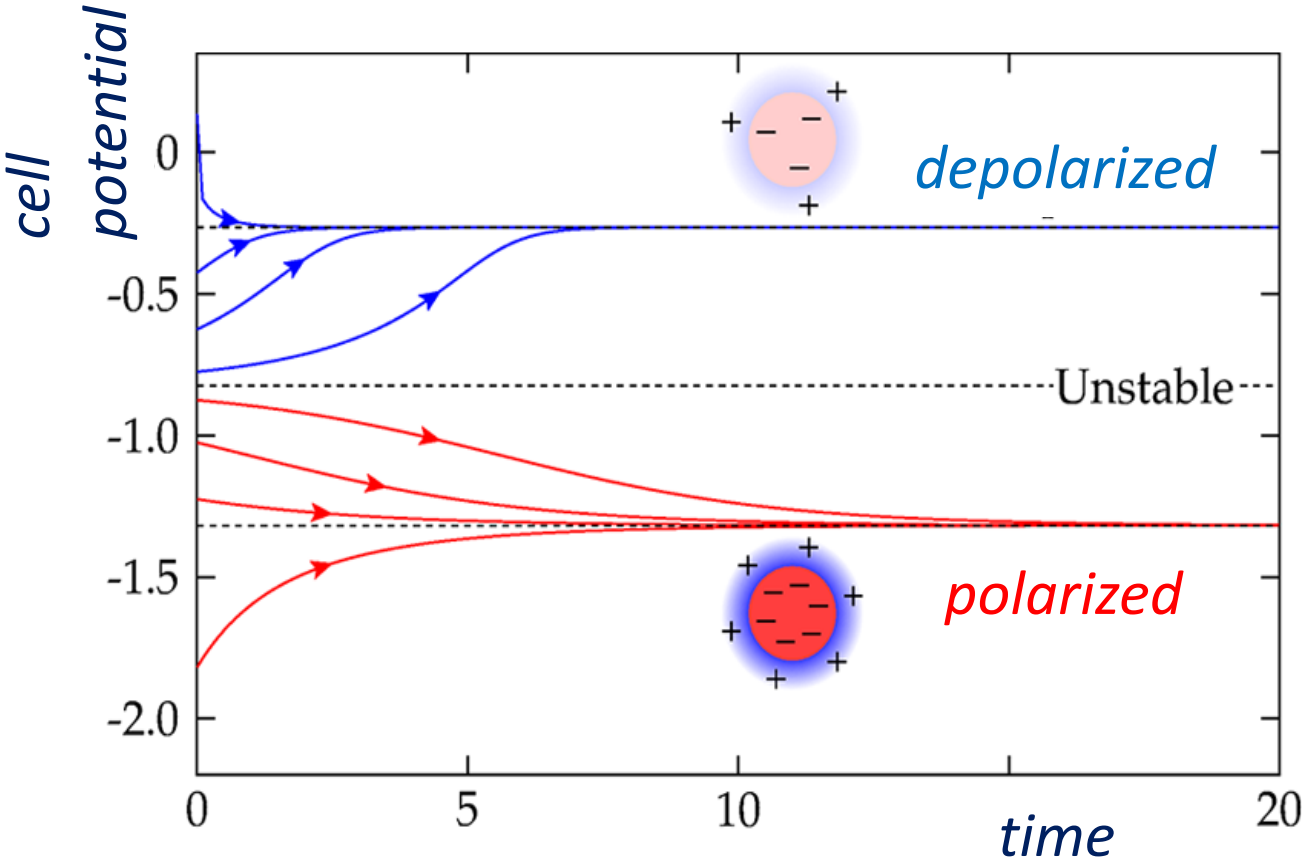
may permit *strong* repolarization (*closure*), thus allowing a

bioelectrical regionalization

J. Phys. Chem. B 2014
10.1021/jp508304h

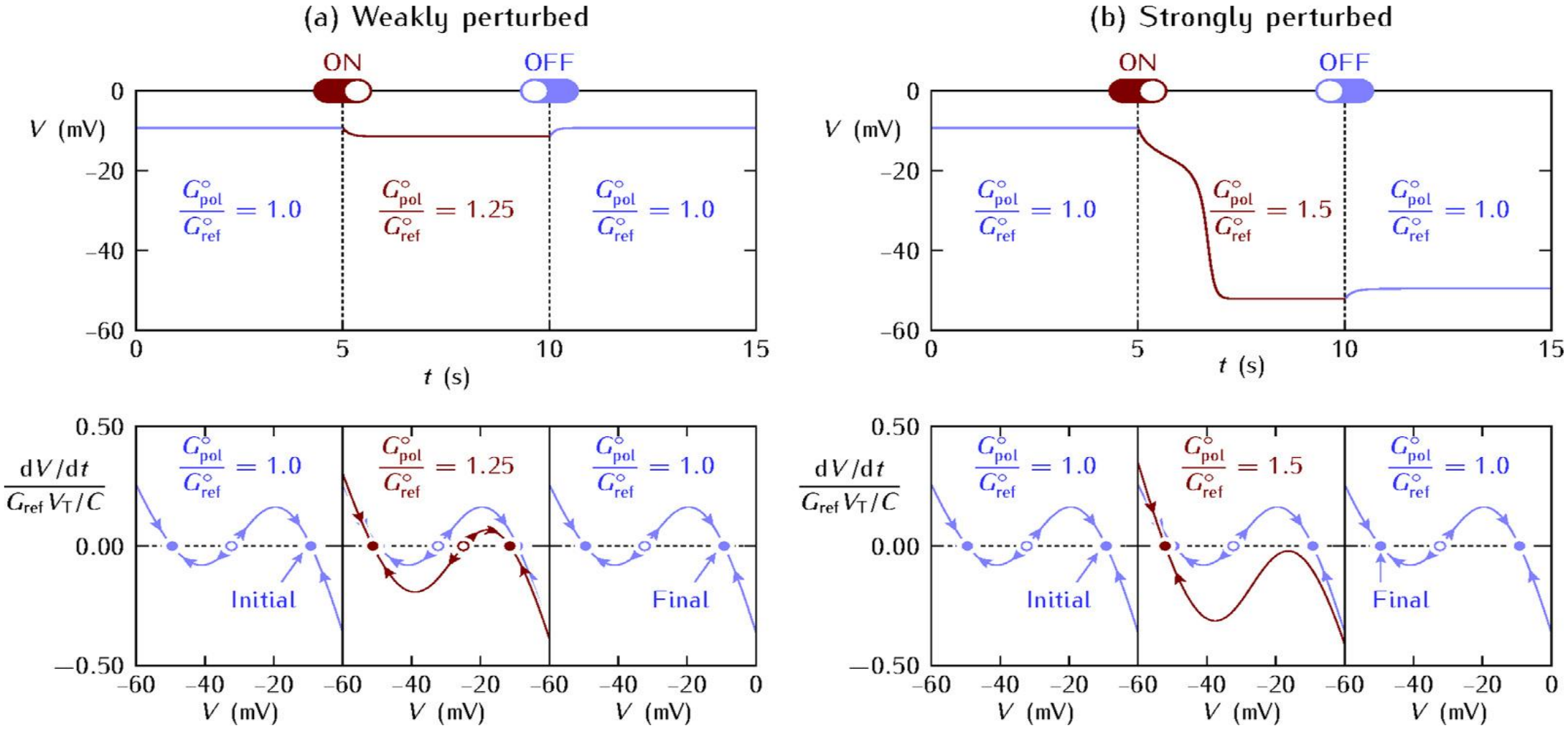
Contrast dynamic sharpening: channels operate over a *wide voltage window* and *polarize* or *depolarize* depending on the particular cell state and the environmental conditions. The *combined action* of the two generic channels *pol/dep* may give a cell potential *bi-stability* that allows the *contrast sharpening* characteristic of *bioelectrical regionalization*.





Thus, channels may support *stable single-cell memories* based on membrane potentials. A *perturbation* or *disruptive* event (e.g. the over-expression or blocking of a specific channel) changes the state of a cell with capacitance *C only* if it is sufficiently *strong*. Instructive changes can thus be established by temporary *short time* actions, as observed experimentally.

Prog. Biophys. Mol. Biol. 2019 10.1016/j.pbiolmolbio.2019.06.004

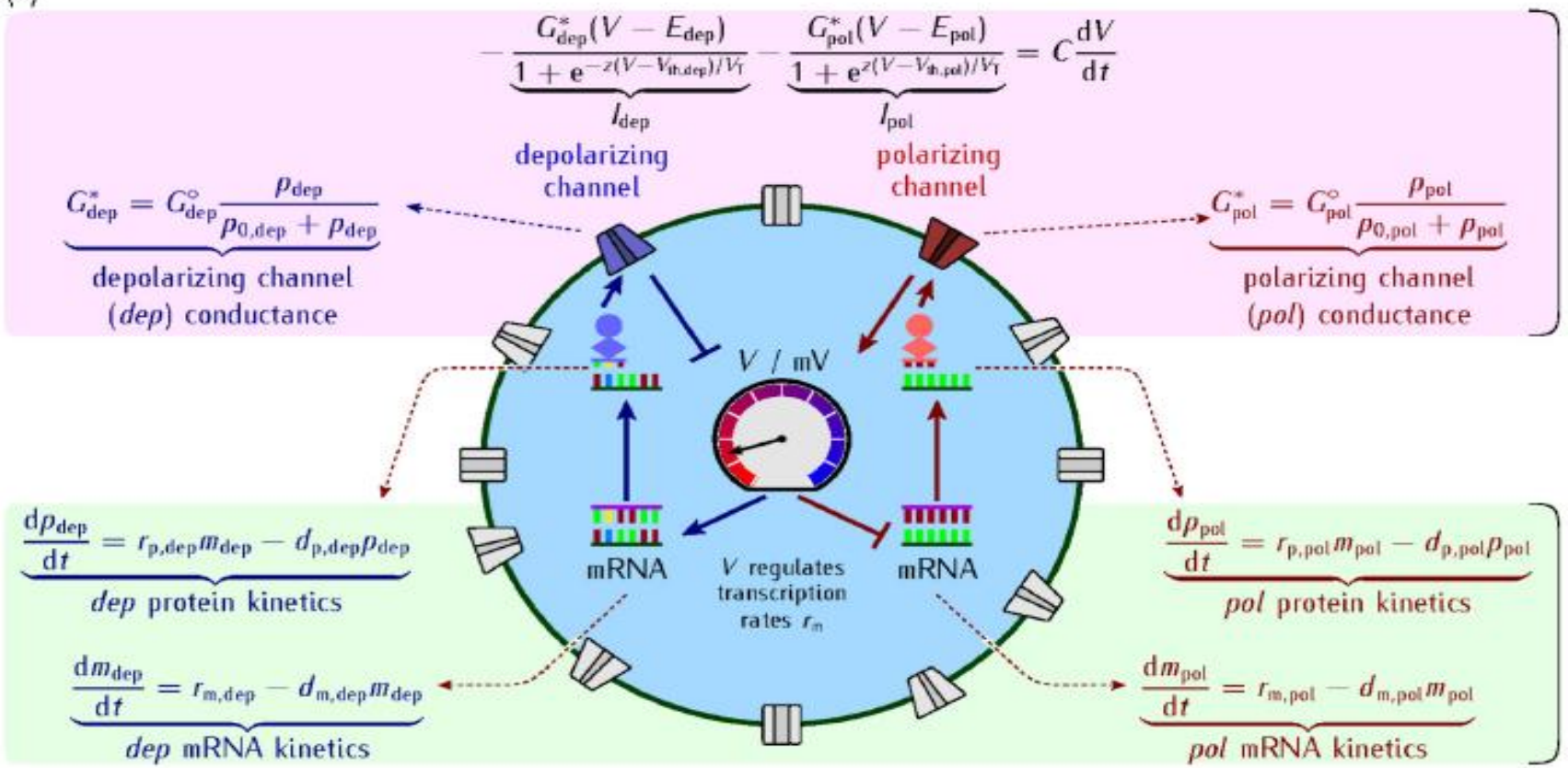


$$C(dV/dt) = -I_{pol} - I_{dep}$$

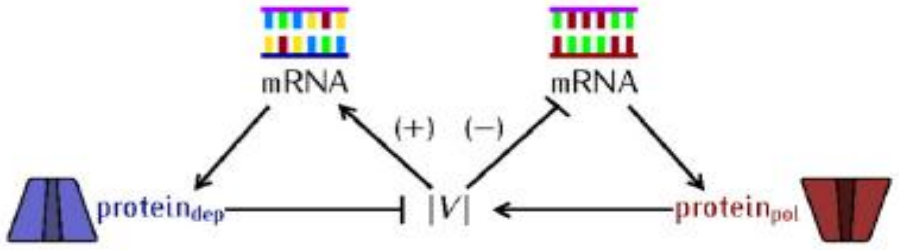
4b Coupling between bioelectricity and transcription

Sci. Rep. 2016
10.1038/srep35201
J. Phys. Chem. B 2019
10.1021/acs.jpcb.9b01717

(a)



(b)



$(+): \frac{r_{m,dep}}{r^\circ_{m,dep}} = \frac{(S_{dep}/S_{0,dep})}{1+(S_{dep}/S_{0,dep})} = \frac{e^{|V|/V_T}}{1+e^{|V|/V_T}}$ $\quad (-): \frac{r_{m,pol}}{r^\circ_{m,pol}} = \frac{1}{1+(S_{pol}/S_{0,pol})} = \frac{1}{1+e^{|V|/V_T}}$

Bioelectrical network

Transcriptional network

The *cell membrane potential V* can influence *transcription via second-messengers*, e.g. calcium: its cytoplasm *concentration S* may show a *positive* (*dep* channel here) or *negative* (*pol* channel here) regulation on *V*.

Sci. Rep. 2016
10.1038/srep35201
J. Phys. Chem. Lett. 2020
10.1021/acs.jpclett.0c00641

In this bioelectrical model:

- the transcription and translation of the *dep* and *pol* channel proteins is described in terms of the *mRNA* (m_{dep} and m_{pol}) and *protein* (p_{dep} and p_{pol}) *concentrations*;

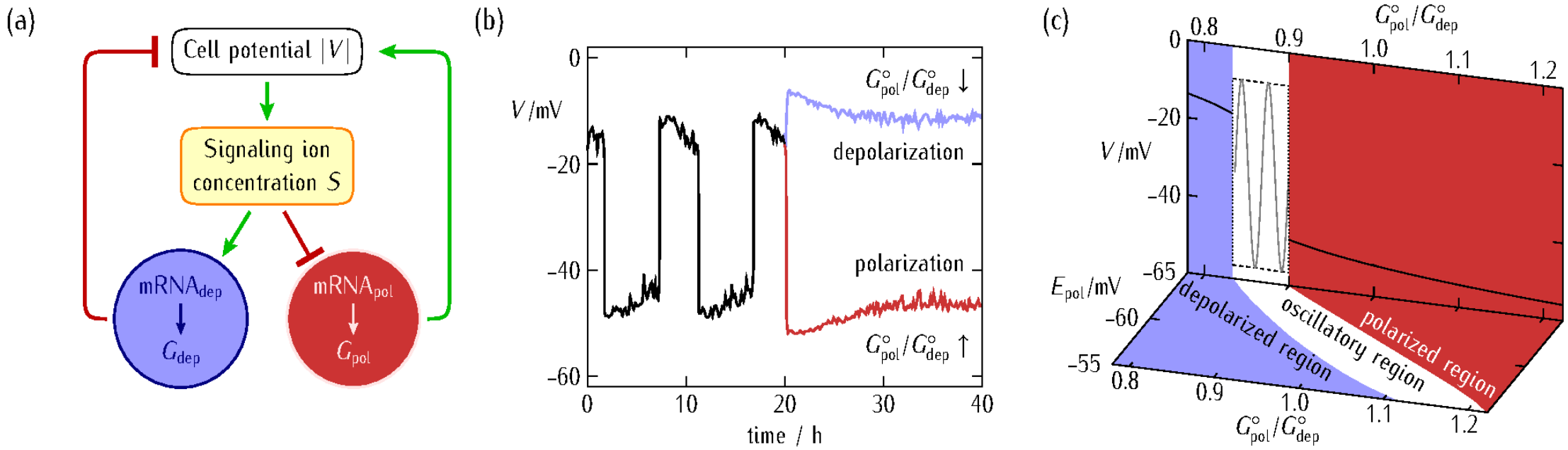
- the *rate constants* $r_{m,k}$ and $r_{p,k}$ (k = *dep*, *pol*) regulate the mRNA transcription and protein translation processes, respectively, which occur with the respective *degradations rates* $d_{m,k}$ and $d_{p,k}$ (k = *dep*, *pol*);

- the *conductances* $G^*_k = G^o_k\, p_k/(p_{0,k} + p_k)$ (k = *dep*, *pol*) follow a *Hill kinetics* with the respective protein concentrations p_k, where G^o_k is the *maximum conductance* and $p_{o,k}$ is the *reference protein concentration* which gives the half-maximum conductance. This kinetic saturation of the *dep* and *pol* channel conductances reflects the finite values of the transcription and translation rates and may also incorporate the limits imposed by the protein trafficking to and insertion in the cell membrane. The action of specific blockers can also be introduced by decreasing the effective values of the maximum conductance G^o_k; and

- the *coupling* between transcription and bioelectricity results in cell potential *V*-dependent mRNA transcription rates $r_{m,k}(V)$ where $r^o_{m,k}$ are the reference values (k = *dep*, *pol*). In this way, *V* modulates the protein concentrations and the resulting channel conductances through the transcription while the channel conductances modulate *V* through the post-translational bioelectricity. Note that this coupling should be *system specific*.

J. Phys. Chem. Lett. 2020
10.1021/acs.jpclett.0c00641

the model allows bi-stable and oscillatory single-cell cell potentials

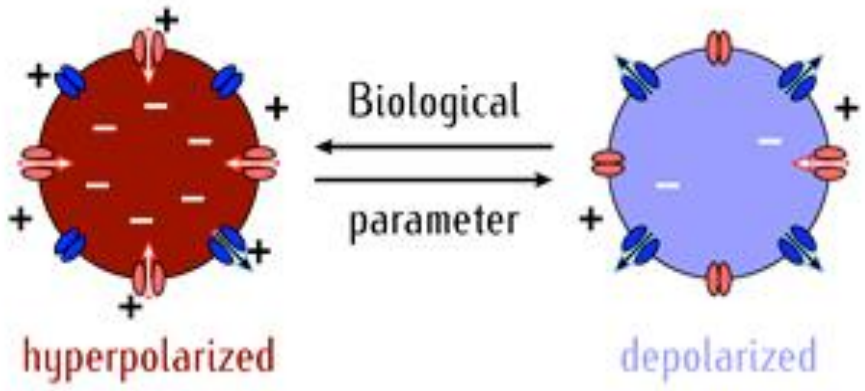


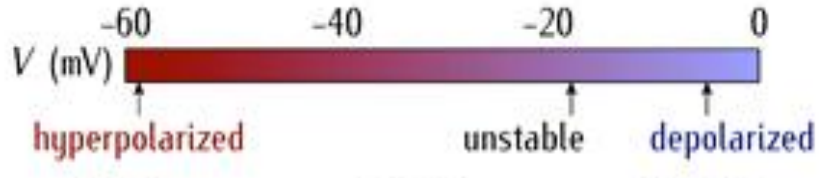
(a) The *cell potential V* characterizing the *pol* and *dep* states, regulated by the *bioelectrical* and *transcriptional feedback*, for the case of a *signaling ion* of *concentration S*. (b) The coupling between bioelectricity and transcription leads to *pol* and *dep stable* and *oscillatory* cell potentials, modulated by the maximum conductances ratio and the equilibrium potentials, when the channel protein regulation is *negative* for the *pol* channel and *positive* for the *dep* channel. (c) The conductance ratio and equilibrium potential define a *configurational space* for the bioelectrical transitions between cell states.

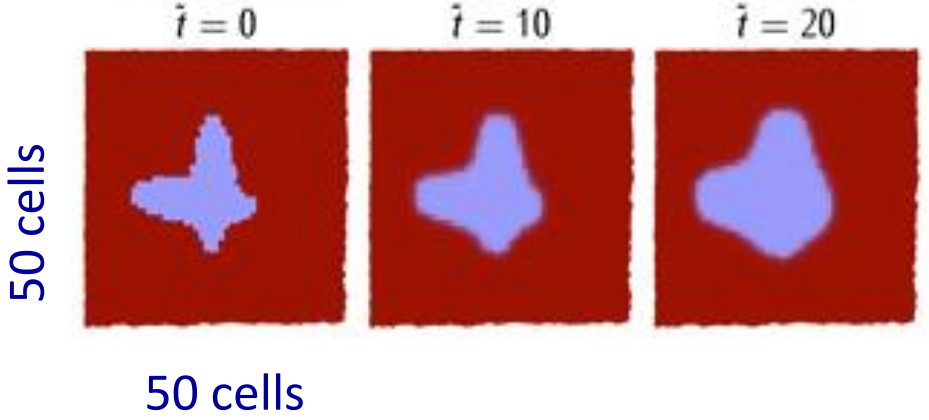
the cell as a bioelectric dynamical system

J. Phys. Chem. B 2014, 2015, 2017
10.1021/jp508304h
10.1021/jp512900x
10.1021/acs.jpcb.7b04774
Sci. Rep. 2016, 2016
10.1038/srep20403
10.1038/srep35201

Low (*depolarized*) and high (*polarized*) cell potential *states* and *transitions* between them.







Transitions are induced by changes in:

1) *ionic concentrations* (equilibrium potentials) and

2) relative values of the *polarizing* and *depolarizing* channels *conductances*.

However, *single-cell* states are coupled at the *multicellular* level. Thus, an *extended* model that includes the *intercellular gap junction* conductance is needed. These junctions may convert cell states into multicellular states characterized by *spatio-temporal maps* of electrical potentials.

limitations of the single-cell model

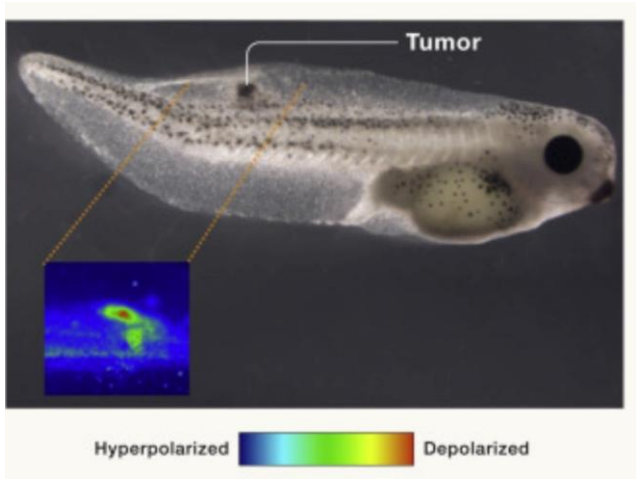
The *bioelectrical-transcriptional coupling* is *system-dependent;* thus, theoretical models may not be universal and should be modified according to the *different channels and mechanisms* relevant to each *experimental case*, as shown in the previous examples. Also, different *time windows* should exist for distinct bioelectrical mechanisms.

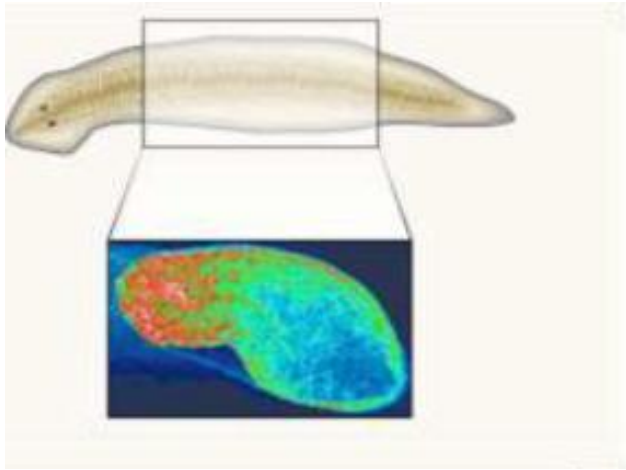
Because channels *polarize* or *depolarize* the cell depending on the particular *cell state* and the *environmental conditions*, externally-induced bioelectrical actions are not only *system-dependent* but also *context-dependent*.

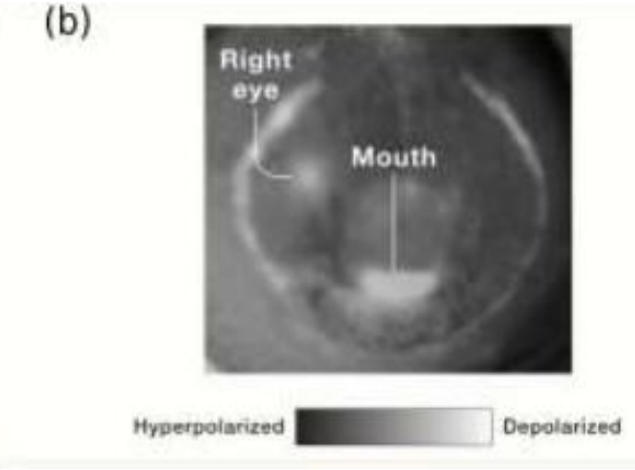
These *serious limitations* should be taken into account when attempting to describe *cell potentials* in specific experimental cases.

4c Extension to the multicelular case: intercellular gap junctions (with José A. Manzanares)

Because of intercellular connectivity, the above *single-cell* coupling between bioelectricity and transcription should be extended to the *multicellular level*. In this way, *average potentials* could either *collectively counteract* disruptive local perturbations in cell polarization or *facilitate* morphologically instructive global transitions, as needed in *different biological stages*.





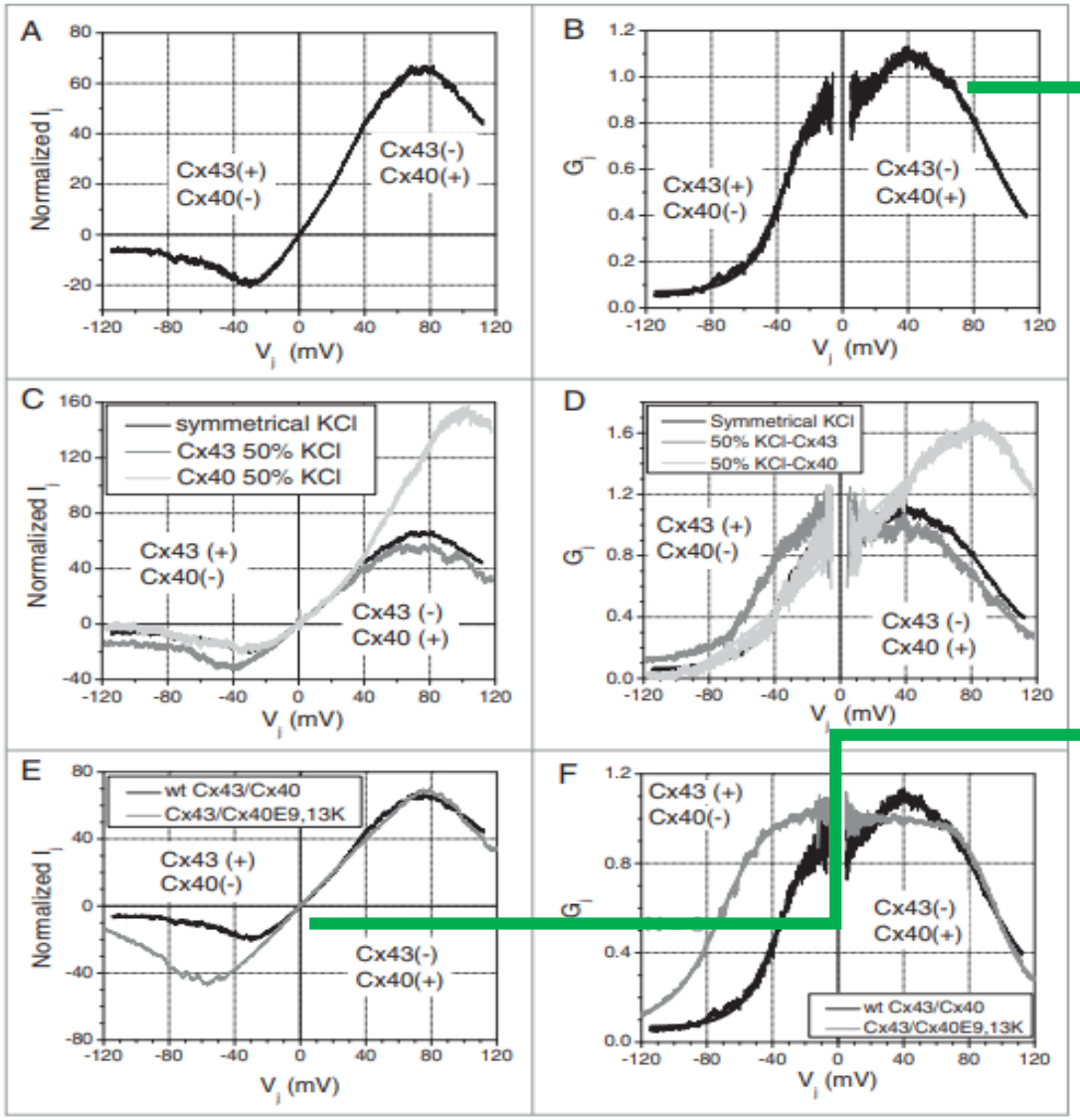


Experimentally, the *junction conductance* G_{ij} established between two neighboring cells *i* and *j* depends on the *potential difference* $V_i - V_j$. This fact contributes to the *bioelectrical regionalization* of the multicellular ensemble in *polarized/depolarized* regions, thus converting *single-cell* into *multicellular states.*

Channels 2014 10.4161/19336950.2014.949188
J. Phys. Chem. B 2019 10.1021/acs.jpcb.9b01717

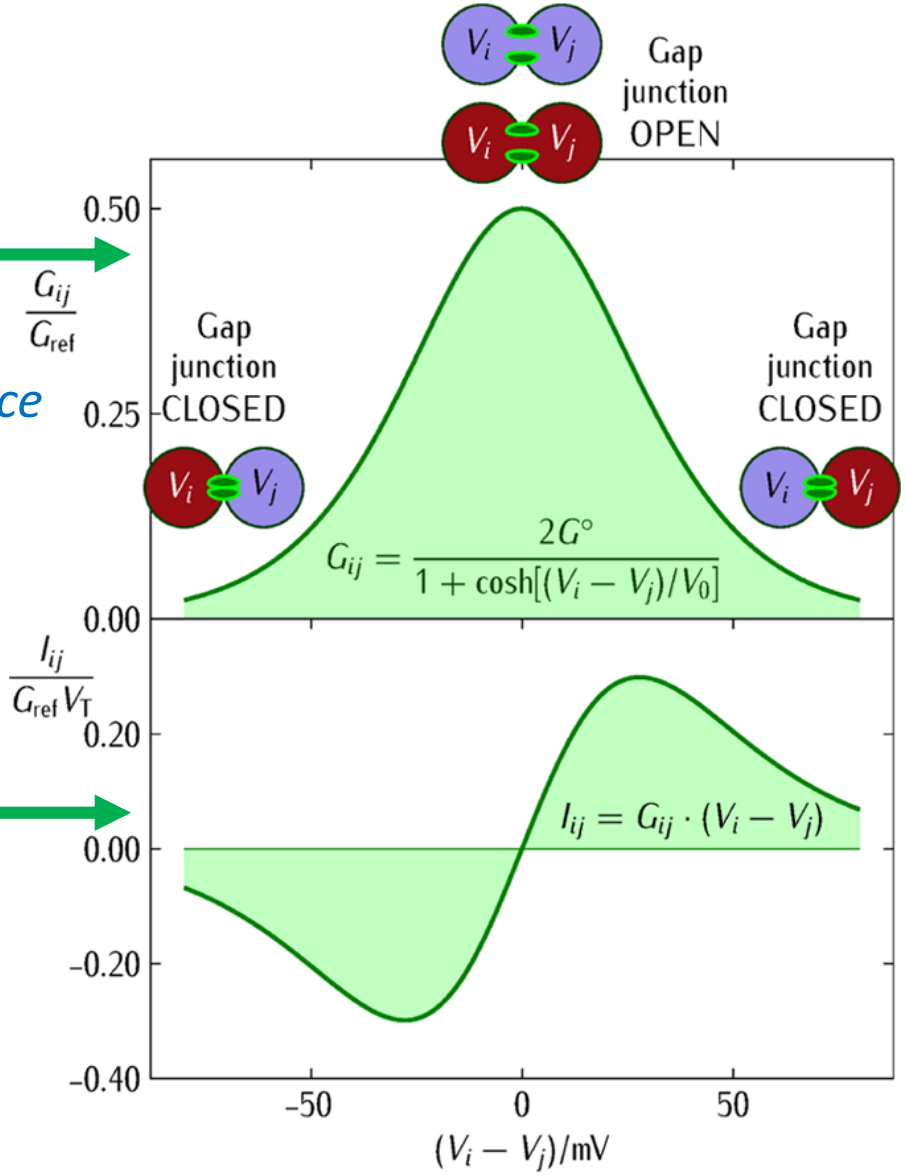
experimental junction current

experimental junction conductance



model conductance

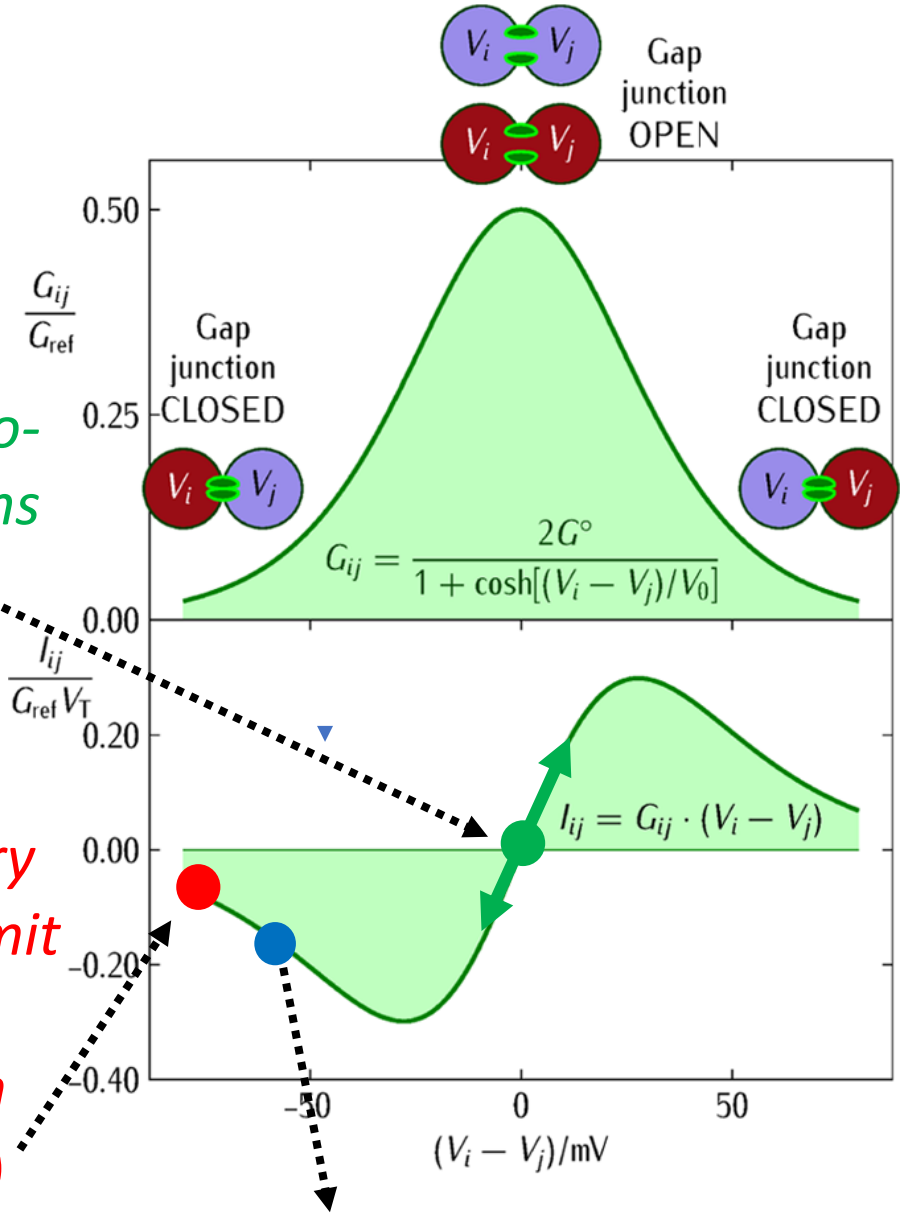
model current



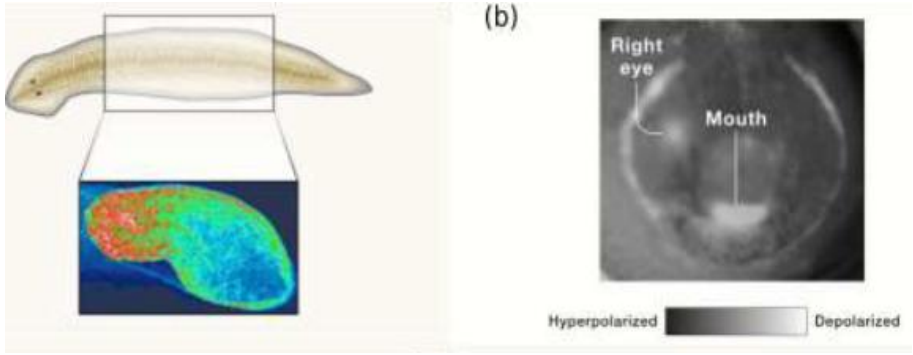
J. Phys. Chem. B 2019
10.1021/acs.jpcb.9b01717
Prog. Biophys. Mol. Biol. 2019
10.1016/j.pbiolmolbio.2019.06.004

***open**: keeps iso-potential regions (**homeostasis**)*

***closed**: inhibitory connections permit bioelectrical regionalization (**development**)*

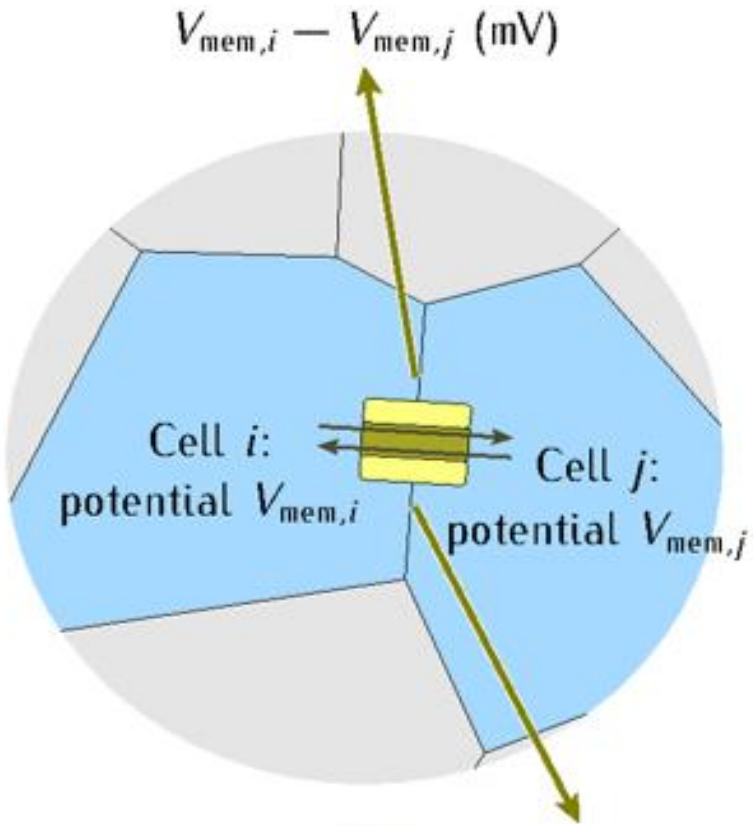


***Contrast dynamic sharpening**: gap junctions operate over a wide voltage window: they may polarize or depolarize the cell depending on the bioelectrical neighborhood and environmental conditions, which contributes to the spatio-temporal contrast needed for bioelectrical regionalization.*



Negative Differential Resistance (NDR) region

J. Phys. Chem. B 2019
10.1021/acs.jpcb.9b01717
Prog. Biophys. Mol. Biol. 2019
10.1016/j.pbiolmolbio.2019.06.004

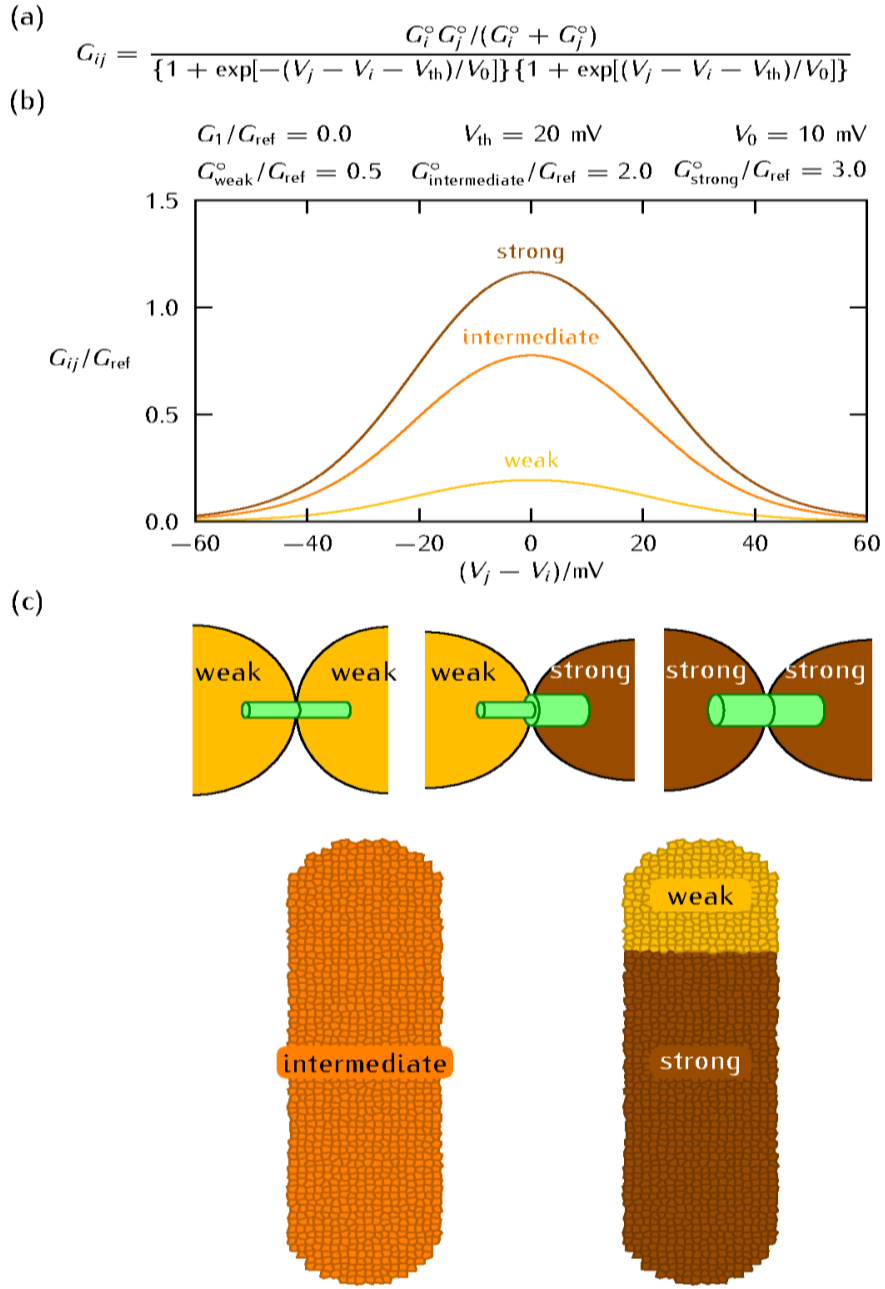


$$C_i \frac{dV_{mem,i}}{dt} = \underbrace{-I_{pol} - I_{dep}}_{\text{single cell currents to extracellular environment}} + \underbrace{\sum_{j \in nn} G_{ij} \cdot (V_{mem,j} - V_{mem,i})}_{\text{gap junction coupling current between neighboring cells}}$$

The membrane potential V_{mem} of cell i changes because of the channel currents I_{pol} and I_{dep} and the *intercellular coupling current* I_{ij} between cell i and all neighboring cells j.

Thus, the junction allows the cytoplasm-coupled cells of a spatial module to form an *isopotential region* that can be *bioelectrically regulated as a whole*, as suggested by the experimental patterns of electrical potentials.

Cancers 2021
10.3390/cancers13215300
Phys. Rev. E 2020
10.1103/PhysRevE.102.052412



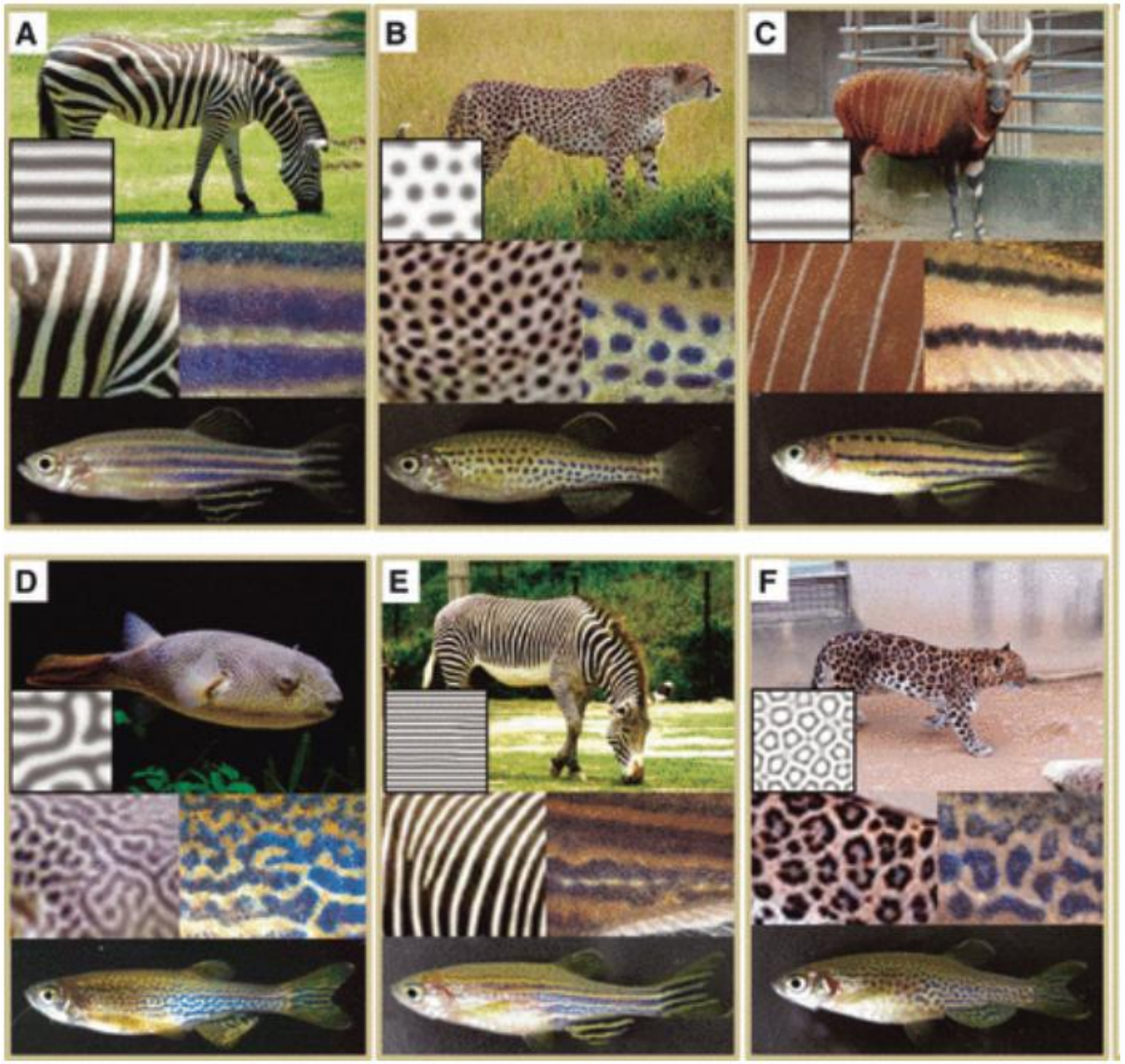
(a) Junction conductances of different connexin proteins obtained with the equation and parameters shown. Experimentally, the effective *junction conductance* can depend on the *potential difference* between *neighboring cells*.

(b) The cells *i* and *j* may also have maximum conductances that depend on the *individual polarization state* of the cell, which regulates the expression of the junction protein.

(c) As a model extension, the system can display a homogeneous spatial distribution of junction conductances (*left*) or be *regionalized* into *different modules* showing distinct conductances and intercellular couplings (*right*) because of the particular conductances prevailing in each module. Together, these facts show a *rich network connectivity dynamics*, as suggested by the variety of bioelectric patterns found experimentally.

other experimental systems: gap junction-assisted changing of skin patterns

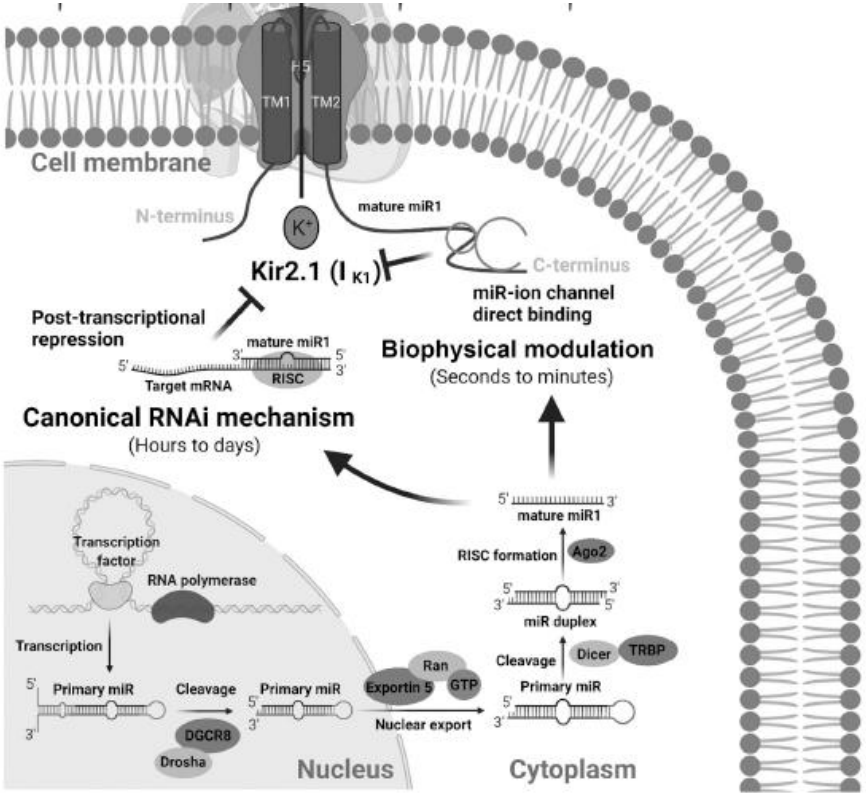
In a different, *biochemically-related context*, it has been suggested that gap junctions tune the *reaction-diffusion* (Turing type) processes responsible for the *skin patterns* observed in zebrafish, where slight junction changes lead to pattern diversity. However, it is difficult to identify the molecules that are specific to particular cellular processes because of the unselective properties of the gap-junction channels.

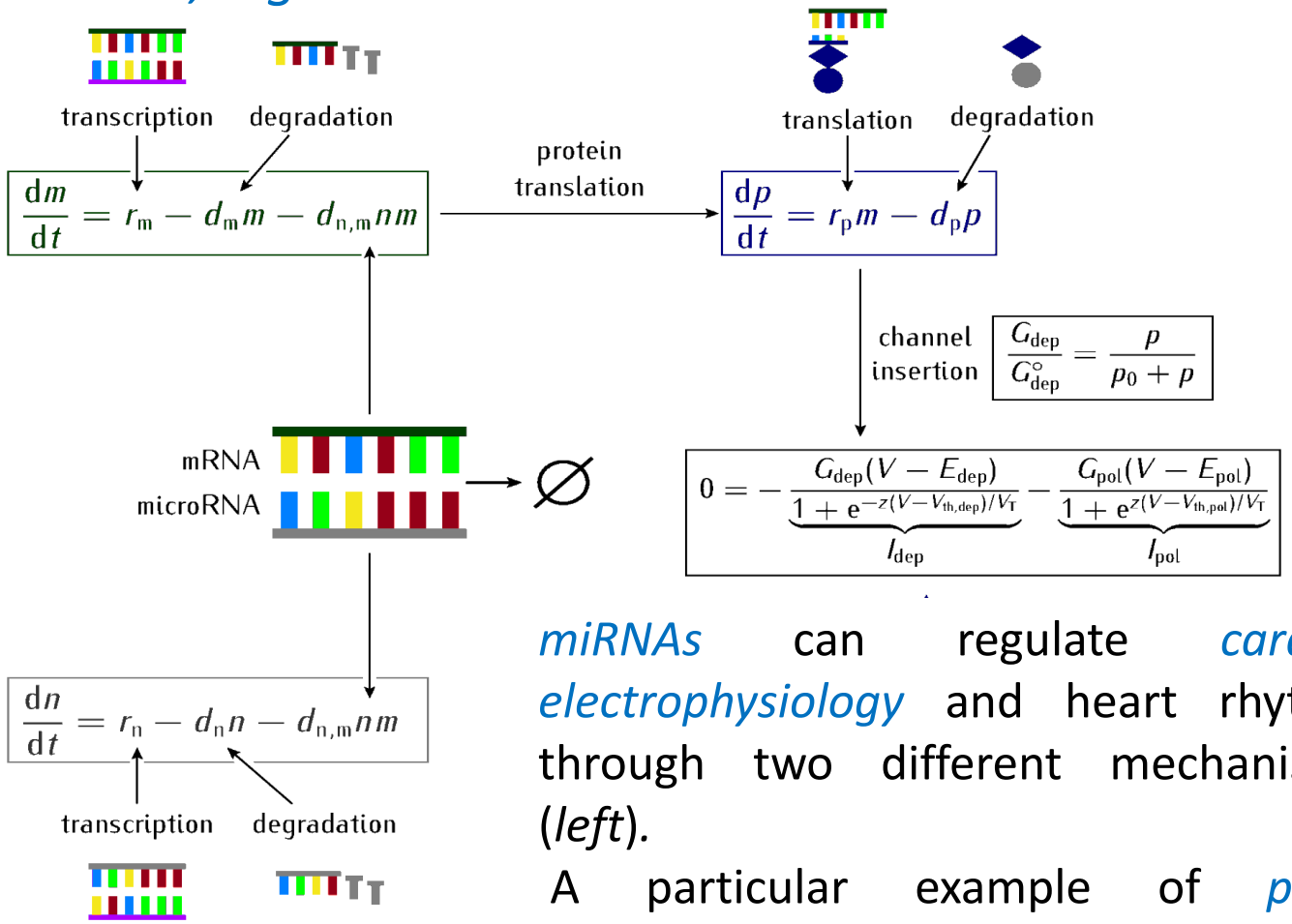


Pigment Cell Melanoma Res. 2012
10.1111/j.1755-148X.2012.00984.x
J. Biol. Chem. 2016
10.1074/jbc.M115.673129

not only intercellular currents are relevant: gap junction-mediated transfer of signaling ions/molecules, e.g. microRNA

J. Phys. Chem. B 2017
10.1021/acs.jpcb.7b04774



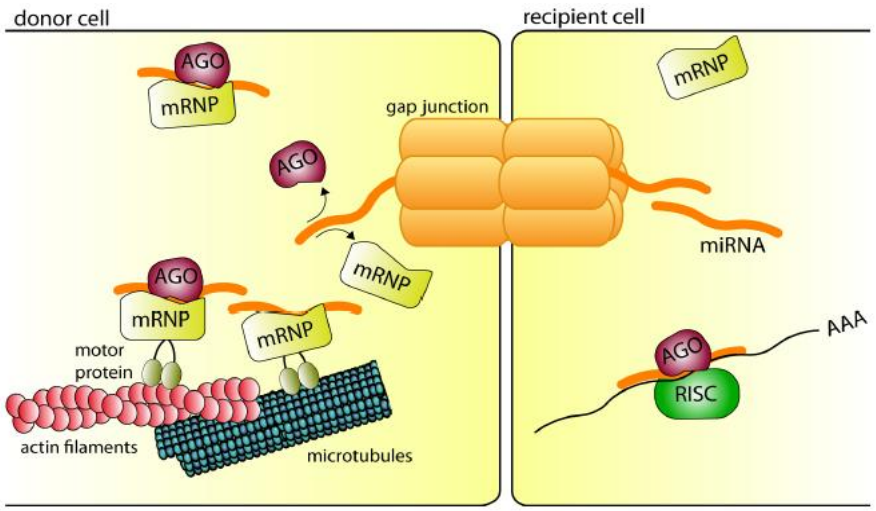


miRNAs can regulate *cardiac electrophysiology* and heart rhythm through two different mechanisms (*left*).

A particular example of *post-transcriptional RNAi mechanism* to regulate the *expression of ion channels* (*right*) and the biophysical modulation of ion channels.

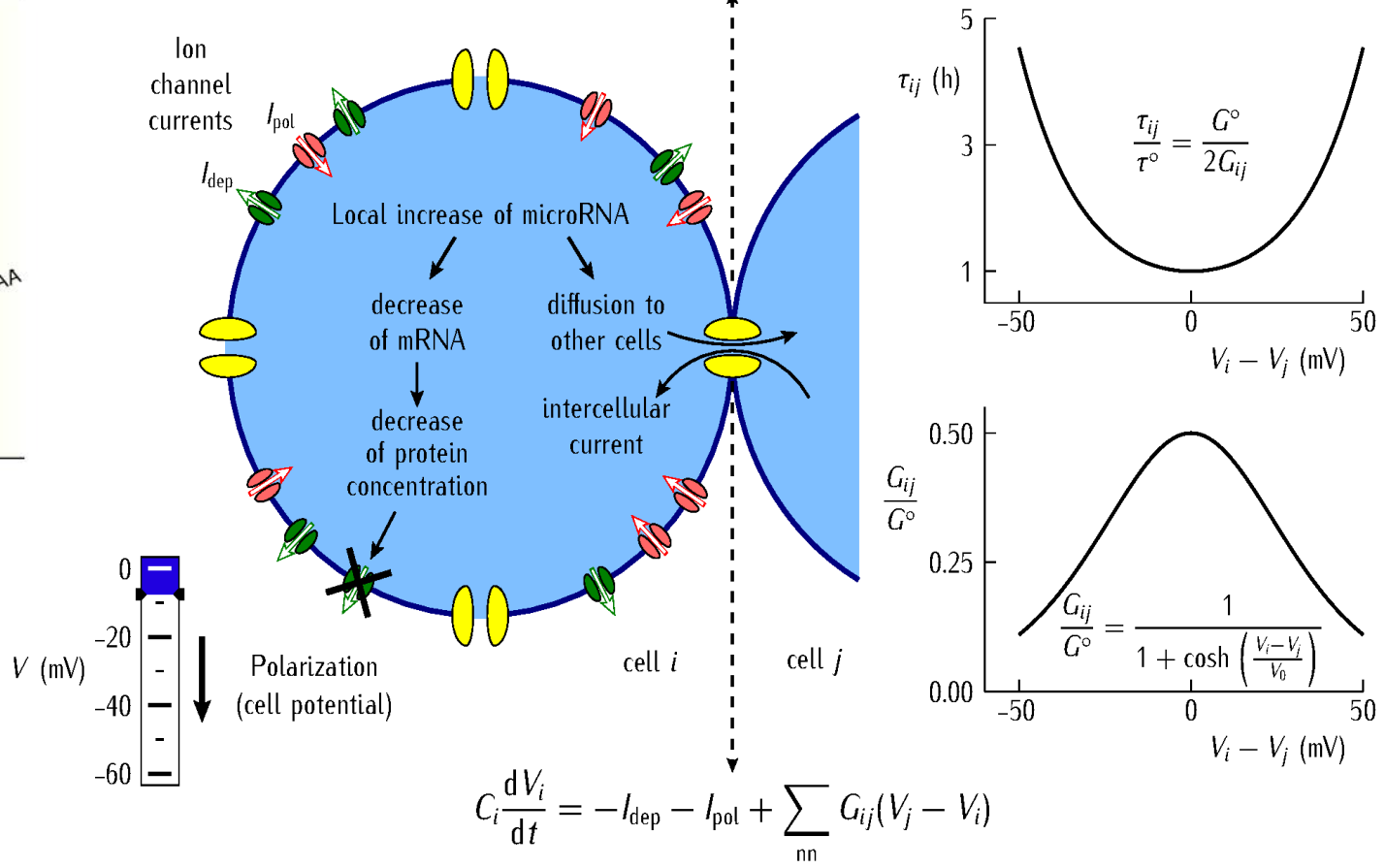
extended model for microRNA intercellular transference

J. Phys. Chem. B 2017
10.1021/acs.jpcb.7b04774
Traffic. 2018
10.1111/tra.12606



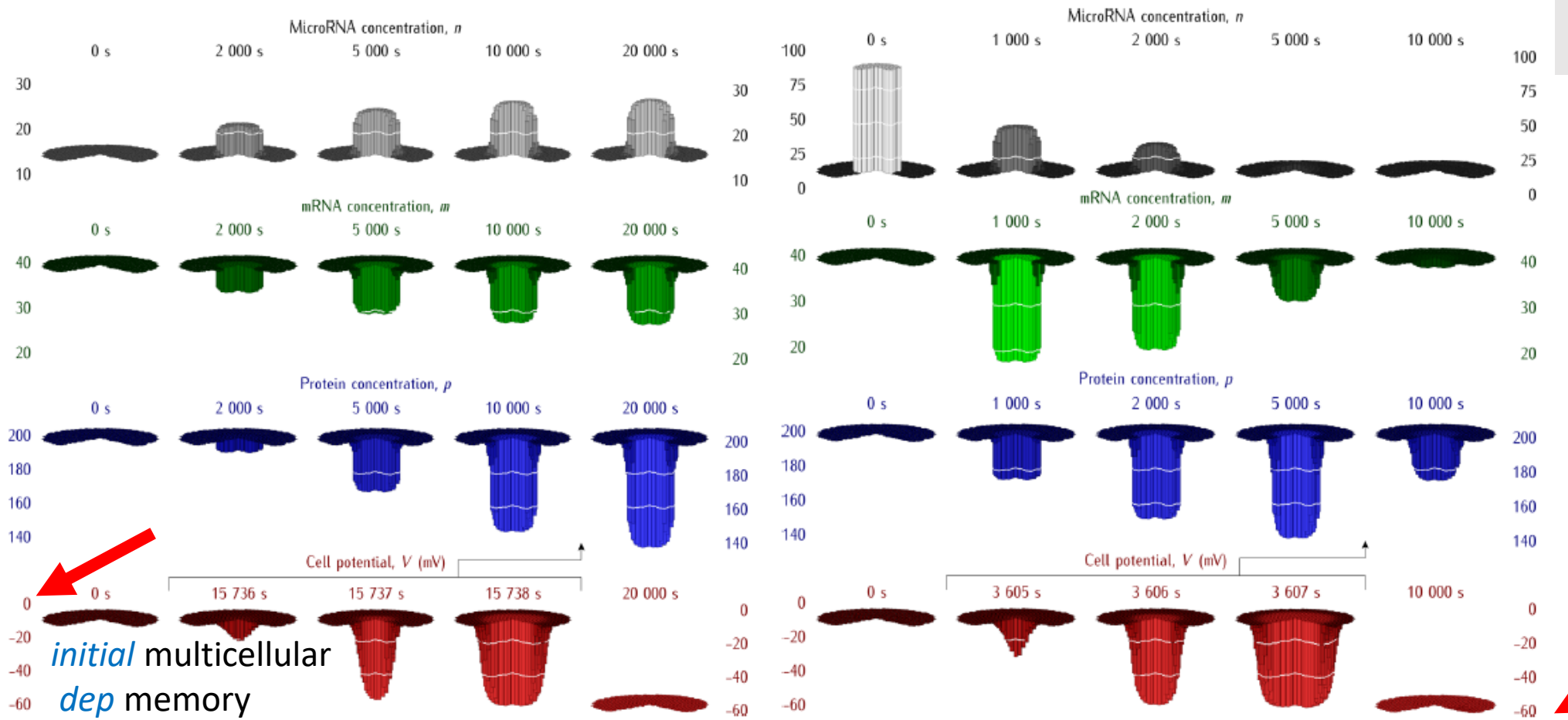
Translocation of miRNA to *gap junctional* cell-cell contacts could be driven by passive diffusion of miRNA molecules, e.g. bound to AGO proteins or other non-AGO proteins (mRNPs) in the cell.

$$\frac{dn_i}{dt} = r_n - d_n n_i - d_{nm} m_i n_i + \sum_{nn} \frac{1}{\tau_{ij}}(n_j - n_i)$$



$$C_i \frac{dV_i}{dt} = -I_{dep} - I_{pol} + \sum_{nn} G_{ij}(V_j - V_i)$$

J. Phys. Chem. B 2017
10.1021/acs.jpcb.7b04774



initial multicellular *dep* memory

final multicellular *pol* memory

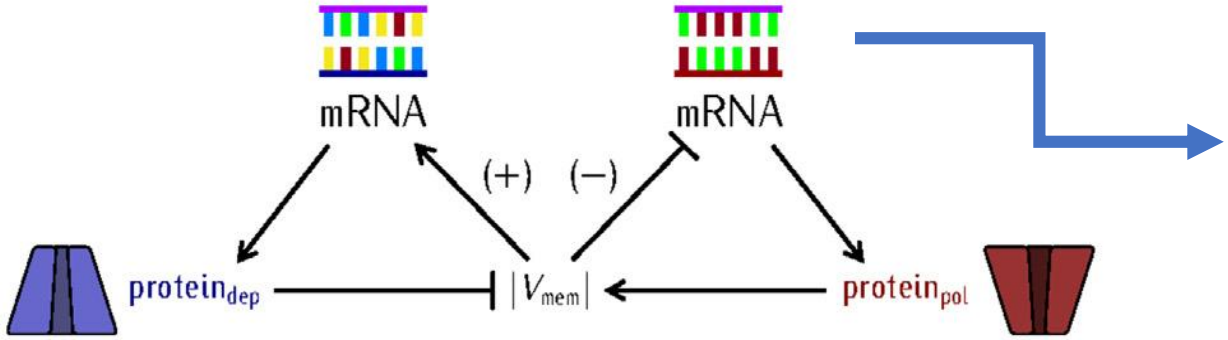
miRNA could change a multicellular bioelectrical memory

Time changes of *dep* channel *miRNA*, *mRNA*, and *protein* concentrations and the *cell potential* after the local increase of the miRNA transcription rate from 1.5 min^{-1} (*external* multicellular domain) to 2.0 min^{-1} (*central* domain). The miRNA transference across the intercellular junctions changes the d*ep* channel protein concentration, forcing the *repolarization* from the *initially depolarized state* (*left*). Time changes following a *local transfer of miRNA* over the central domain. Now, the initial *mRNA* and *protein concentrations* are *recovered at long times* because of the finite amount of miRNA transferred but *repolarization is maintained* (*right*).

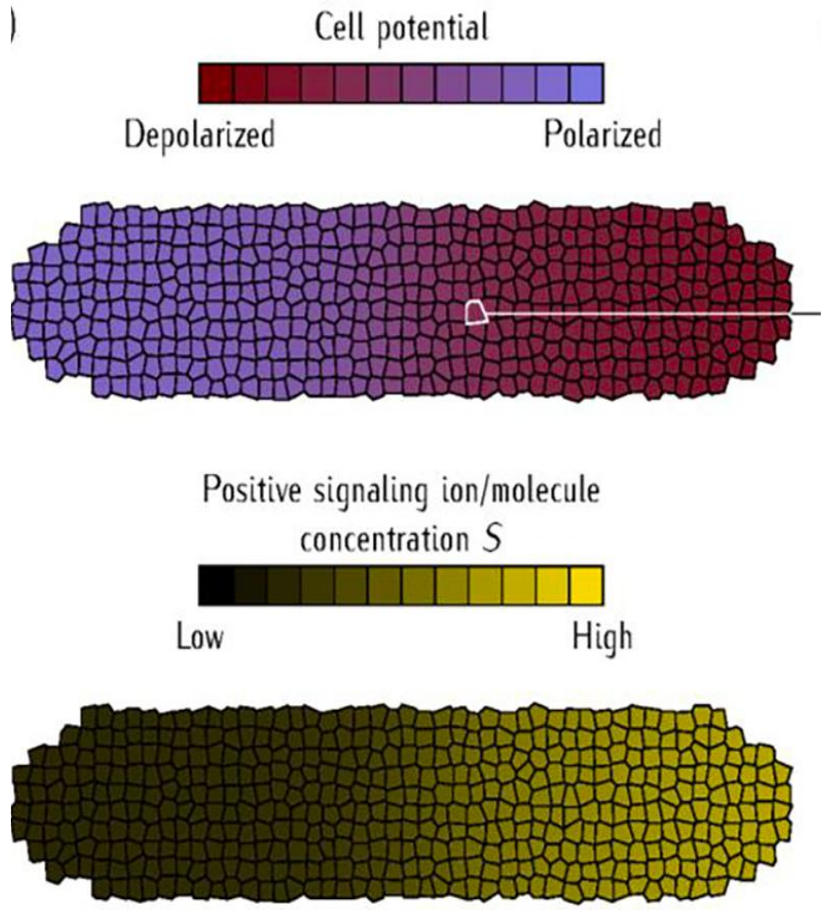
summary: intercellular junctions can extend single-cell to multicelular bioelectricity

Phys. Rep. 2023
10.1016/j.physrep.2022.12.0030370-1573

It is the *difference* between the *multicellular potentials* that may give *distinct downstream gene expression patterns*: cells are sensitive to *spatio-temporal bioelectrical patterns* to develop specific programs.



$$(+): \frac{r_{m,dep}}{r^{\circ}_{m,dep}} = \frac{(S_{dep}/S_{0,dep})}{1+(S_{dep}/S_{0,dep})} = \frac{e^{|V_{mem}|/V_T}}{1+e^{|V_{mem}|/V_T}} \qquad (-): \frac{r_{m,pol}}{r^{\circ}_{m,pol}} = \frac{1}{1+(S_{pol}/S_{0,pol})} = \frac{1}{1+e^{|V_{mem}|/V_T}}$$



In this example, the *cell potential* modulates the local concentration *S* of the *signaling ion/molecule* that regulates in turn the channel protein *transcription*. This *feedback* between the *cell potential* and the *transcription* can be *positive* or *negative*, depending on the specific system. The model suggests that *changes* in *cell potentials* modify the expression and functions of *channels* and *gap junctions* in the *multicellular* aggregate.

5. Theoretical results of qualitative relevance

The progress from an *individual cell* to *multicellularity* leads to *average biophysical fields* that influence *development* and *regeneration* on *multi-scale processes*, from cell biology to tissue organization. They offer useful *higher levels of abstraction*.

In *Bioelectricity*, an initial

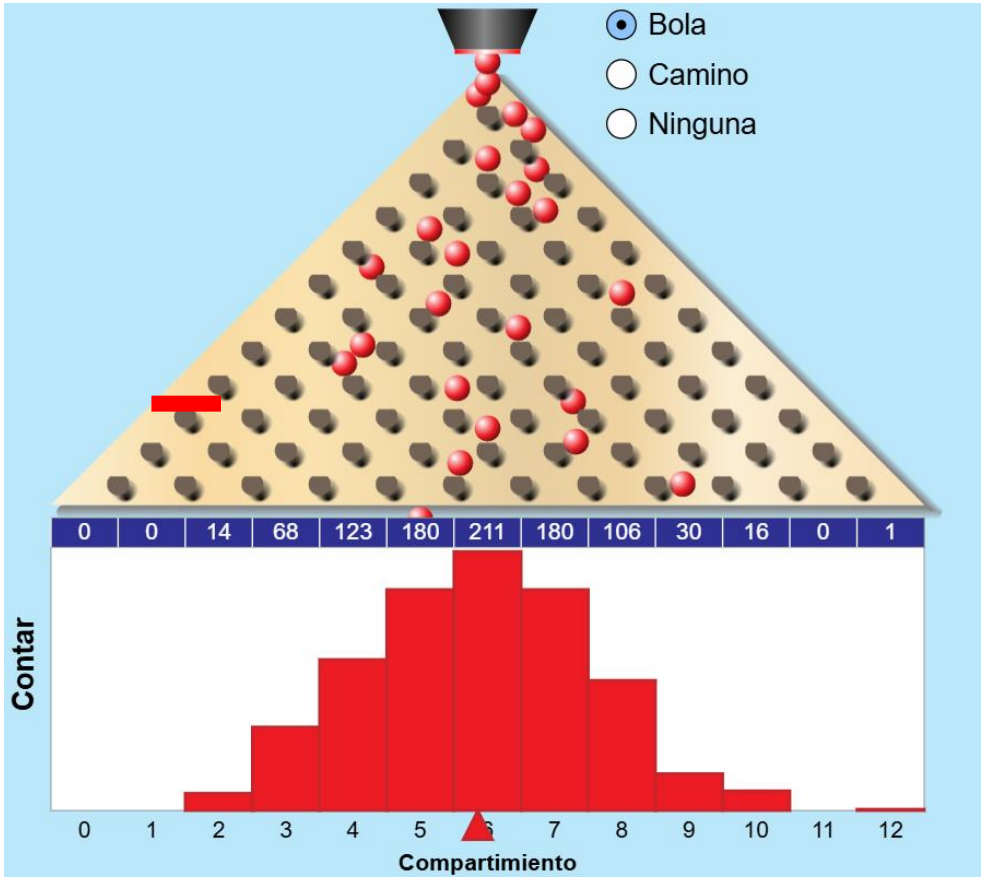
small asymmetry, *local disruptive event*, *change in environmental conditions*, etc.

can trigger subsequent processes where the coupling between *bioelectricity* and *transcription* acts to *oppose* or *amplify* the initial perturbation. The resulting processes can *reestablish previous* or *establish new* multicellular instructive programs.

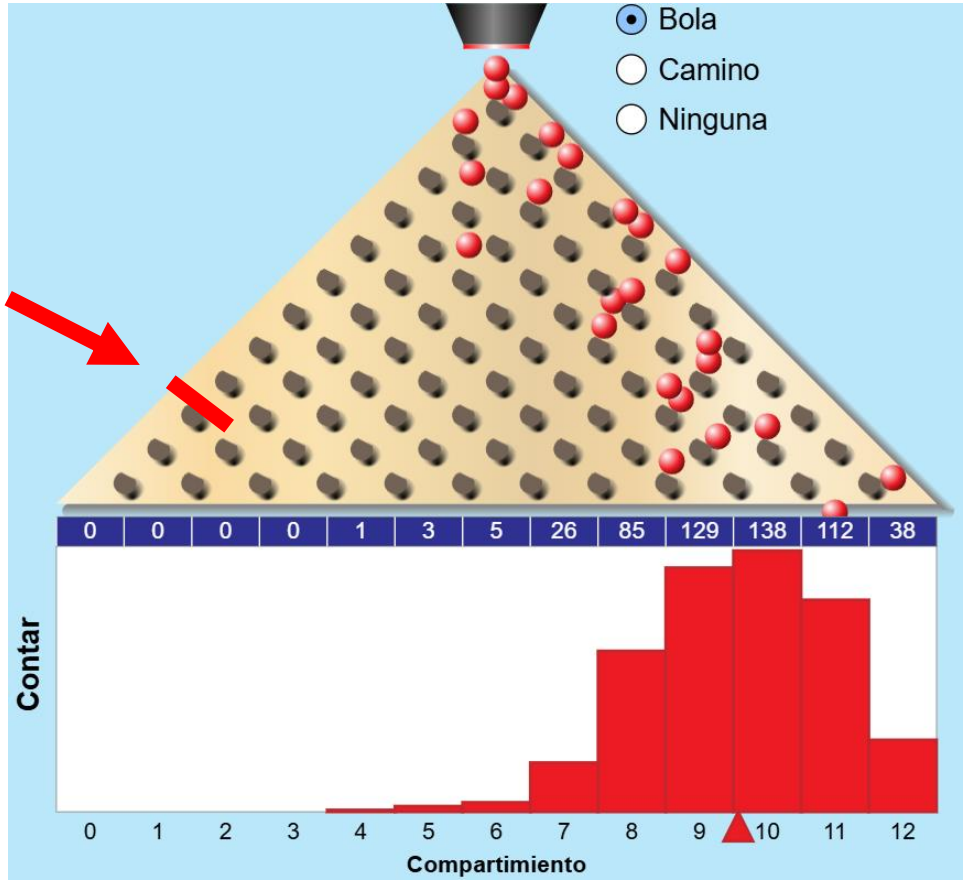
The following *model simulations* explore the *qualitative consequences* of different *symmetry-breaking* and *disruptive* phenomena, with an emphasis on *bioelectrical concepts*. Admittedly, additional *biochemical* and *biomechanical* processes not included here should also be important in real systems.

PhET Interactive Sims. Univ. Colorado at Boulder 2024
https://phet.colorado.edu/es/simulations/plinko-probability

A previous observation: *complex phenomena* such as *symmetry-breaking* and *shape regulation* can emerge in relatively *simple systems* composed by multiple units.



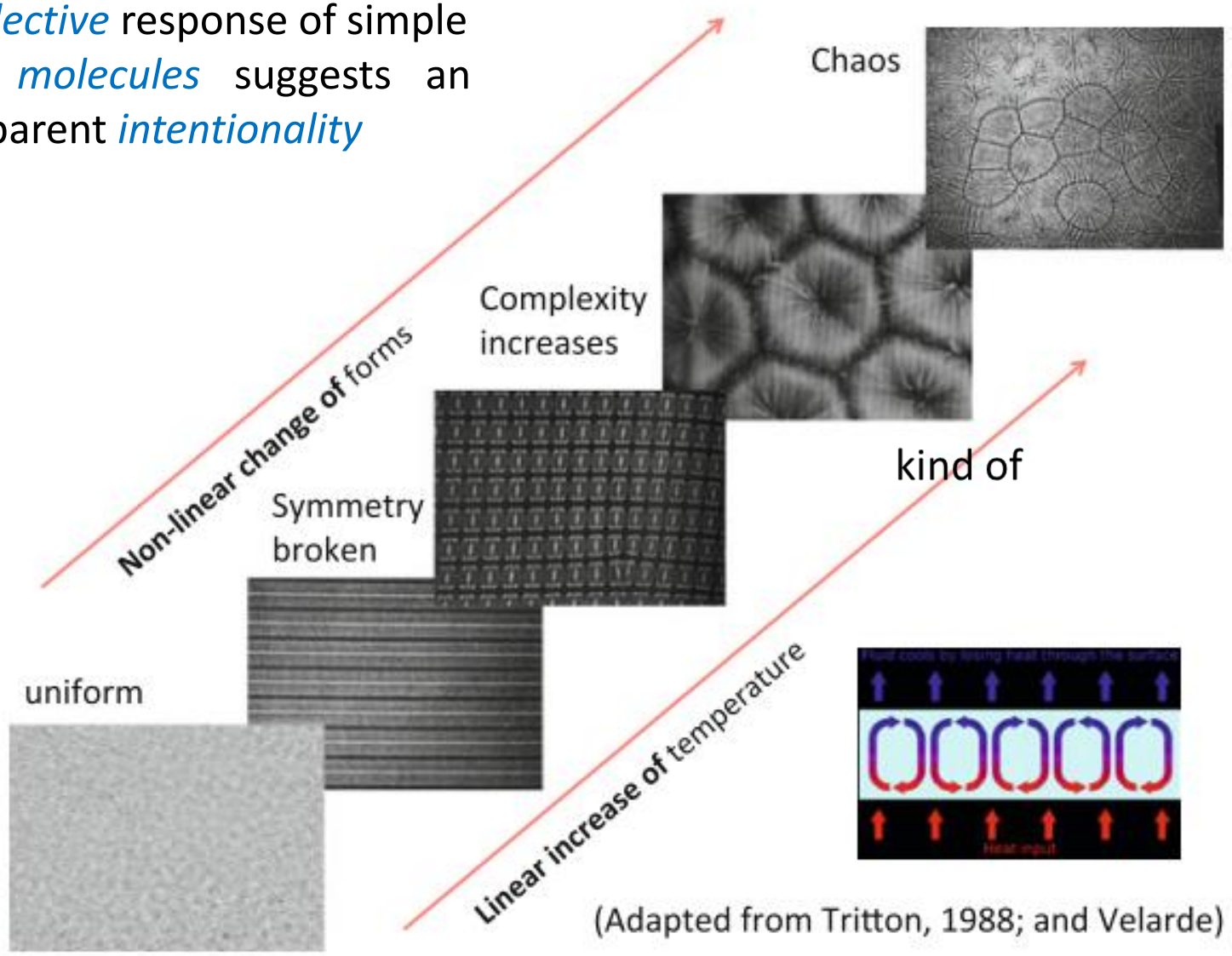
central



left-right

The Frontiers Collection (Springer Verlag) 2013
10.1007/978-3-642-36086-2_9

Rayleigh-Bénard cells: the *collective* response of simple *oil molecules* suggests an apparent *intentionality*



When heating from below a thin layer of oil spread out on a plane surface, *convection currents* with peculiar geometric shapes (lines or polygons) *self-organize*. The shapes change abruptly when the temperature gradient goes over particular *thresholds*. This non-linearity may *mimic* self-organization in *living systems* [Photos adapted from Tritton (1988); and Manuel Velarde, Universidad Complutense, Madrid].

Sci. Rep. 2016
10.1038/srep204031
Ageing Res. Rev. 2024
10.1016/j.arr.2024.102310

In contrast to the universality of equilibrium phase transitions, *no general theory* could predict what organization may emerge far from equilibrium in *biological systems* because every system has *highly specific details* that matter. In particular, *Bioelectricity* shows the *complexity* and *diversity* of living systems over broad *spatial* and *temporal scales*.

However, two *bioelectrical features* are central to our model:

Contrast dynamic sharpening: *channels* and *gap junctions* operate over *wide voltage windows*; they may *polarize* or *depolarize* depending on the cell state and the neighboring conditions. This fact can contribute to the spatio-temporal contrast needed for *bioelectrical regionalization*.

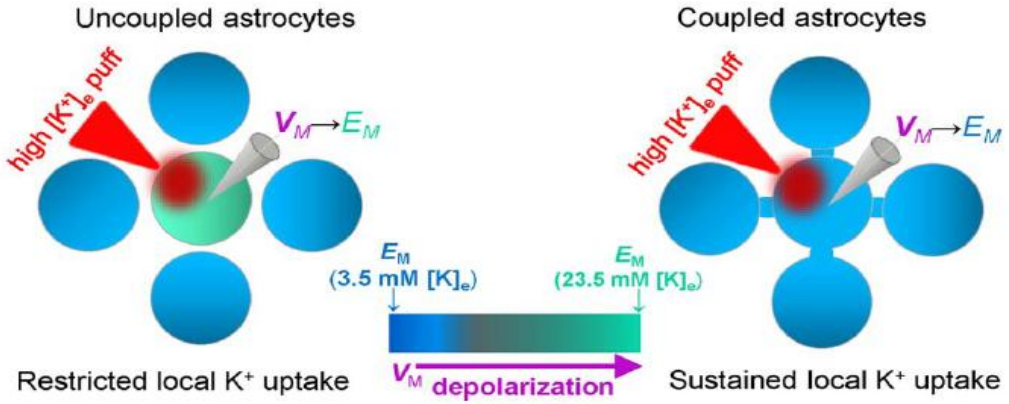
Degraded pattern recovering: "corrupted images" due to a *local depolarization/ polarization* (single-cell failure) or a *decrease of connectivity* (multicellular failure), can still be recovered by *single-cell* (e.g. channel redundancy re-establishing a cell state) or *multicellular* (e.g. community effects re-establishing intercellular communication) mechanisms.

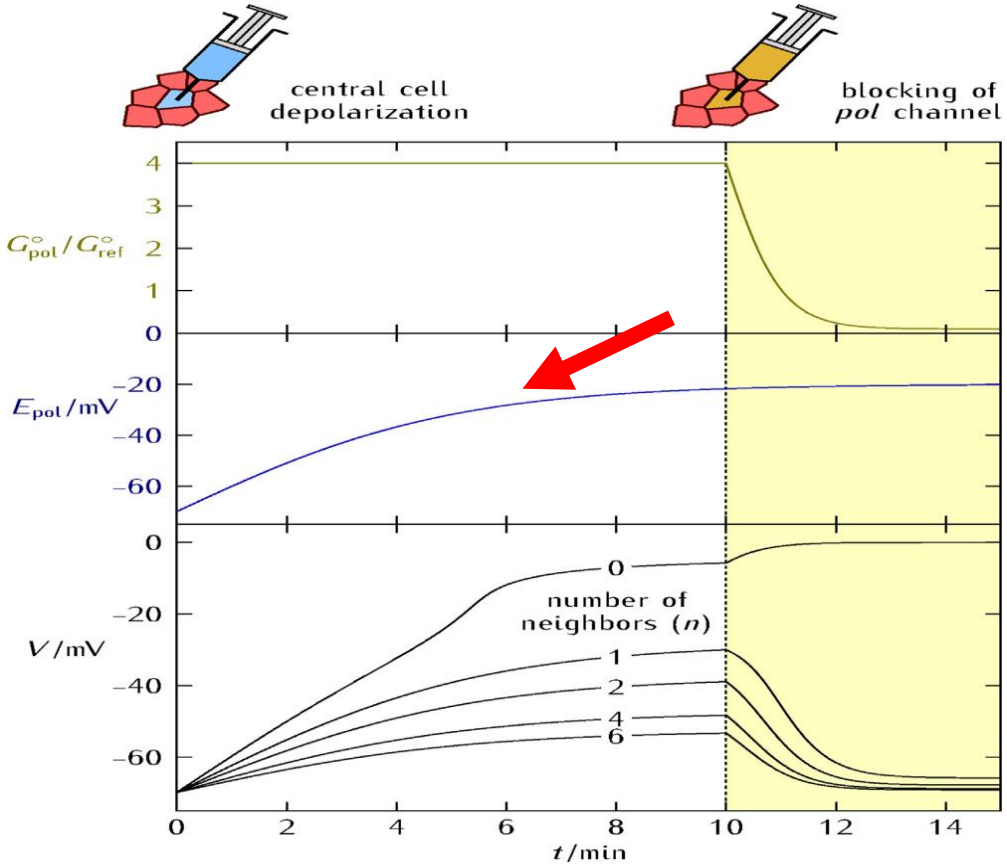
the bioelectrical community effect

The *gap junction-mediated* transfer of *electrical* and *chemical* signals within a *multicellular community* provides a *feeling of belonging* to a whole cluster that may inhibit increased proliferation and migration.

Also, the resulting *buffer effect* due to the *neighboring cells* can *correct* a perturbation, which is caused here by the *abnormal depolarization* of the *central* cell due to a local change in E_{pol}. Note that a *pol* channel *blocking* causing this depolarization could also be collectively counteracted.

As expected, the community effect *opposing* the *disruptive* event increases with the *number of neighbors* and the *junction conductance*.

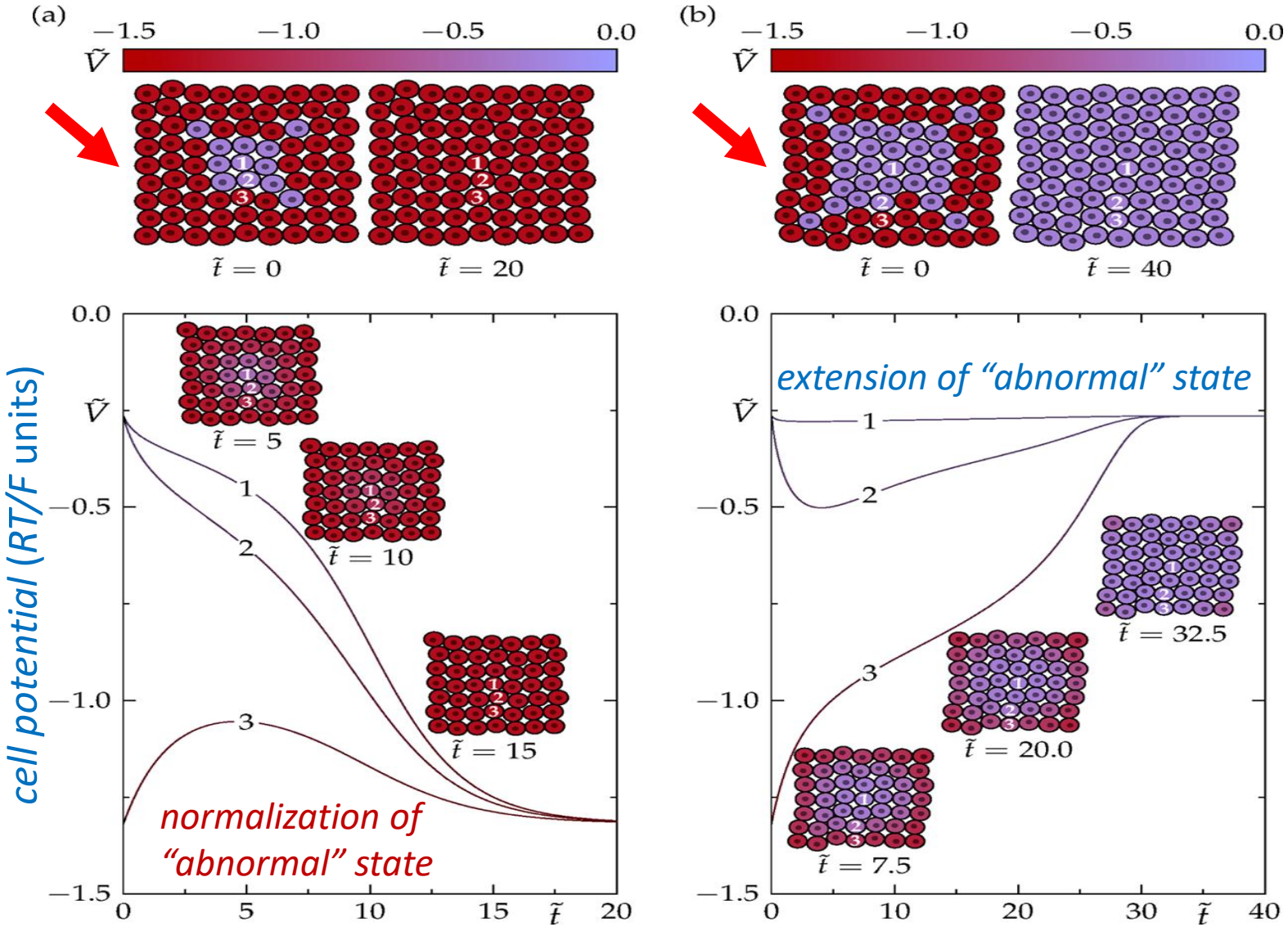




Glia 2015
10.1002/glia.22924
Prog. Biophys. Mol. Biol. 2019
10.1016/j.pbiolmolbio.2019.06.004

"kiss of life" or "kiss of death" as a community effect

J. Phys. Chem. B 2015
10.1021/jp512900x
Cancers 2021
10.3390/cancers13215300

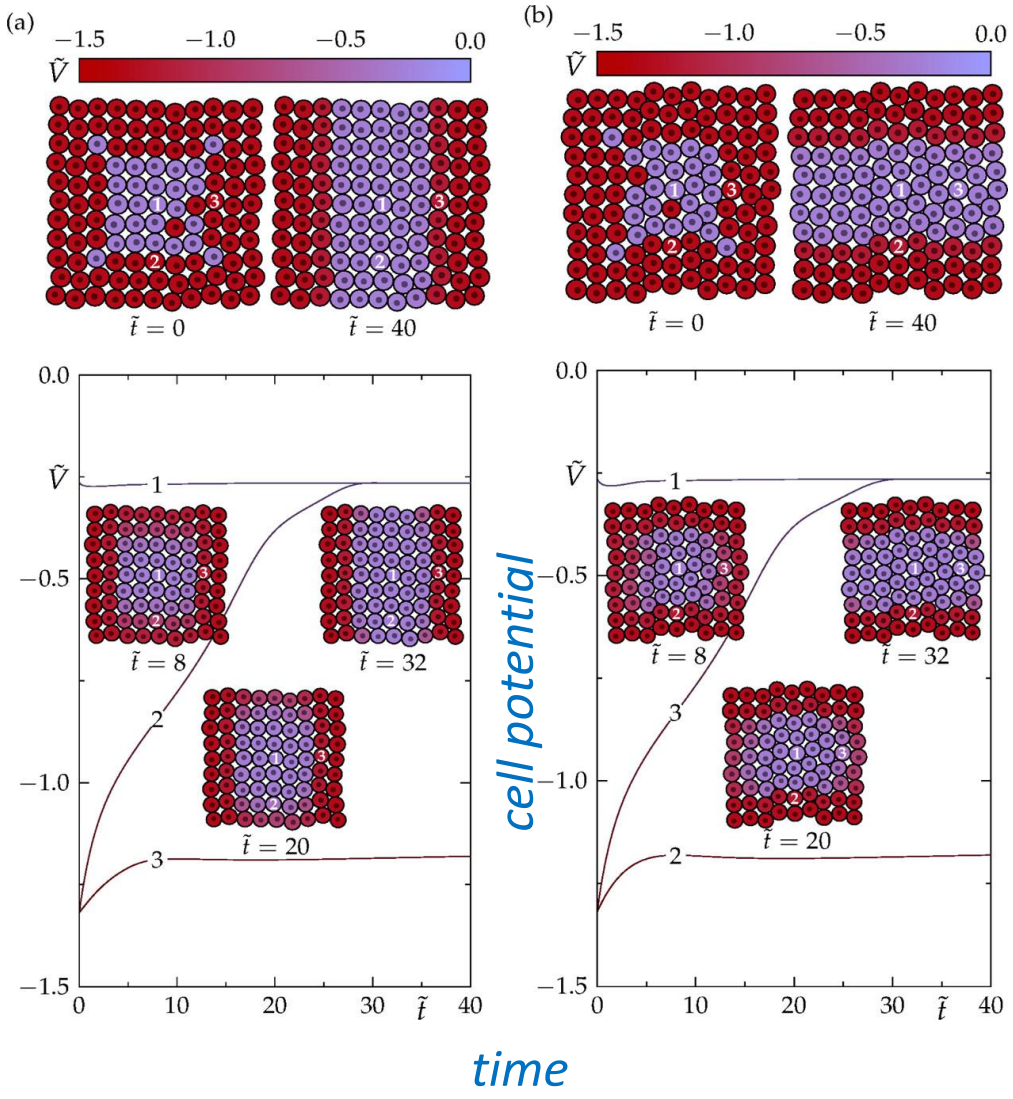


Bioelectrically *suppressing* (*left*) or *enhancing* (*right*) a disruptive local *depolarization* event:

for *small regions* of "*abnormal*" cells, the community effect allows a *normalization* of the bioelectrical cell state: *dep* cells *lose* memory (*left*). This normalization is *not possible* for *large "abnormal" regions: dep* cells *keep* memory (*right*).

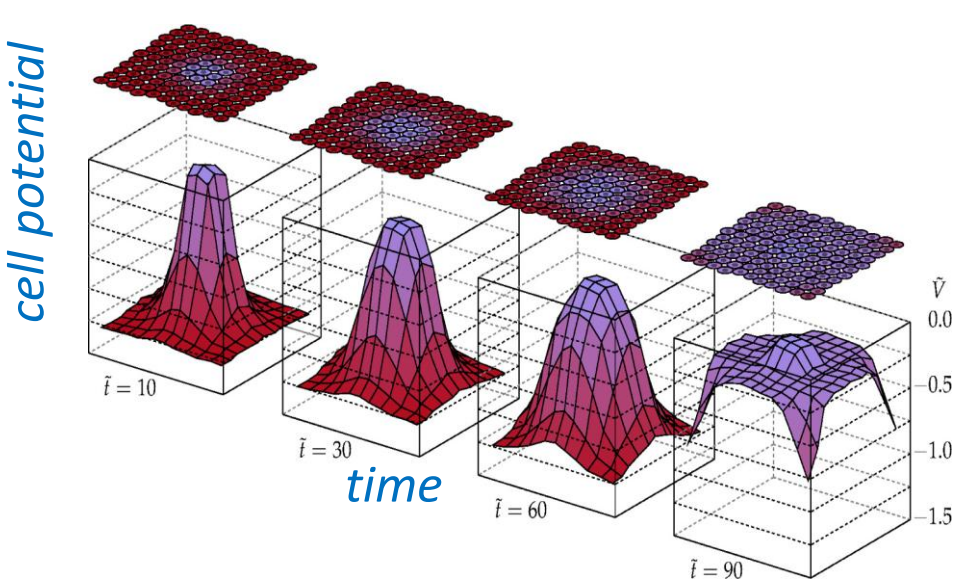
Thus, the *intercellular connectivity* could normalize small regions ("*kiss of life*") but also spread the abnormal cell state in the case of large enough regions ("*kiss of death*").

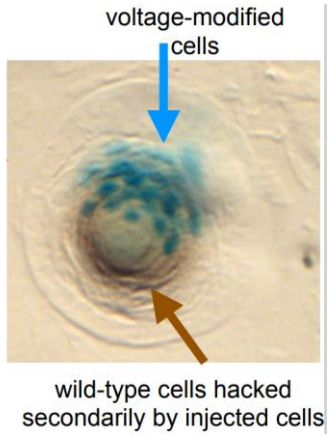
A *pre-pattern* of intercellular *gap junctions* allows imprinting a *directionality* to the tissue *re-writing*.



J. Phys. Chem. B 2015
10.1021/jp512900x
Commun. Biol. 2024
10.1038/s42003-024-06037-4

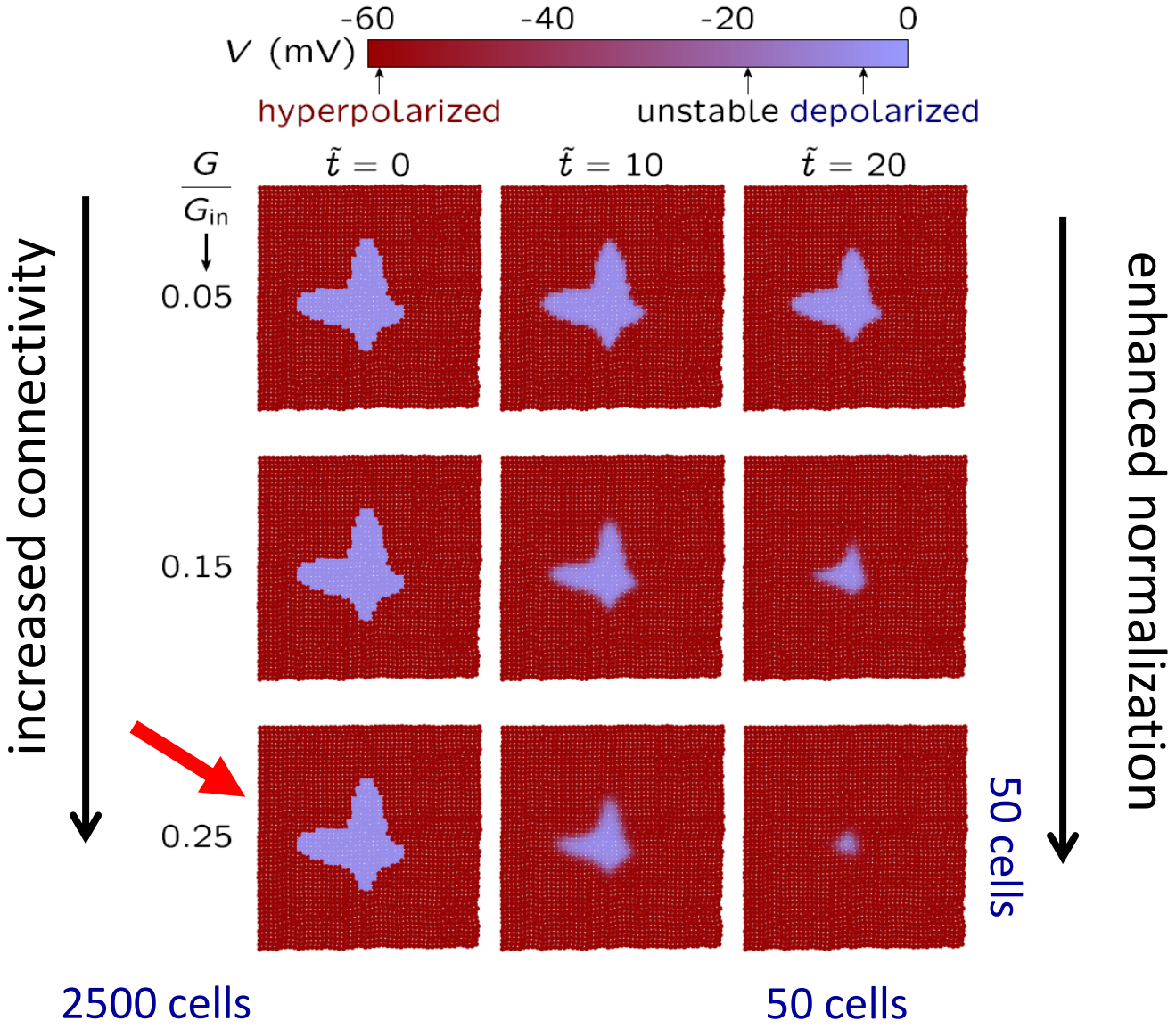
cell bioelectrical recruiting?





A bioelectrically *enhanced* disruptive event: because of bi-stability, a *reduced number of cells* in a *dominant* depolarized (*blue*) state, established by a pulse current, mis-expression or blocking of a *pol* potassium channel, could recruit the initially polarized (*red*) neighbors to *re-write* a *bioelectric pattern.* However, this recruiting is *not* always possible: it depends crucially on the *relative stability* of *pol/dep* cell states.

Sci. Rep. 2016
10.1038/srep204031
Cancers 2021
10.3390/cancers13215300



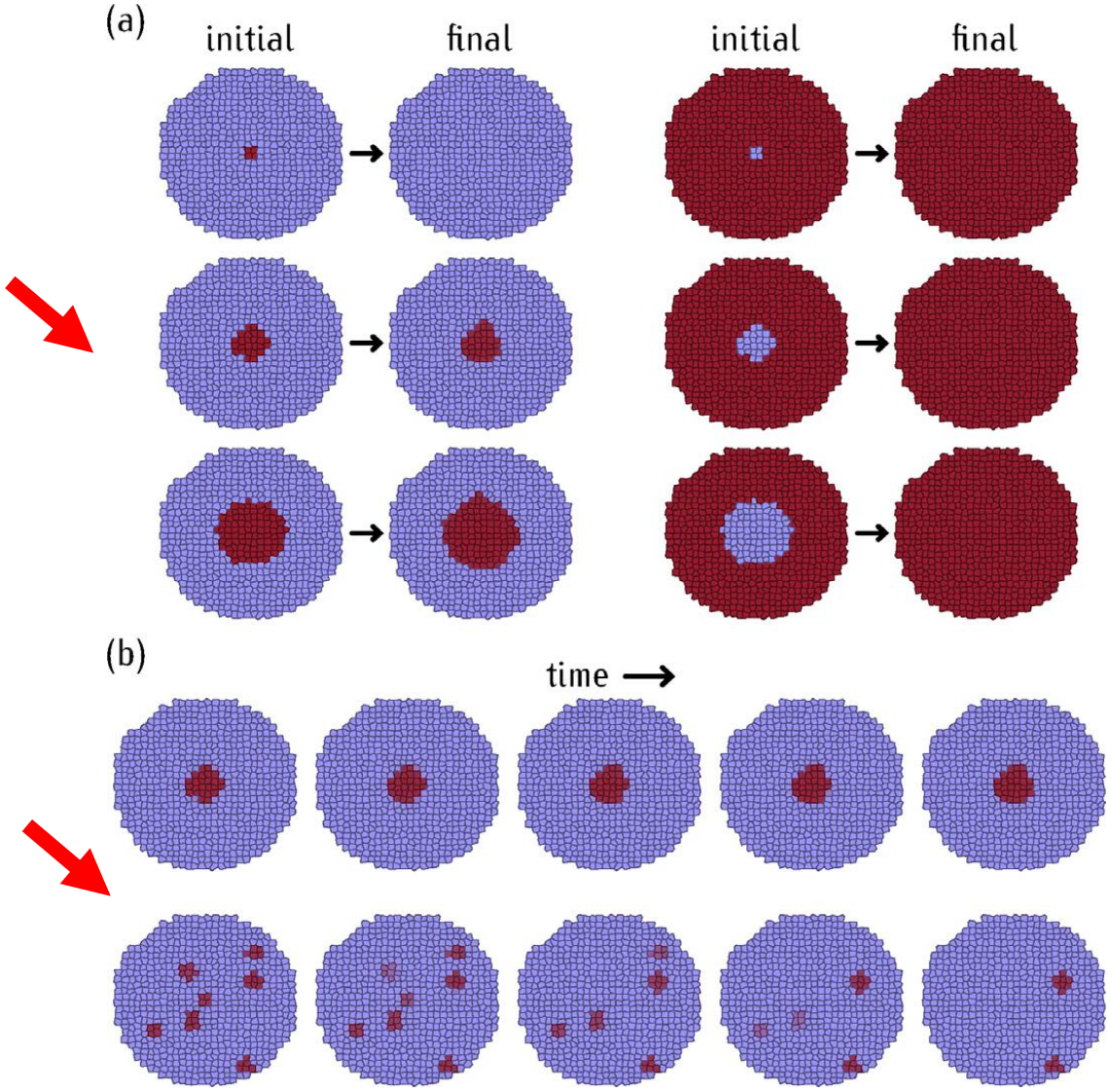
stage-dependent behavior of intercellular junctions:

- at *early* stages, a disruptive event could be *bioelectrically suppressed* by *increasing* the connectivity of the *small abnormal* region with the normal neighborhood; however,

- at *final* rather than initial stages, the disruptive event should be *suppressed* by *decreasing* rather than increasing the *internal* connectivity (*cohesion*) of the now *large abnormal* region, as suggested in 10.1103/PhysRevE.102.052412).

Experimental limitations: 1) not only *electrical* but also *chemical* signals are transferred through gap junctions; and 2) bioelectrical *suppression* or *enhancement* of a disruptive event depends on the *relative pol/del* cell state stability.

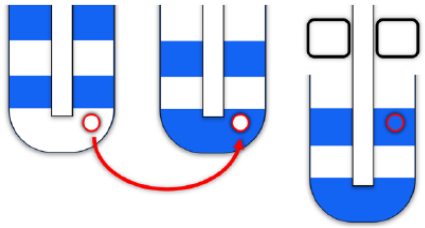
Phys. Rev. E 2020
10.1103/PhysRevE.102.052412
Commun. Biol. 2024
10.1038/s42003-024-06037-4



(a) The *community* effect is *size-sensitive* and depends also on which *isolated cell state* is dominant at the *single-cell* level: an "abnormal" *initially depolarized central region* can be *normalized* (*right*) but a polarized central region could resist the community normalization effect (*left*) because the *pol* state is dominant over *dep* state in this simulation.

(b) A *big patch* of cells shows a *stronger community* effect than many *small patches*: small bioelectrically "abnormal" regions hardly progress within a *highly-connected* multicellular ensemble.

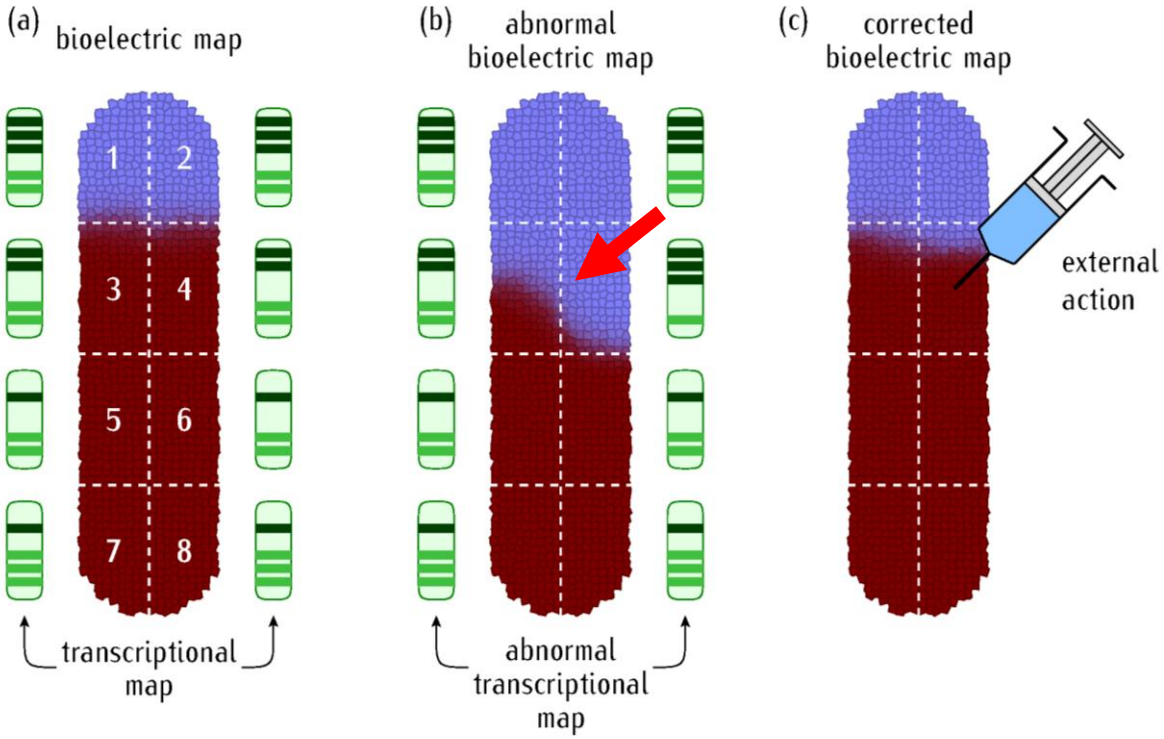
A *biochemical* reminiscence (cell grafting in *segmentation*)?

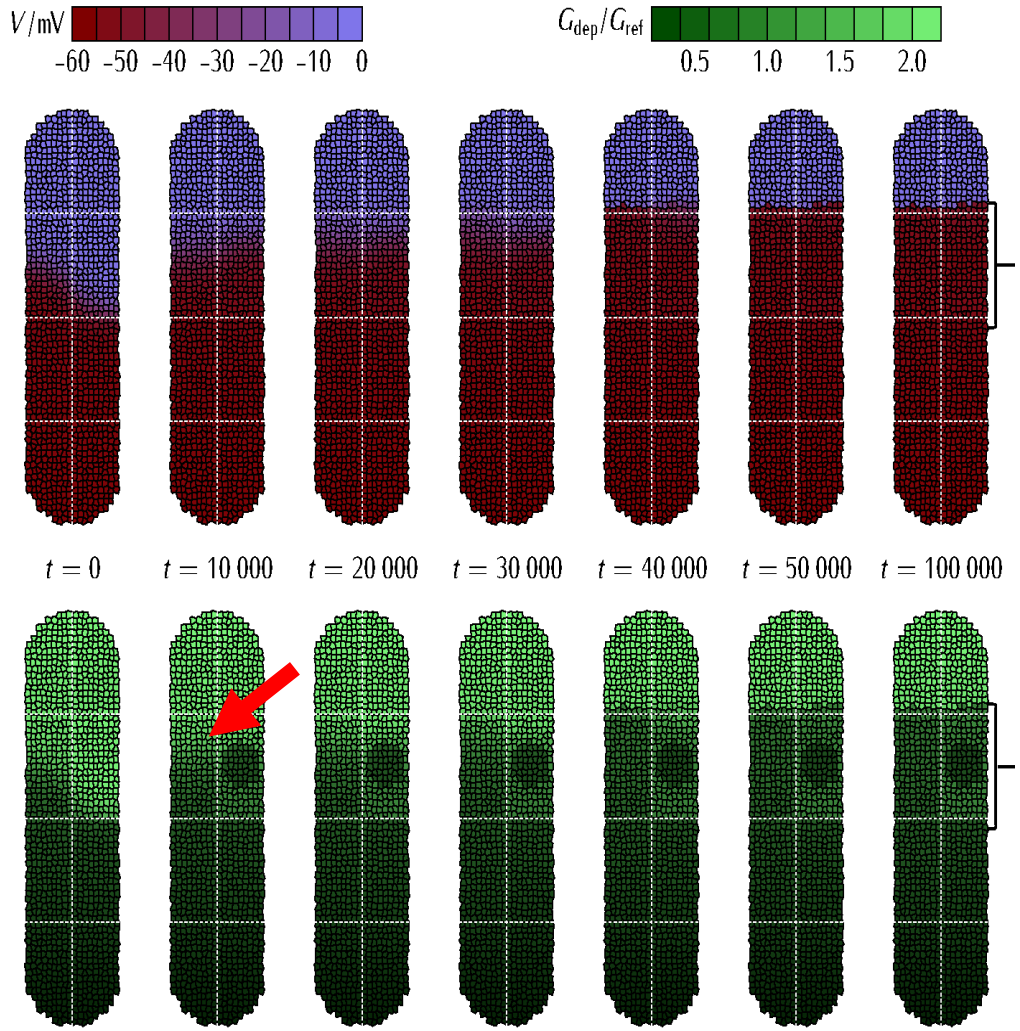


Biochim. Biophys. Acta 2023
10.1016/j.bbagen.2023.130440

correction and completion: can locally degraded patterns be restored?

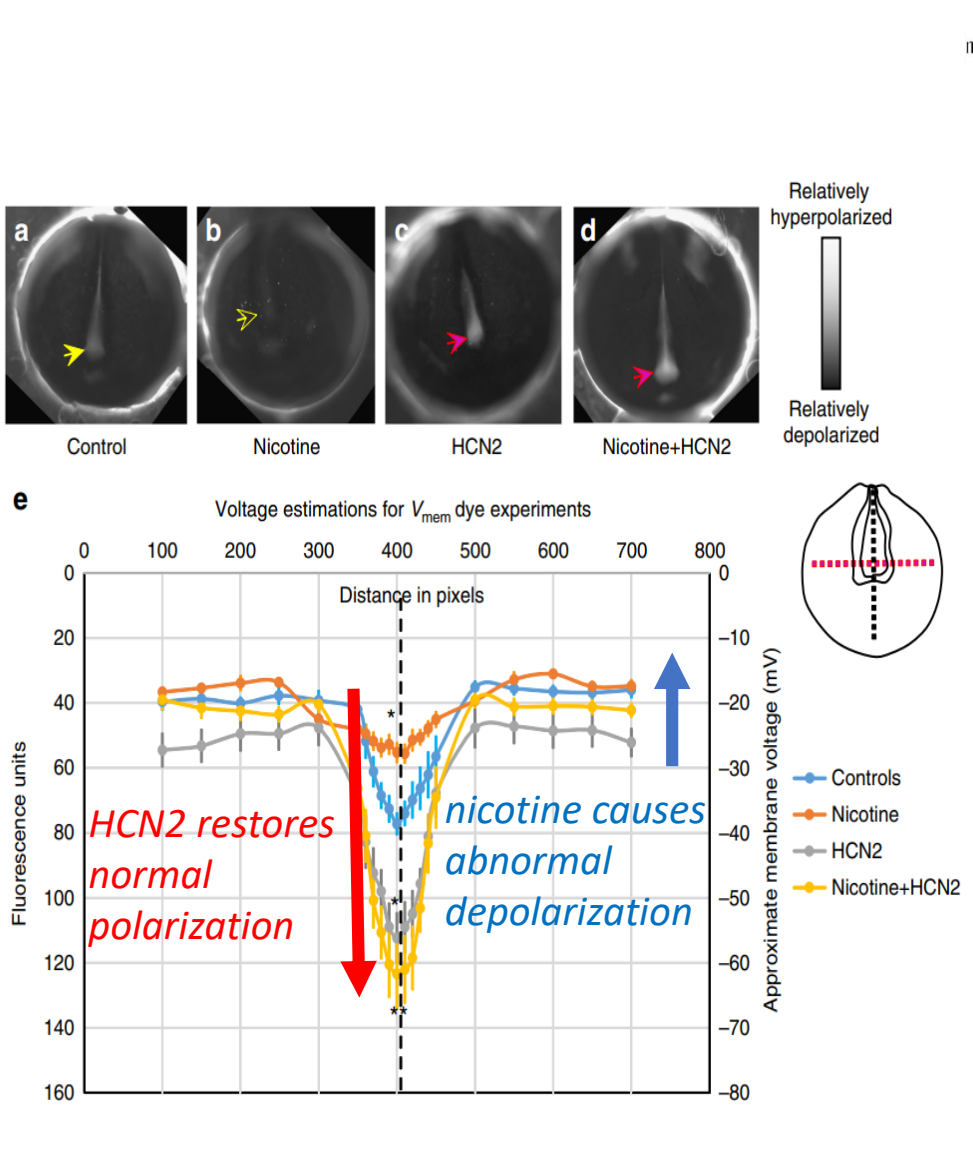
Because of the *coupling* between *bioelectricity* and *transcription*, the simulations suggest that *corrupted spatio-temporal maps* could be *corrected* by *external actions:* the *blocking* of an *incorrect* (*dep*) channel (*here*) or the *upregulation* of a *rescue* (*pol*) channel *restores* the *correct pattern*.

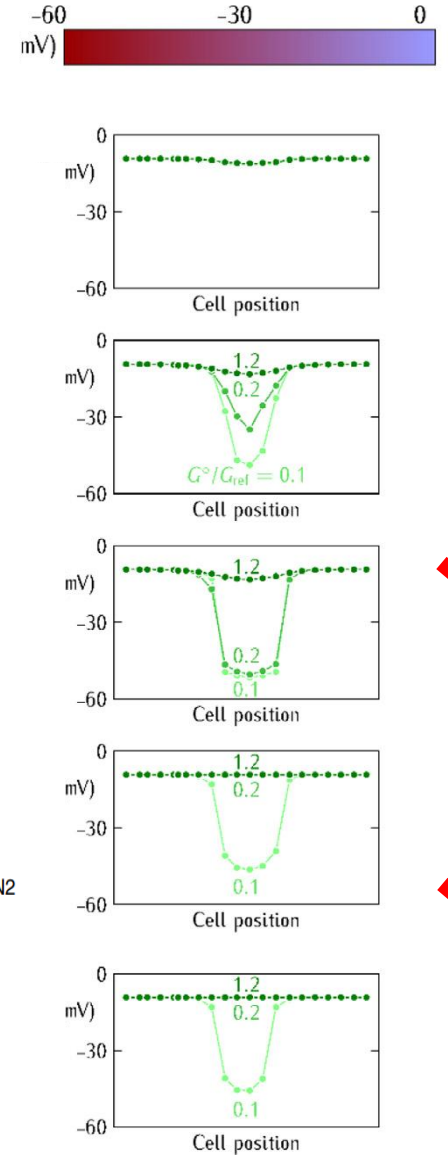




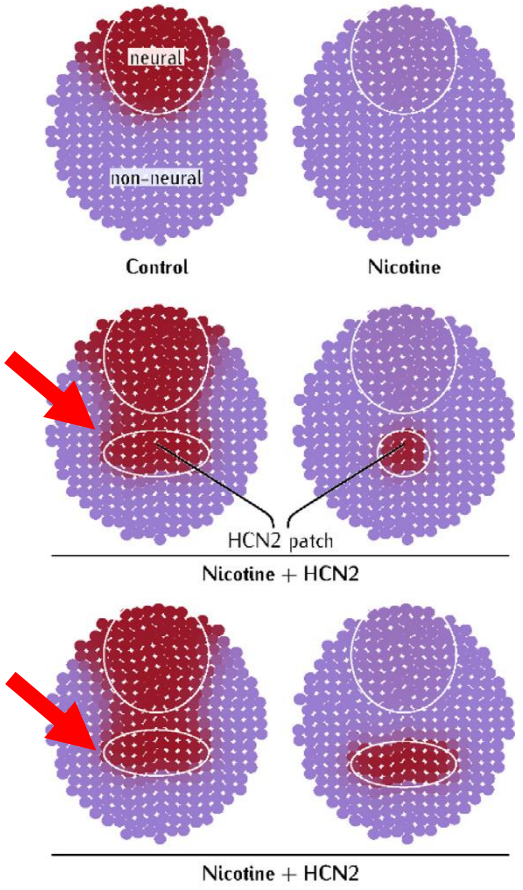
relative potentials between separated cell groups influence transcriptional patterns: are distant actions possible (polarization waves)?

Nat. Commun. 2018
10.1038/s41467-018-03334-5
Front. Cell. Neurosci. 2020
10.3389/fncel.2020.00136





distant cell bioelectrical recruiting

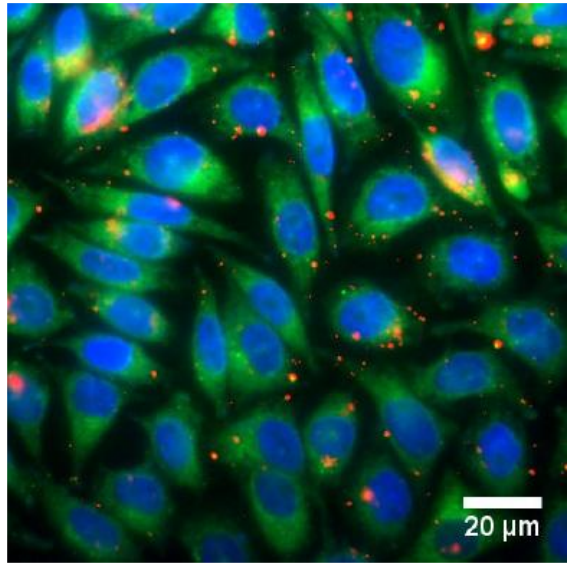


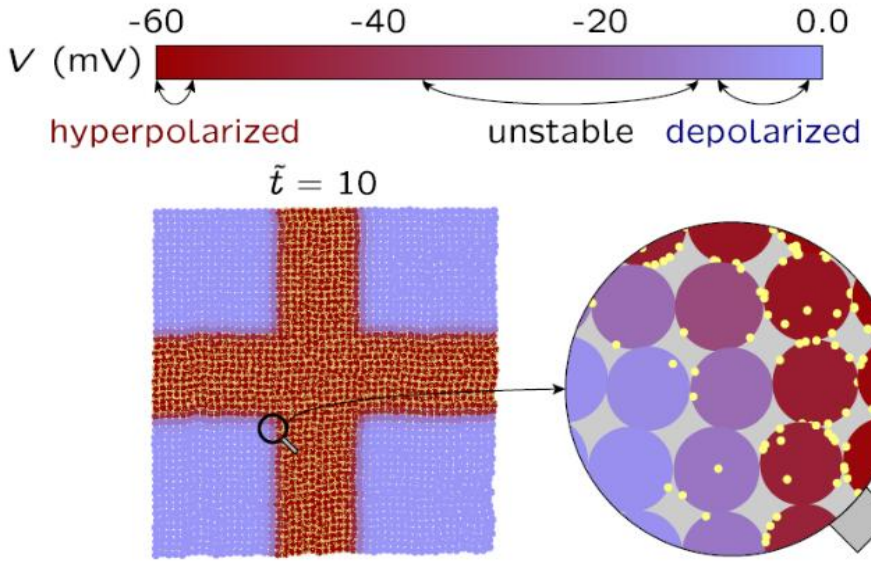
Incorporation of a *polarizing channel* (*HCN2*) partially recovers, *at distance*, the *potential pre-pattern* partly distorted by previous nicotine exposition.

Size and *distance* effects influence the *repolarization* outcome.

are other correcting actions possible (nanoparticles seeding)?

Nanoscale 2013
10.1039/C3NR01667F
RSC Adv. 2015
10.1039/C4RA15727C
Small 2024
10.1002/smll.202404152
Sci. Rep. 2016
10.1038/srep20403
Phys. Rep. 2023
0.1016/j.physrep.2022.12.003





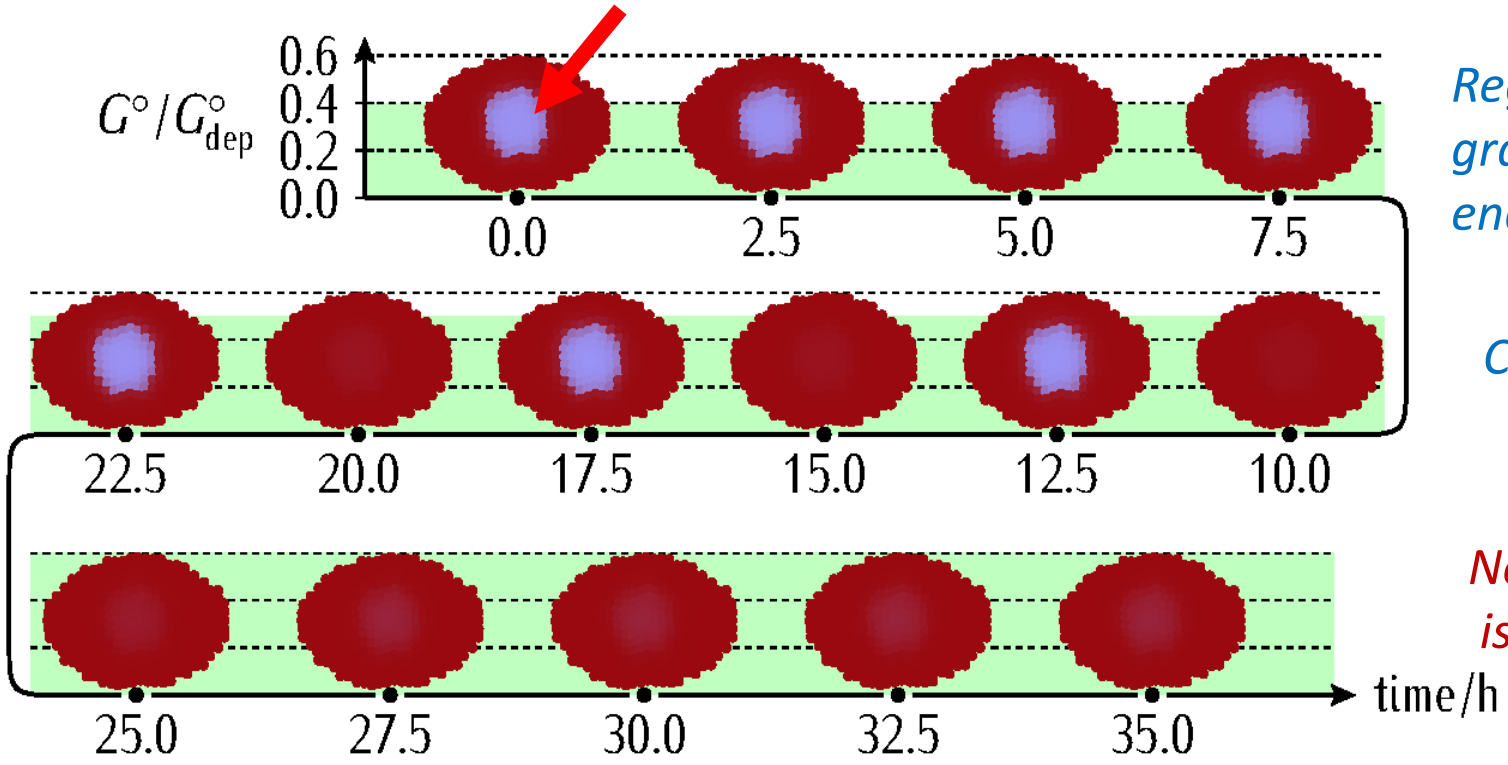
The 60 nm *amine-modified polystyrene nanoparticles* (*small red circles*) bound to the *Chinese Hamster Ovary* (CHO) *cell membranes* can be observed by flux cytometry, dyes, and fluorescence microscopy techniques (*up*).

The bioelectrical simulation suggests that the *spatial distribution* of *positively charged nanoparticles* (*small yellow circles*) may follow the *cell potential pattern*: particle accumulation is more significant around polarized (*more negative*) cells than around depolarized cells. For clarity, the inset zooms a small region of the multicellular system (*down*).

An experimental *limitation*: not only electrical but also *hydrophobic/hydrophilic* contributions to *nanoparticle binding* should be experimentally relevant.

Phys. Rep. 2023
0.1016/j.physrep.2022.12.003

acting on the intercellular connectivity: emergence of oscillations at intermediate connectivity



increasing connectivity

Regionalization by exogenous grafted region or endogenous disruptive event

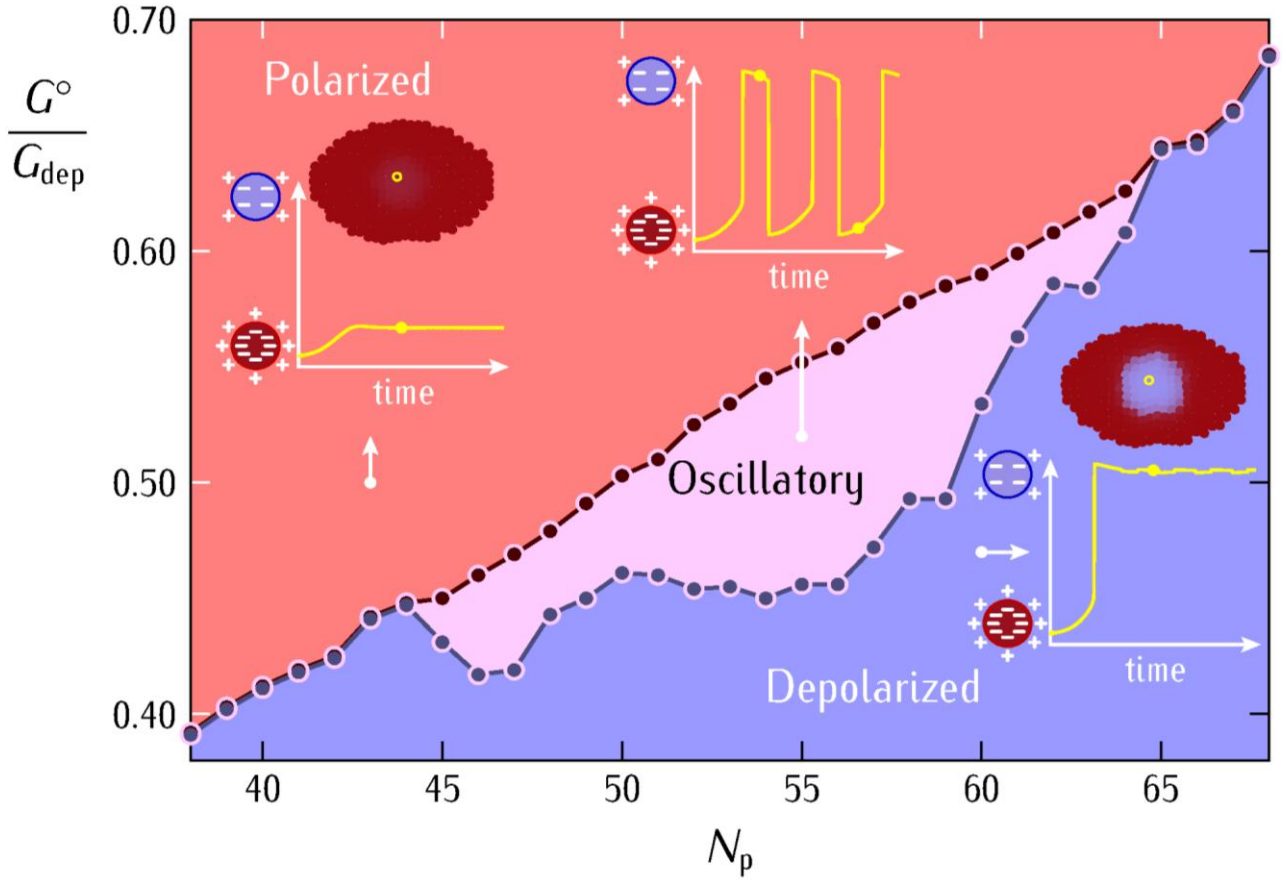
Collective Oscillation

Normalization: isopotential system

In the central region, the simulations show transitions between *depolarized* and *polarized* multicellular states, including *collective oscillations*, that can be modulated by the *intercellular connectivity*. Note that *all three* possible bioelectrical states can be realized in the central region, which suggests that external *instructive actions* on the *gap junctions* should be possible.

configuration phase space for central region: different bioelectrical states can be established by changing intercellular connectivity

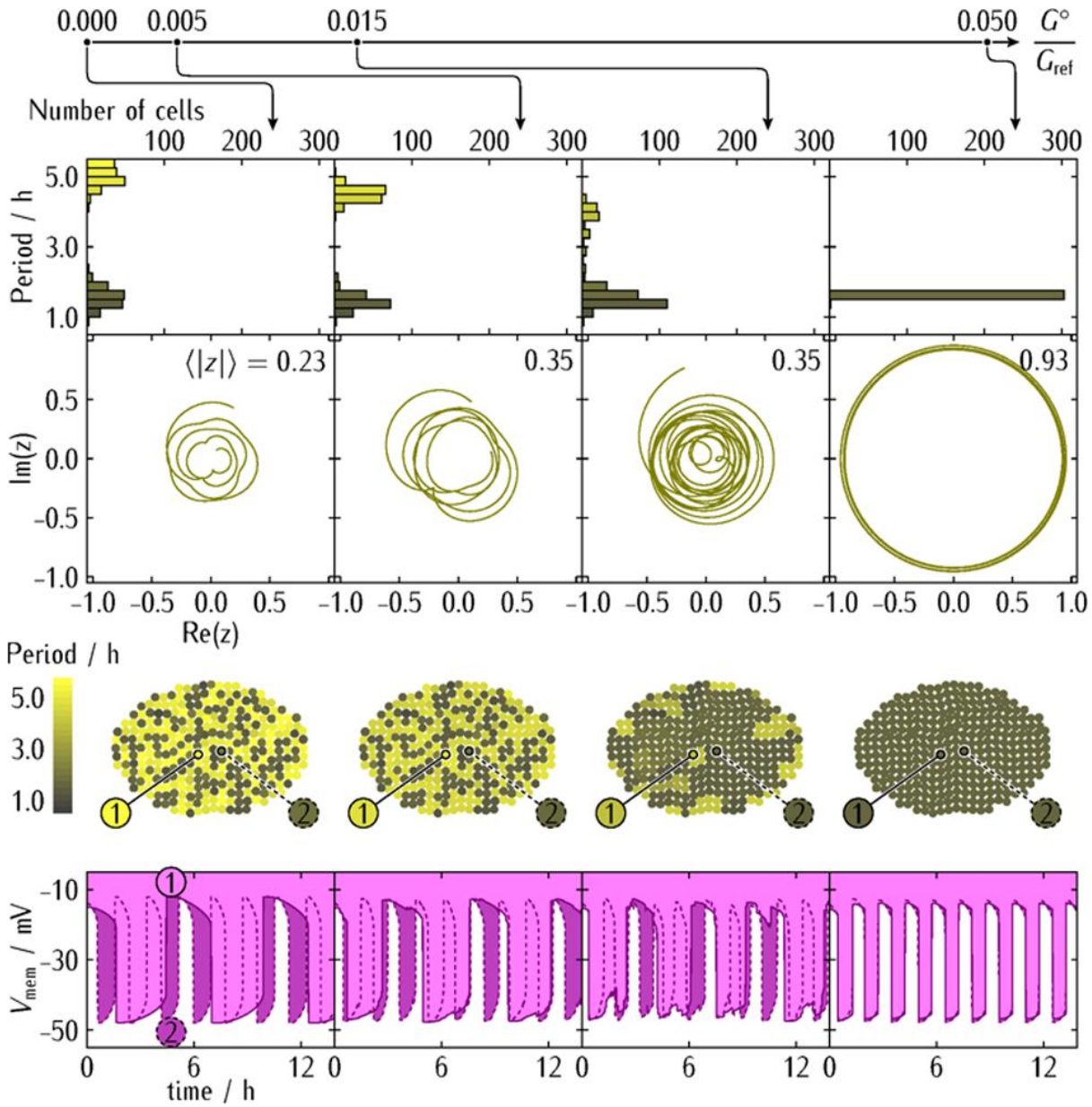
Phys. Rep. 2023
0.1016/j.physrep.2022.12.003



Polarized, depolarized and oscillatory *multicellular states* as a function of the *intercellular junction conductance* and the *number of cells* in the central region. *Environmental* changes and external *actions* might explore first the *bioelectrical* rather than *genetic* phase space.

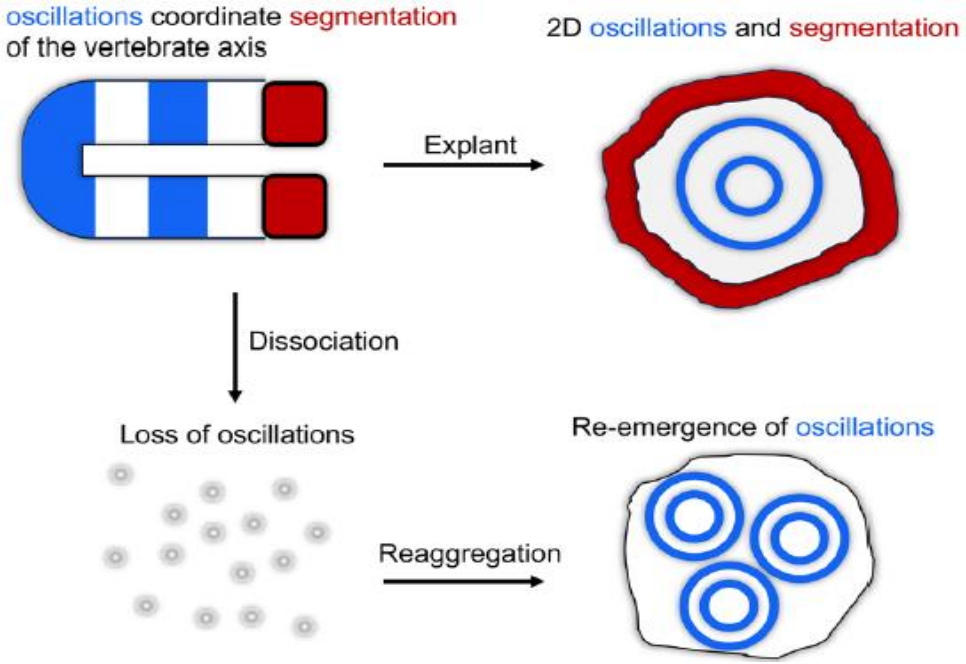
J. Phys. Chem. B 2019
10.1021/acs.jpcb.9b01717
Commun. Biol. 2024
10.1038/s42003-024-06037-4

increasing intercellular connectivity →



Synchronization phenomena: the modulation of the *junction conductance* can establish *regions* with the same *oscillatory phase* of *bioelectricity* and *transcription* (*left*).

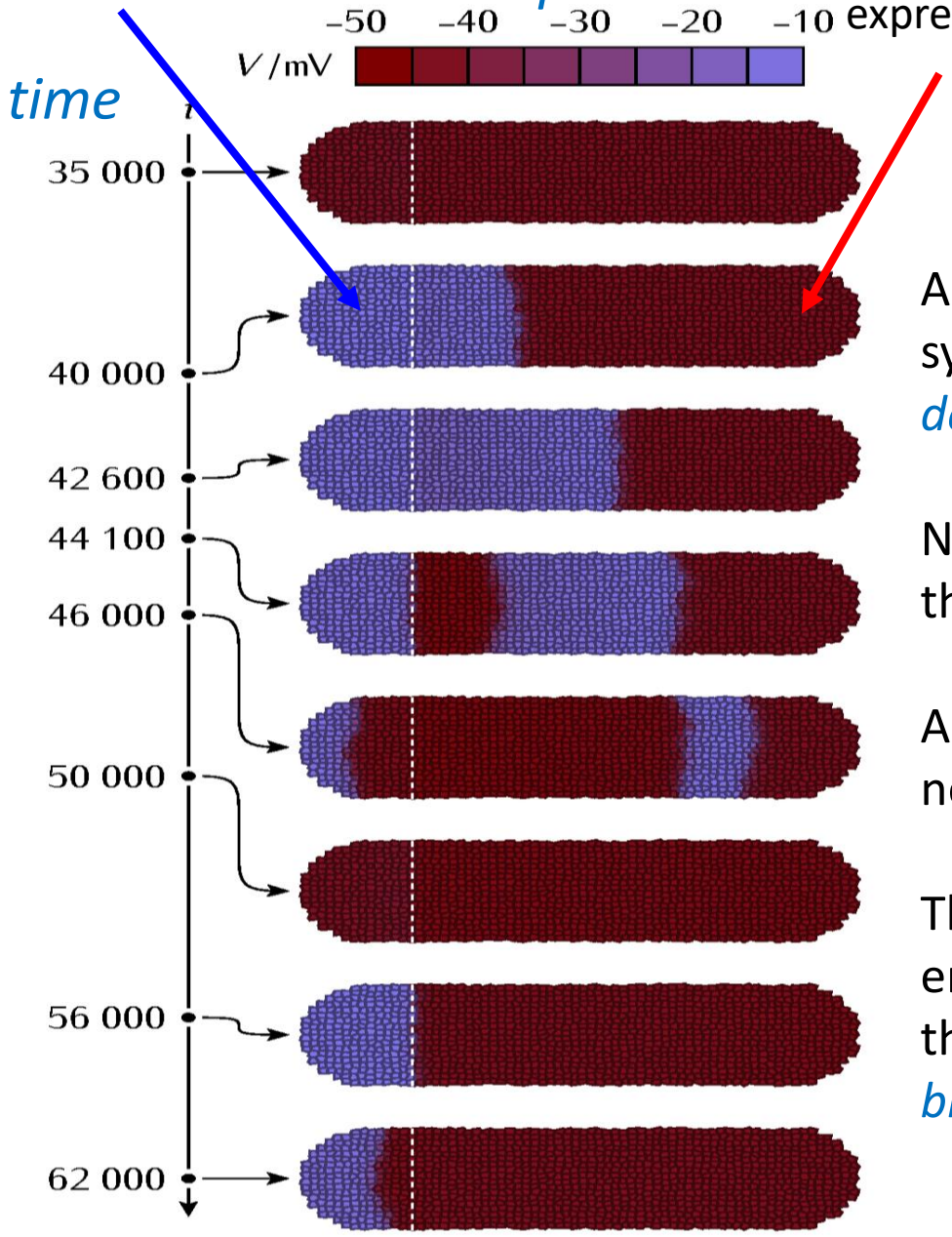
A *biochemical* reminiscence?: when the tissue is dissociated, cells stop oscillating, but coordinated oscillations emerge by re-aggregation (*bottom*).



Comp. Biol. Med. 2024
10.1016/j.compbiomed.2024.108964

low pol channel
expression region: **cell # 1**

high pol channel
expression region: **cell # 2**



symmetry breaking can be amplified: bioelectrical-transcriptional waves

A *depolarization wave* can propagate across the multicellular system, as evidenced by the *spatio-temporal bands* of alternate *dep* and *pol* regions.

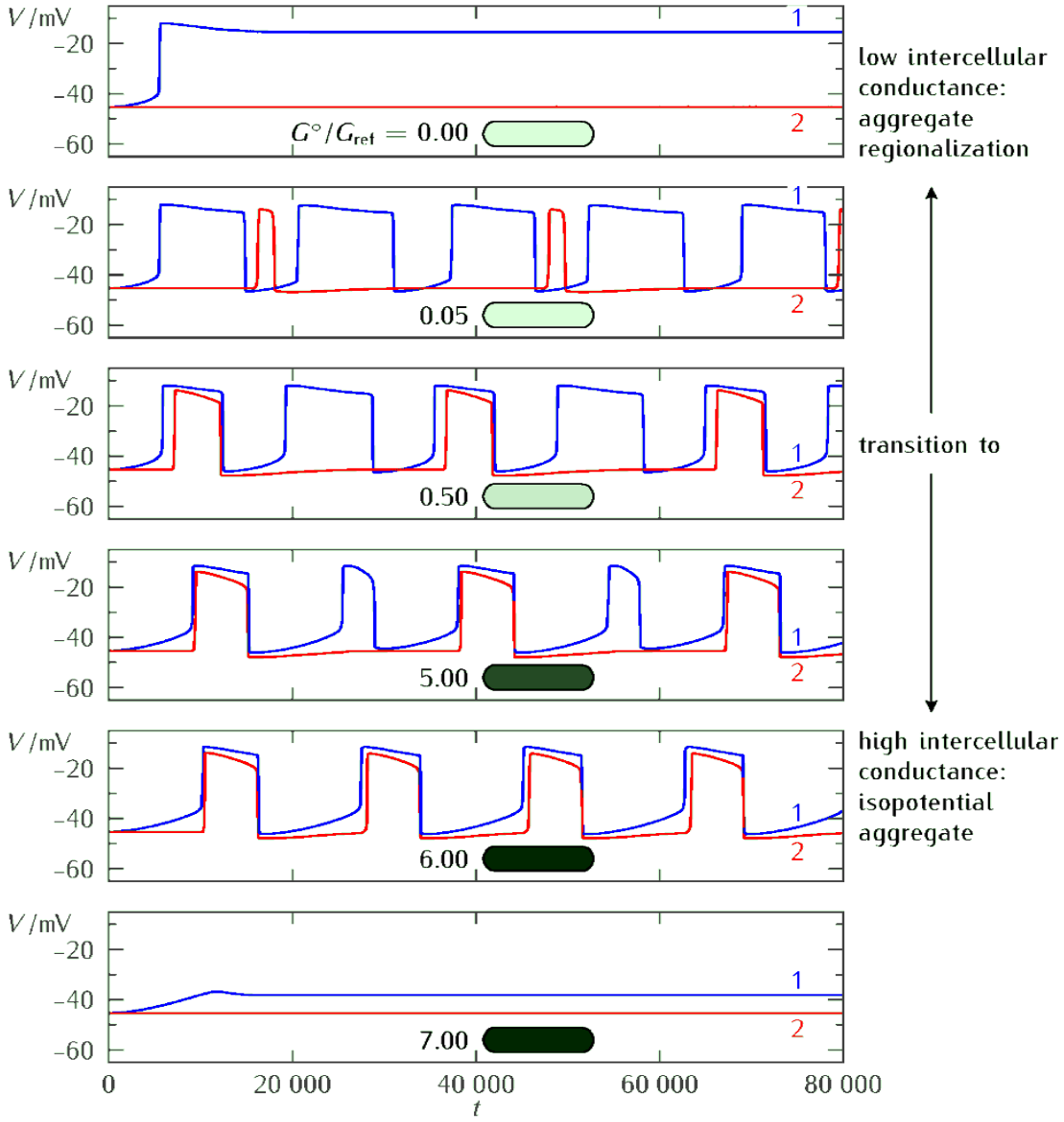
Note the coexistence of *phase* and *antiphase regions.* Could these regions be *developmentally* relevant?

A *polarized central region* between two depolarized neighboring regions can *arise* and *expand* at long times.

The *coupling* between *bioelectricity* and *transcription* can encode *spatio-temporal information* (e.g. segmentation) through the *response* of *multicellular potentials* to a *symmetry-breaking* (the gradient of *pol* channel expression here).

transitions between bioelectrical states regulated by intercellular gap junctions

Comp. Biol. Med. 2024
10.1016/j.compbiomed.2024.108964

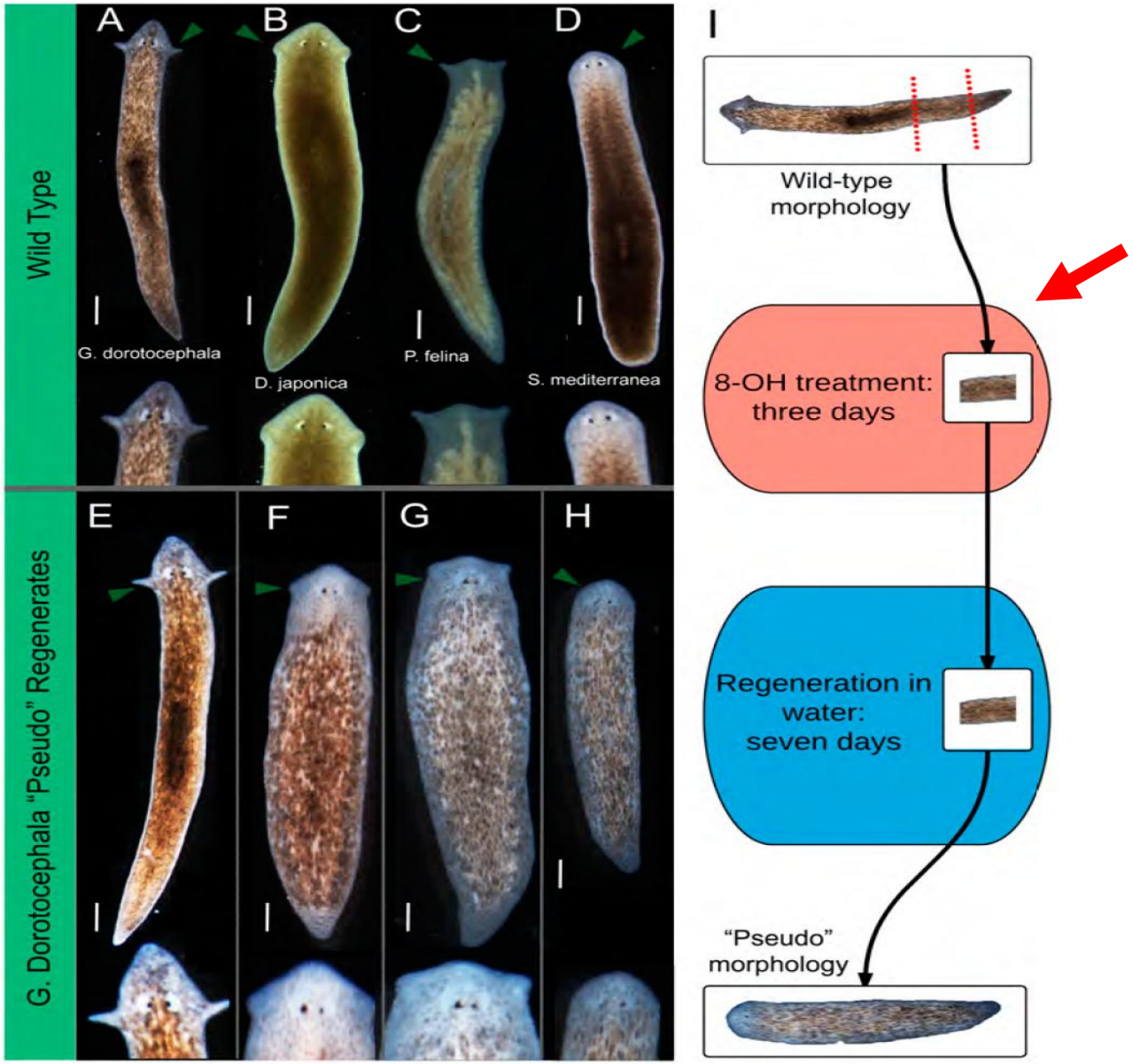


At *zero connectivity* (*top*), the *depolarized* (cell # 1, *left* region) and *polarized* (cell # 2, *right* region) are *independent* and show different potentials.

Intermediate connectivity couples the *left* and *right* regions in *phase* and *antiphase* oscillations, leading to a rich dynamics and the eventual *synchronization* of *distant* cell potentials.

At *high connectivity* (*bottom*), an *iso-potential* system is obtained.

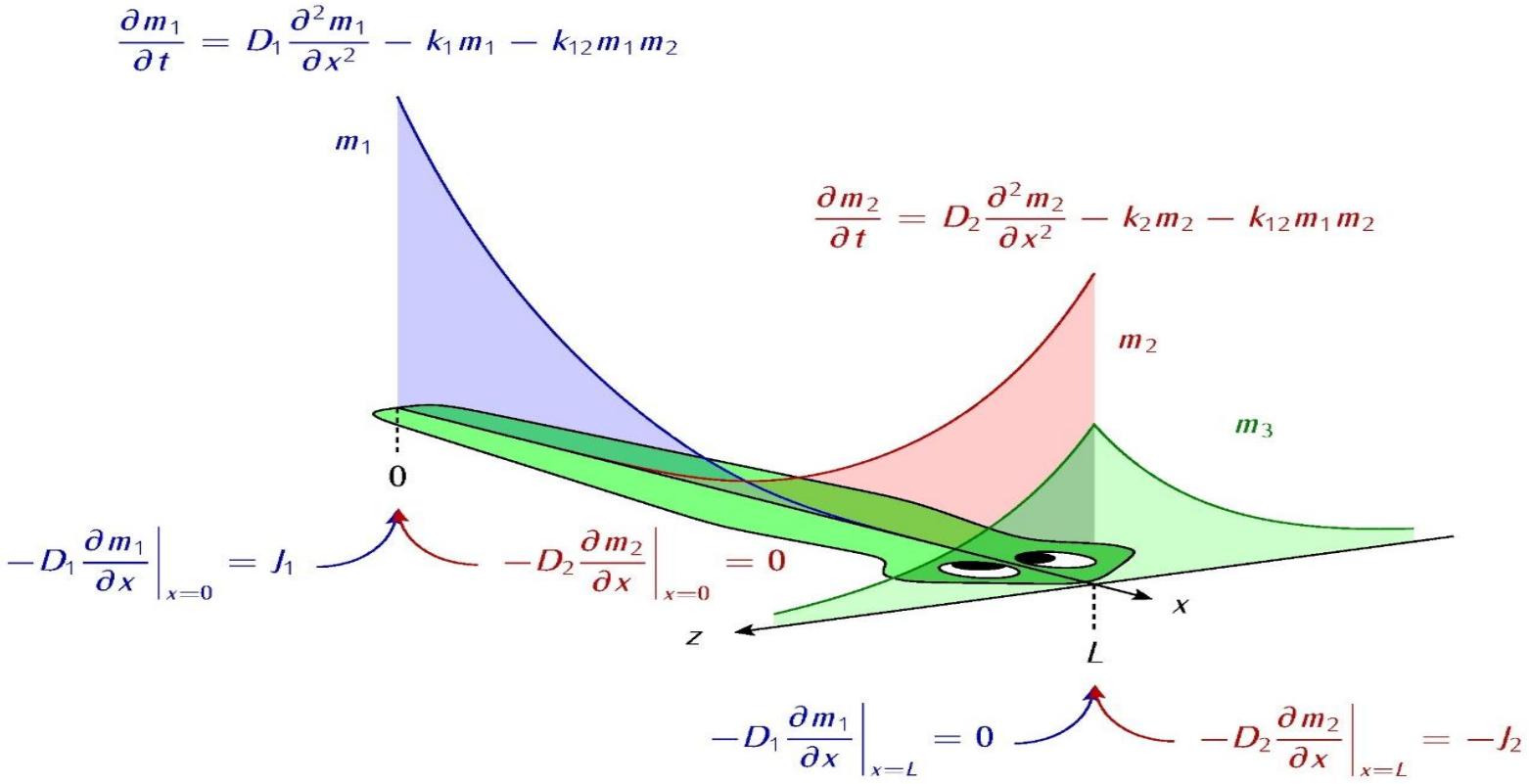
Int. J. Mol. Sci. 2015
10.3390/ijms161126065



more actions on gap junctions: octanol blocking may change planaria head morphology

Different *head morphologies* observed after *octanol* treatment of *four species* of *planaria* flatworm (A–D). The green arrows indicate the distinct head shapes (E–H) obtained when the *pre-tail fragments* are *amputated* from G. dorotocephala *worms* and treated according to the experimental procedure shown. The scale bar corresponds to 0.5 mm.

Biosystems 2021
10.1016/j.biosystems.2021.104511

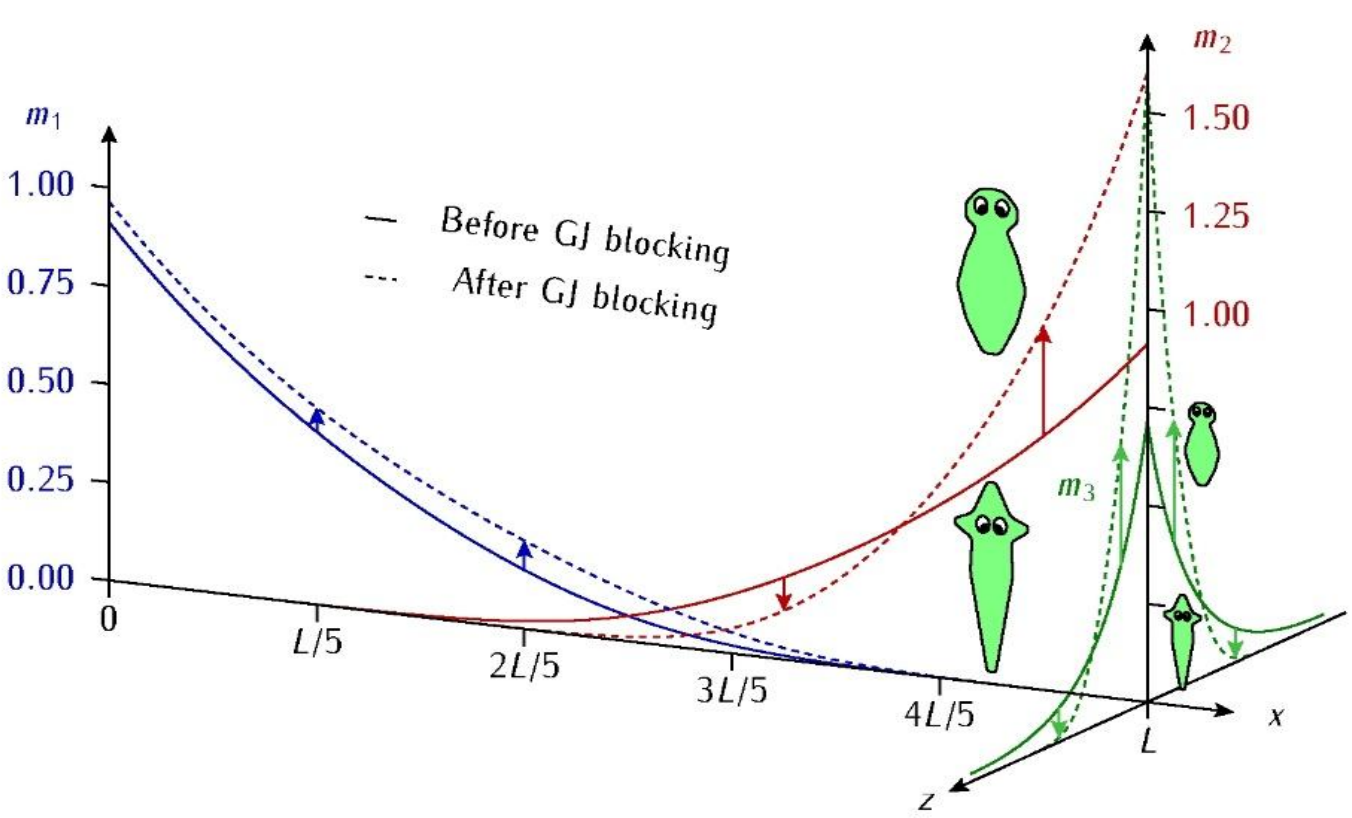


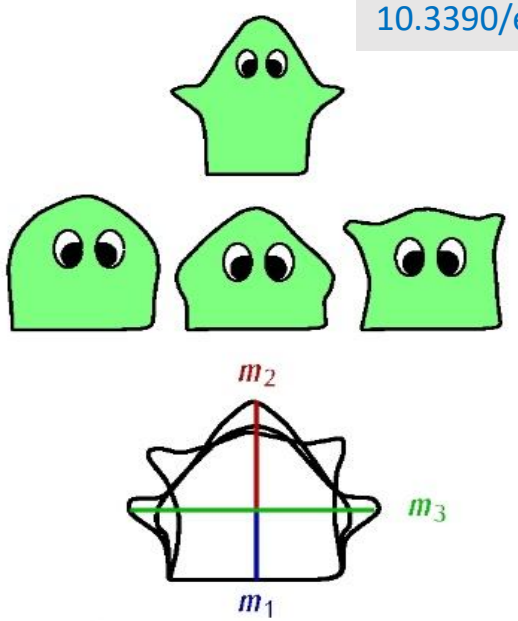
model equations for planaria head morphology

Reaction-diffusion equations with boundary conditions at x = 0 (*tail*) and L (*head*). The *spatial regionalization* of two *morphogen concentrations* m_1 and m_2 along the x axis establishes the *antero-posterior morphology* of the multicellular system. Because the position of the *central wavefront* between the two concentrations is regulated by the diffusion coefficients D_1 and D_2, the *gap junction blocking* eventually impacts on the *spatial patterns*. A third morphogen of concentration m_3 that diffuses independently along the orthogonal direction (z axis) may also influence lateral morphology.

Biosystems 2021
10.1016/j.biosystems.2021.104511
Entropy 2022
10.3390/e24060819

model qualitative results





Anteroposterior and *auricular* morphogen concentrations modulate *shape morphology*

The simulations suggest that changes in the *instructive patterns* of the antero-posterior morphogens 1 and 2 and the auricular morphogen 3, which result from the *gap junction blocking*, may lead to different *expression patterns* and *head shapes*. This result shows the importance of identifying *biological morphospaces* defined by experimentally accessible magnitudes.

a bioelectrical configuration space for flatworm morphology: the head and tail modules contribute to pattern completion

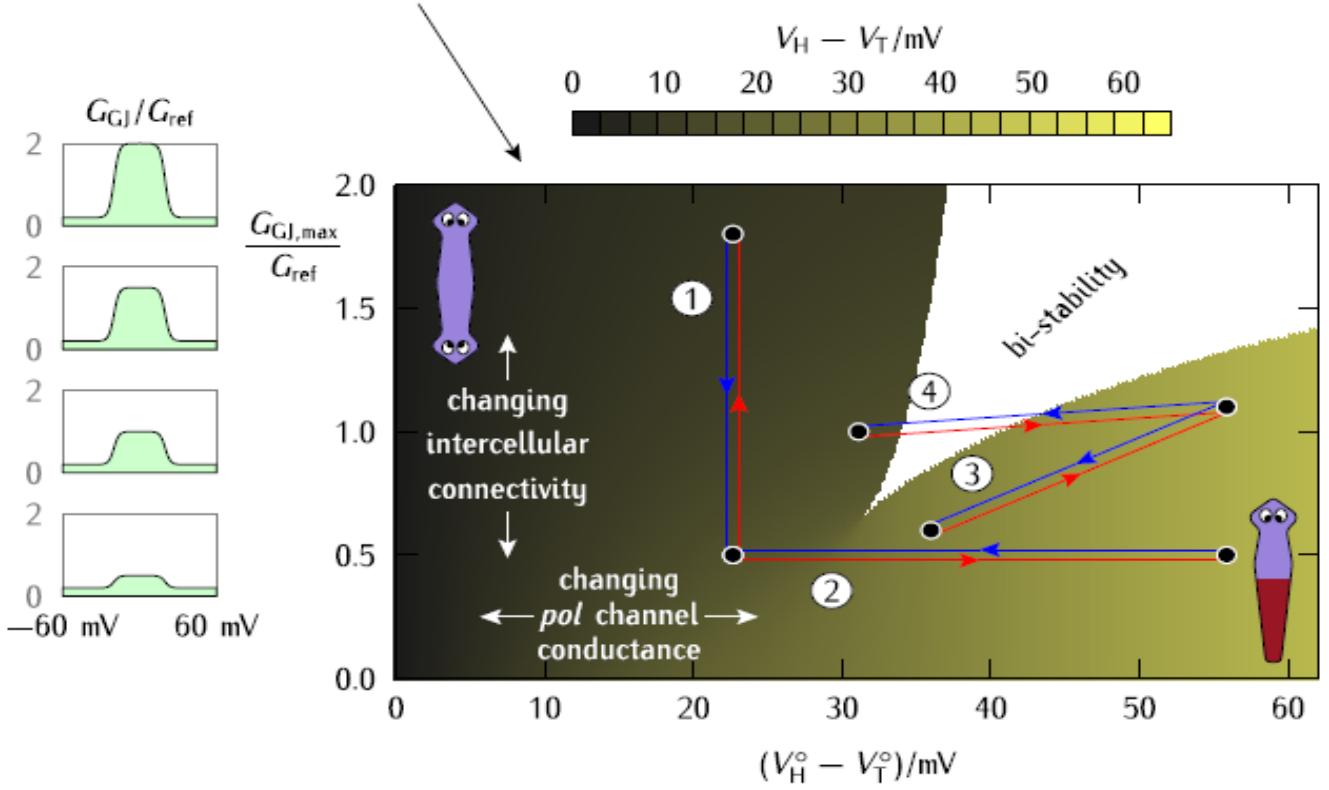
Bioelectrochem. 2020
10.1016/j.bioelechem.2019.107410

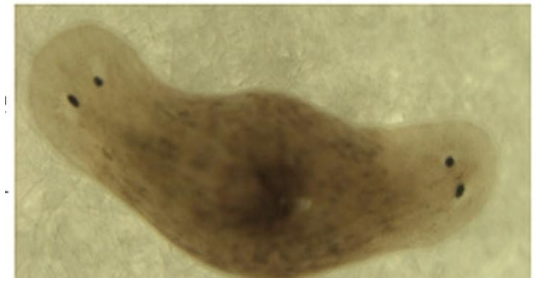
Steady-state solution for isolated cells:

$$V_H^\circ \equiv \frac{G^\circ_{pol,H}E_{pol,H} + G^\circ_{dep,H}E_{dep,H} - I_{pump,H}}{G^\circ_{pol,H} + G^\circ_{dep,H}}; \quad V_T^\circ \equiv \frac{G^\circ_{pol,T}E_{pol,T} + G^\circ_{dep,T}E_{dep,T} - I_{pump,T}}{G^\circ_{pol,T} + G^\circ_{dep,T}}$$

Steady-state solution for coupled cells:

$$\left[1 + \frac{G_{GJ}(V_H - V_T)}{G^\circ_{pol,H} + G^\circ_{dep,H}} + \frac{G_{GJ}(V_H - V_T)}{G^\circ_{pol,T} + G^\circ_{dep,T}}\right](V_H - V_T) - (V_H^\circ - V_T^\circ) = 0$$

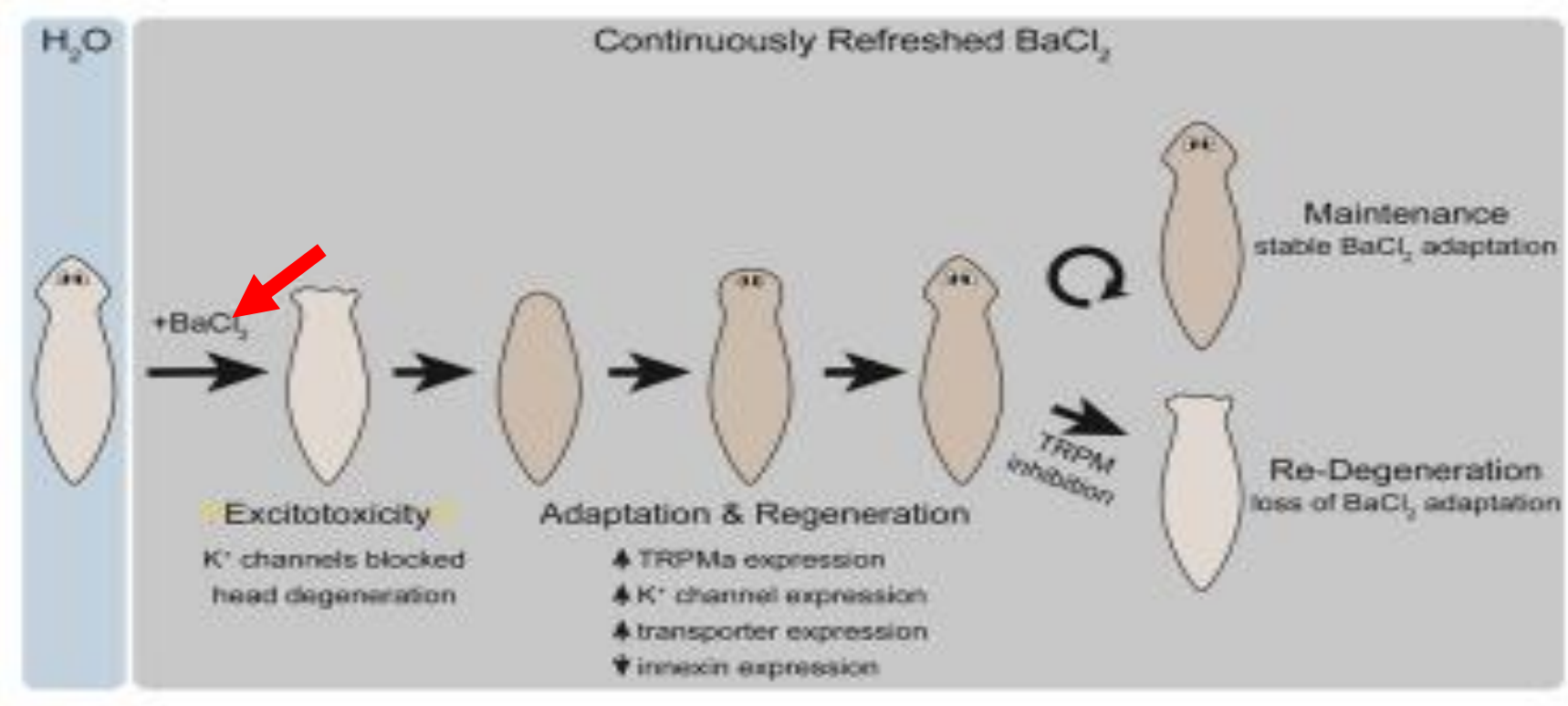




Model equations and resulting *configurational space* for the *head-tail* potential difference $V_H - V_T$ as a function of the *isolated cell* potential difference (*superindex* o) and the *maximum gap junction conductance* $G_{GJ;max}/G_{ref}$. The color gradient indicates the different *monostable solutions* while the central white zone corresponds to the *bi-stability region*. The arrows show the trajectories of different bioelectric processes.

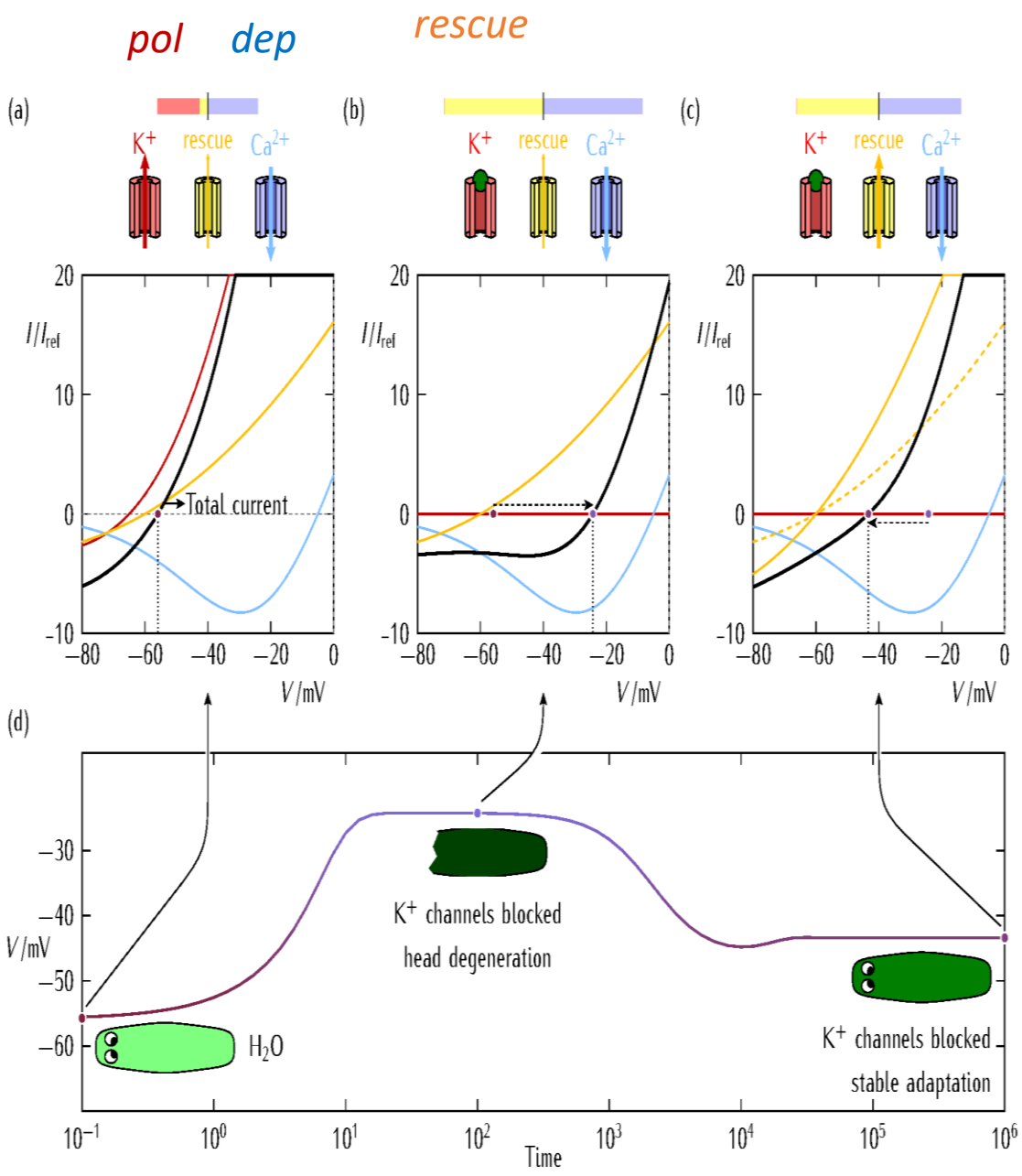
iScience 2019
10.1016/j.isci.2019.11.014

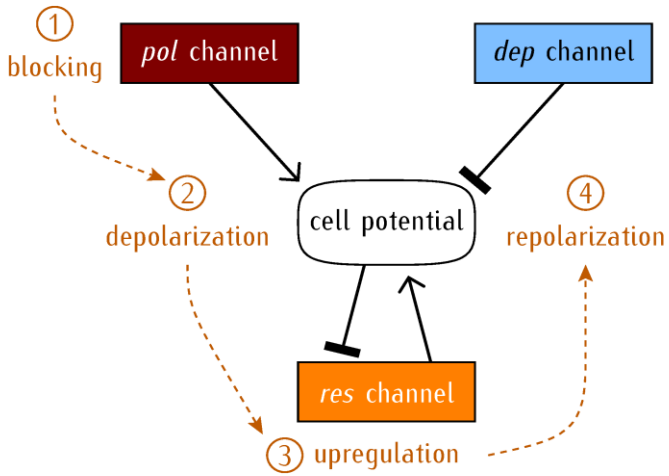
planarian regeneration as a model for acquired tolerance to toxic environments



Exposure to barium chloride ($BaCl_2$) provokes a rapid degeneration of anterior tissue in *Dugesia japonica* but continued exposure to fresh solution of $BaCl_2$ results in regeneration of heads that are insensitive to $BaCl_2$. A model of *adaptation to toxicity* shows the central role of *ion channels* in *adaptive plasticity*, which may provide a target for *biomedical* strategies.

Sci. Rep. 2024
10.1038/s41598-024-79087-7

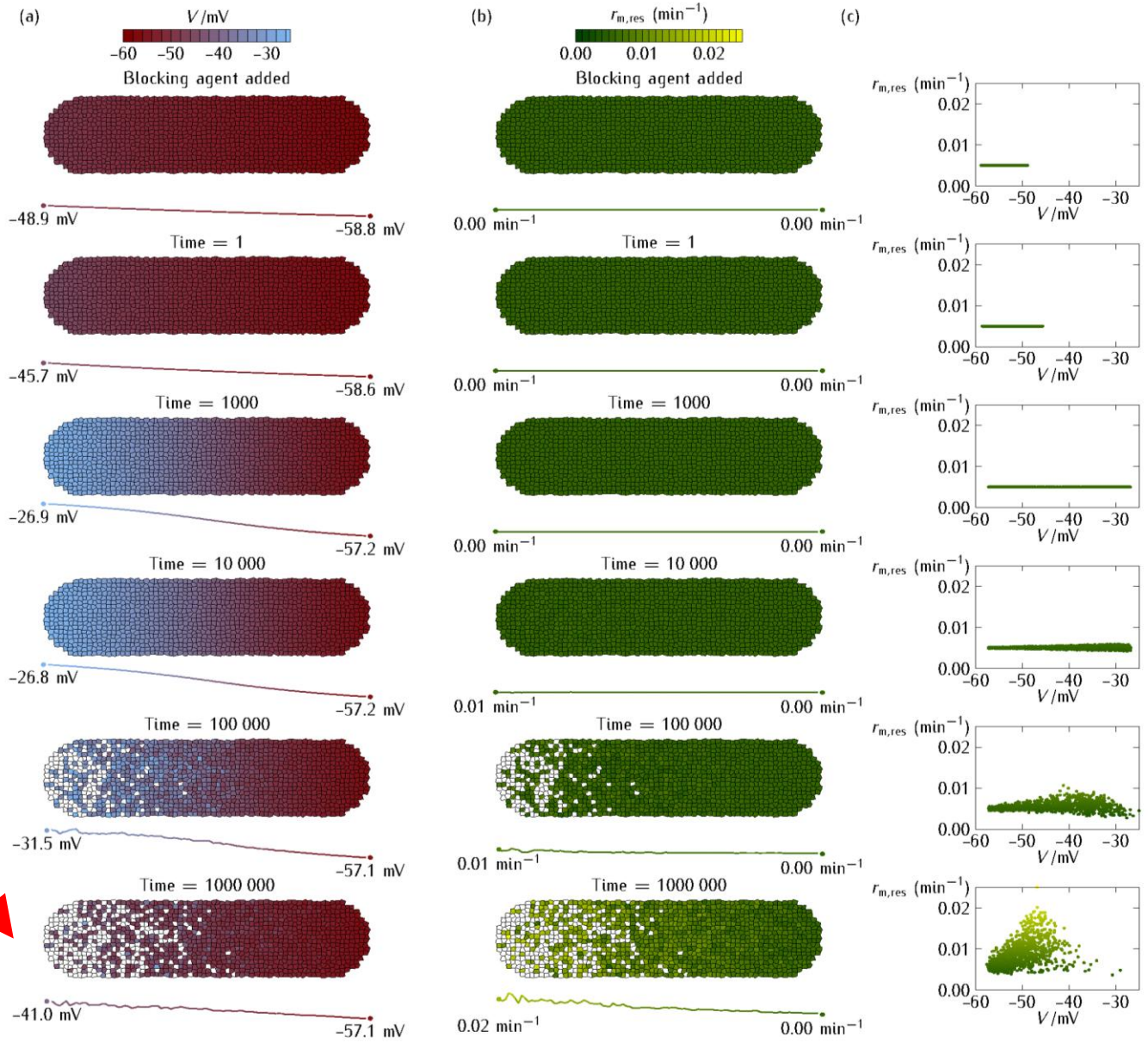




a simplified bioelectrically-focused model

Dominant ion channels and *cell polarization states* (*up*). The cell potential is regulated by two effective *depolarizing* (*dep*) and *polarizing* (*pol* or *rescue*) channels. (a) The *I-V* curves of two opposing channels show the outward (*red*) and inward (*blue*) currents by the potassium and calcium channels, respectively, before blocking. (b) When the potassium channel is blocked, the depolarized cell potential marks the onset of the outward *rescue* channel. (c) Cell physiological *repolarization* can eventually be achieved by the *increased expression* of the *compensatory rescue* channel. (d) The *time trace* of the head cell potential.

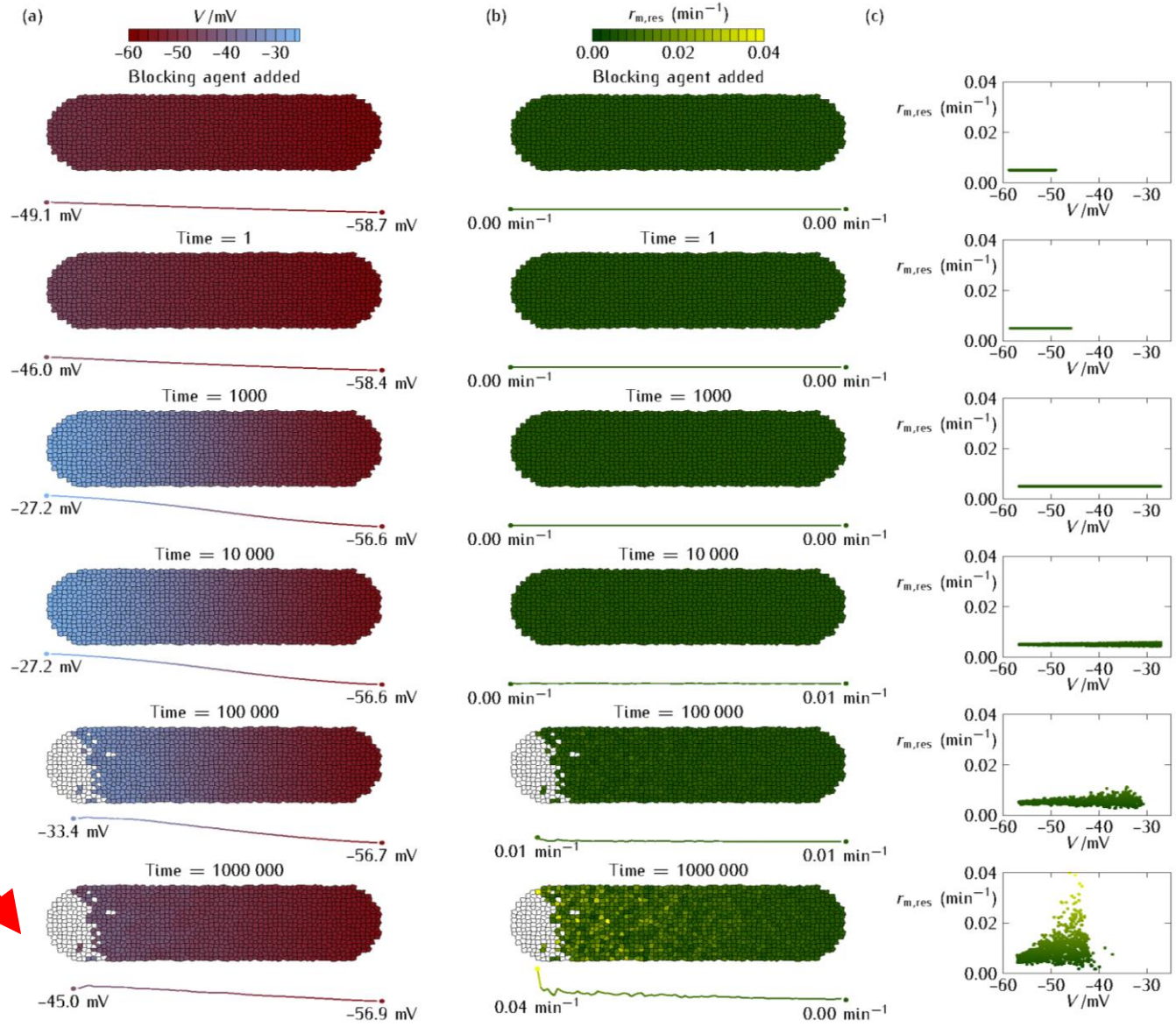
Sci. Rep. 2024
10.1038/s41598-024-79087-7



The single-cell state noisy updating can be *slightly biased* to upregulate the *res* channel that acts to reestablish cell polarization. However, model simulations of the multicellular aggregate at *zero* junction conductance (isolated cells, *no* intercellular connectivity) show the *limited compensatory effect* of the *res* channel: note the significant *extension* of *dead cells* in the head.

(a) Cell potentials *V*.
(b) Cell rate constants of the upregulated *res* channel.
(c) Rate vs. *V* plot for the living cells (*points*).

Sci. Rep. 2024
10.1038/s41598-024-79087-7



The model simulations of the multicellular aggregate at *non-zero* junction conductance (intercellular connectivity) show the significant *compensatory effect* of the *res* channel: note the *limited extension* of *dead cells* in the head. This result suggests a *collective* contribution to the *adaptive response* that may contribute to the bioelectrical *pattern completion* (head re-establishment here).

(a) Cell potentials *V*.
(b) Cell rate constants of the upregulated *res* channel.
(c) Rate vs. *V* plot for the living cells (*points*).

other bioelectrical models: BETSE simulator (Alexis Pietak)

Front. Bioeng. Biotechnol. 2016
10.3389/fbioe.2016.00055
J. R. Soc. Interface 2017
10.1098/rsif.2017.0425

frontiers in Bioengineering and Biotechnology

ORIGINAL RESEARCH
published: 06 July 2016
doi: 10.3389/fbioe.2016.00055

Exploring Instructive Physiological Signaling with the Bioelectric Tissue Simulation Engine

*Alexis Pietak and Michael Levin**

Allen Discovery Center at Tufts University, Medford, MA, USA

Bioelectric cell properties have been revealed as powerful targets for modulating stem cell function, regenerative response, developmental patterning, and tumor reprograming. Spatio-temporal distributions of endogenous resting potential, ion flows, and electric fields are influenced not only by the genome and external signals but also by their own intrinsic dynamics. Ion channels and electrical synapses (gap junctions) both determine, and are themselves gated by, cellular resting potential. Thus, the origin and progression of bioelectric patterns in multicellular tissues is complex, which hampers the rational control of voltage distributions for biomedical interventions. To improve understanding of these dynamics and facilitate the development of bioelectric pattern control strategies, we developed the BioElectric Tissue Simulation Engine (BETSE), a finite volume method multiphysics simulator, which predicts bioelectric patterns and their spatio-temporal dynamics by modeling ion channel and gap junction activity and tracking changes to the fundamental property of ion concentration. We validate performance of the simulator by matching experimentally obtained data on membrane permeability, ion concentration and resting potential to simulated values, and by demonstrating the expected outcomes for a range of well-known cases, such as predicting the correct transmembrane voltage changes for perturbation of single cell membrane states and environmental ion concentrations, in addition to the development of realistic transepithelial potentials and bioelectric wounding signals. *In silico* experiments reveal factors influencing transmembrane potential are significantly different in gap junction-networked cell clusters with tight junctions, and identify non-linear feedback mechanisms capable of generating strong, emergent, cluster-wide resting potential gradients. The BETSE platform will enable a deep understanding of local and long-range bioelectrical dynamics in tissues, and assist the development of specific interventions to achieve greater control of pattern during morphogenesis and remodeling.

Keywords: bioelectric simulation, pattern formation, resting potential, transmembrane voltage

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Ovidiu Radulescu,
Université de Montpellier 2, France

Reviewed by:
Victor P. Andreev,
Arbor Research Collaborative for Health, USA
Leonid Evsey Fridlyand,
University of Chicago, USA

***Correspondence:**
Michael Levin
michael.levin@tufts.edu

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1. INTRODUCTION

1.1. Bioelectricity: Why Model Electrical Activity in Non-Neural Cells?

Explaining and learning to control large-scale pattern is a central unsolved problem, with implications for mitigation of birth defects, and the advancement of regenerative medicine and synthetic bioengineering. The dynamics of signals orchestrating large-scale order *in vivo* are a key area of

INTERFACE

rsif.royalsocietypublishing.org

Research

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http://dx.doi.org/10.1098/rsif.2017.0425

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Subject Category:
Life Sciences–Physics interface

Subject Areas:
biophysics, computational biology, systems biology

Keywords:
bioelectricity, regeneration, gene regulatory networks, *in silico* simulations

Author for correspondence:
Michael Levin
e-mail: michael.levin@tufts.edu

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THE ROYAL SOCIETY PUBLISHING

Bioelectric gene and reaction networks: computational modelling of genetic, biochemical and bioelectrical dynamics in pattern regulation

Alexis Pietak and Michael Levin

Allen Discovery Center, Tufts University, Medford, MA, USA

ML, 0000-0001-7292-8084

Gene regulatory networks (GRNs) describe interactions between gene products and transcription factors that control gene expression. In combination with reaction–diffusion models, GRNs have enhanced comprehension of biological pattern formation. However, although it is well known that biological systems exploit an interplay of genetic and physical mechanisms, instructive factors such as transmembrane potential (V_{mem}) have not been integrated into full GRN models. Here we extend regulatory networks to include bioelectric signalling, developing a novel synthesis: the bioelectricity-integrated gene and reaction (BIGR) network. Using *in silico* simulations, we highlight the capacity for V_{mem} to alter steady-state concentrations of key signalling molecules inside and out of cells. We characterize fundamental feedbacks where V_{mem} both controls, and is in turn regulated by, biochemical signals and thereby demonstrate V_{mem} homeostatic control, V_{mem} memory and V_{mem} controlled state switching. BIGR networks demonstrating hysteresis are identified as a mechanisms through which more complex patterns of stable V_{mem} spots and stripes, along with correlated concentration patterns, can spontaneously emerge. As further proof of principle, we present and analyse a BIGR network model that mechanistically explains key aspects of the remarkable regenerative powers of creatures such as planarian flatworms. The functional properties of BIGR networks generate the first testable, quantitative hypotheses for biophysical mechanisms underlying the stability and adaptive regulation of anatomical bioelectric pattern.

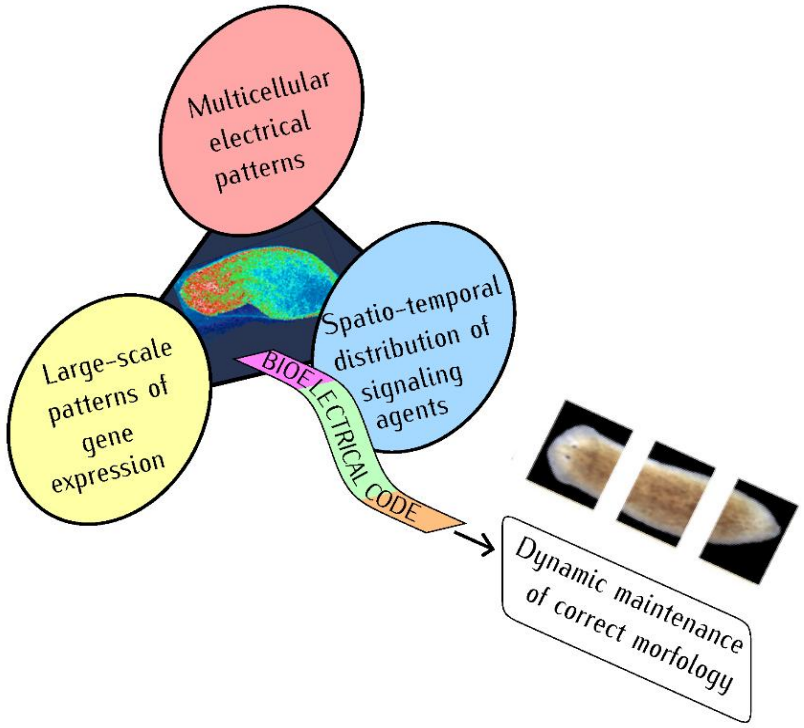
1. Introduction

Large-scale biological patterning in development, regeneration and disease remains among the most fundamental and important questions facing modern biology. Metazoan organisms reliably self-assemble a complex body plan from a single fertilized egg cell; furthermore, many animals, such as salamanders and planaria, are able to repair or remodel their bodies back to the correct shape despite injury and other types of drastic perturbation such as limb amputation [1,2]. Understanding the mechanisms that control the formation and regulation of organism-scale biological patterns may allow us to mitigate birth defects, implement organ regeneration strategies and to prevent, heal or even reprogramme the cancer state [3]. It is crucial to begin to understand and exploit the multicellular algorithms and dynamics that control anatomy and its remodelling, in addition to the details of subcellular signalling pathways.

Biological pattern formation is highly complex, involving numerous biomolecular mechanisms that lead to formation of instructive chemical patterns in a tissue collective, as well as mechanical considerations concerning shape changes and movements of individual cells and the tissue substratum as a whole. From the chemical patterning perspective pioneered by Turing [4] and Wolpert [5] (among others [6–8]), individual cells produce a variety of substances which may: (i) have the capacity to influence the production of other substances via genetic expression

6. Where we go: identifying key bioelectrical steps in biological complexity

6.a Bioelectrical limitations



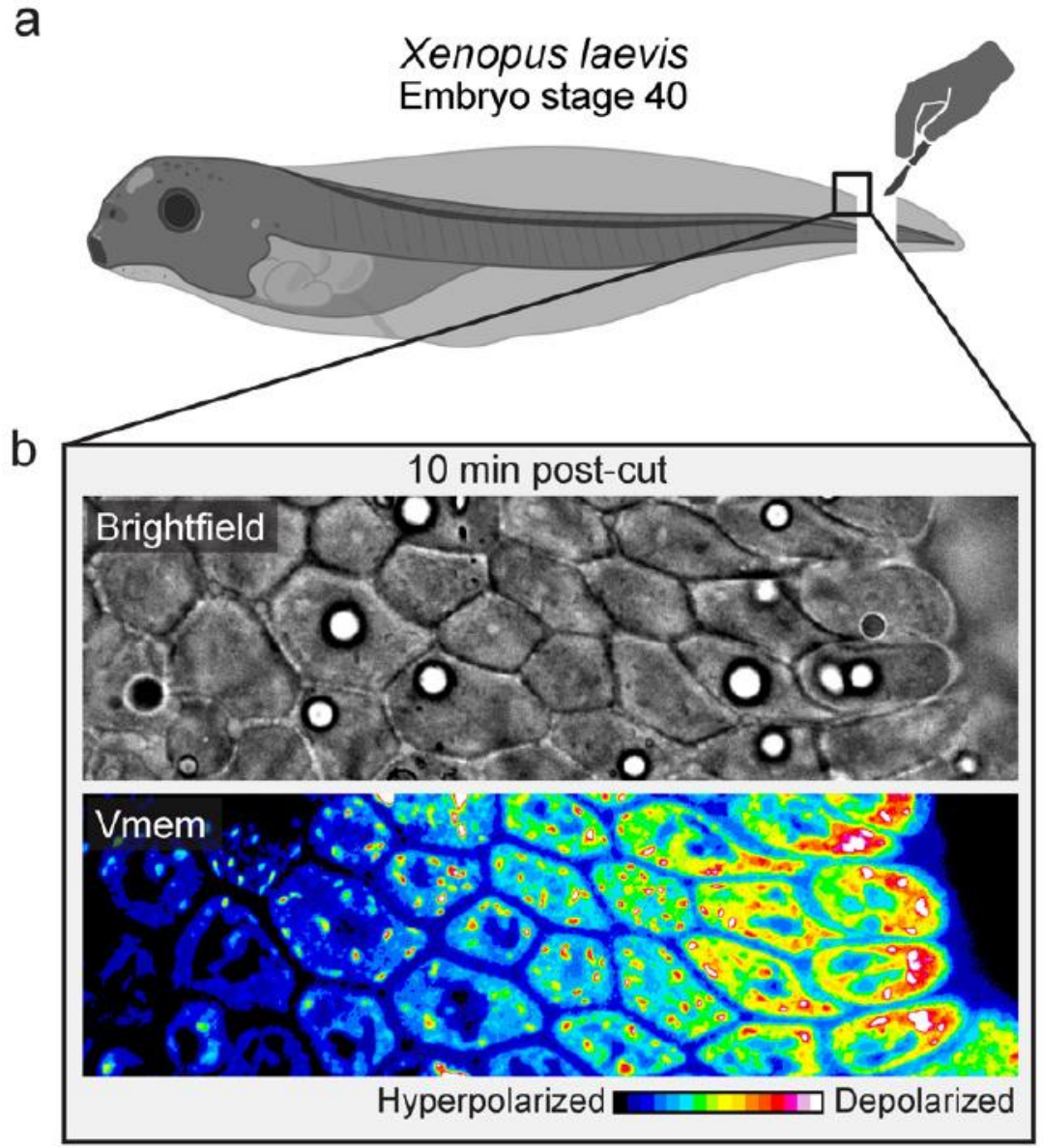
The model simulations explore the *interplay* between *transcription* and *bioelectricity*, modulated by the *single-cell states* and their *intercellular coupling*, in the establishment of *instructive* spatio-temporal patterns at the *multicellular level*. Could *perturbative* changes be *suppressed* or *enhanced* by interrelated *bioelectrical* and *biochemical* processes?

Although the physical model is exceedingly *simple* for quantitative descriptions, it suggests a *rich* phenomenology, based on the *multicellular coupling* between *bioelectricity* and *transcription,* that can be explored further. Also, the core concepts are general enough to permit future *extensions*.

In moving forward, however *realistic* models must incorporate *more contributions* to morphogenesis/regeneration, together with the *coordinated integration* of controlling signals. In particular, concerted *bioelectrical-biomechanical-biochemical* actions should act together to modulate expression patterns, as shown in the following cases.

bioRxiv 2024
10.1101/2023.11.02.565386

membrane potential as master regulator of cellular mechano-transduction



The *interplay of membrane potential and cytoplasmic dilution* at the initiation of*Xenopus* tail regeneration after amputation (a) and the membrane potential (DiSBAC2(3) dye) images of the wound edge (b) suggest that cell layers are progressively depolarized, from the deep tissue towards the wound edge.

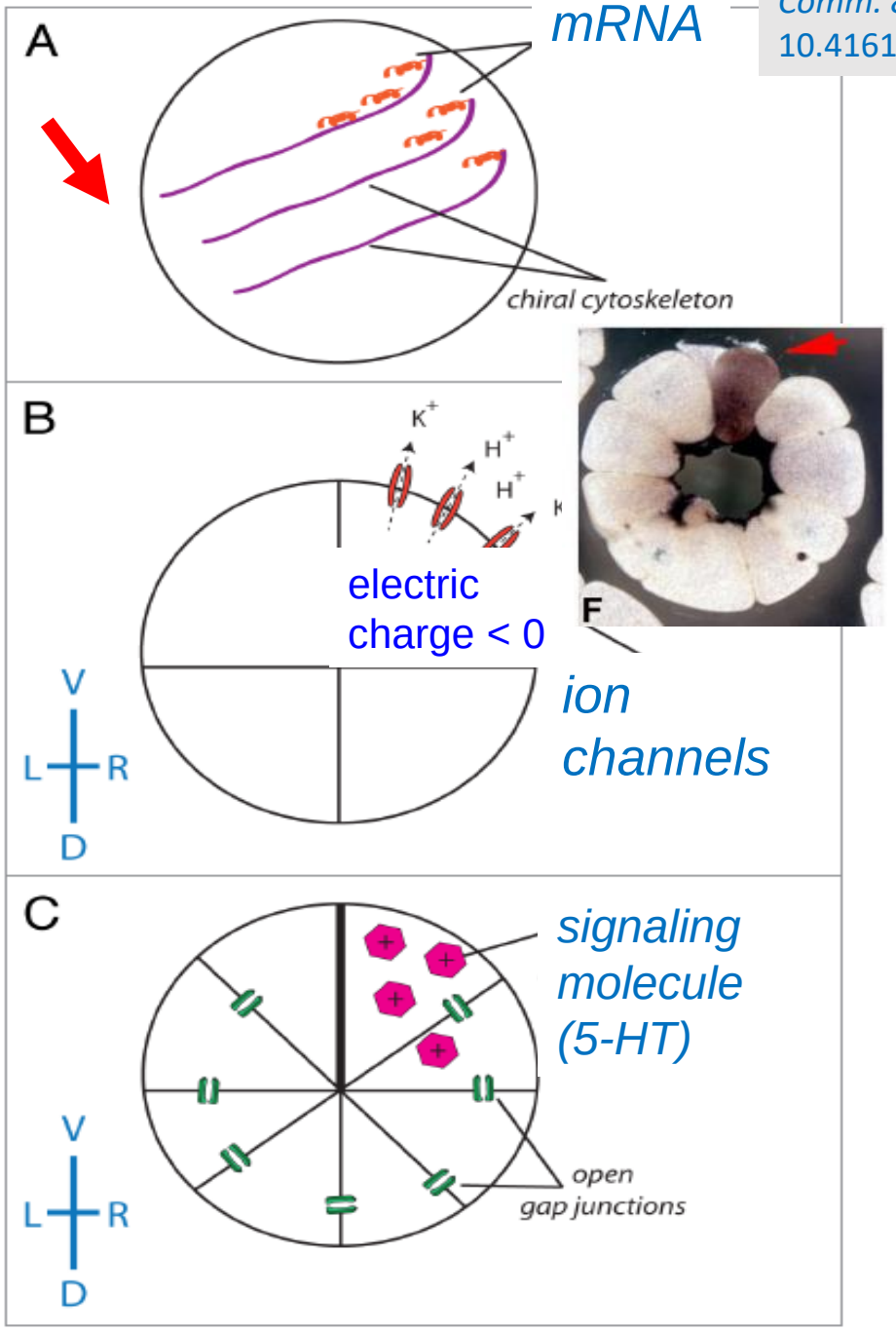
mechano-transduction, bioelectricity and biochemical networks acting together in embryogenesis: the Xenopus frog case

(A) Cytoskeleton *asymmetrically* distributes *mRNAs* encoding *ion transporters.*

(B) The biased expression gives an *electric potential* difference.

(C) Negative potential in the ventral right blastomere yields a *net flow* of positively charged *signaling molecules* (serotonin).

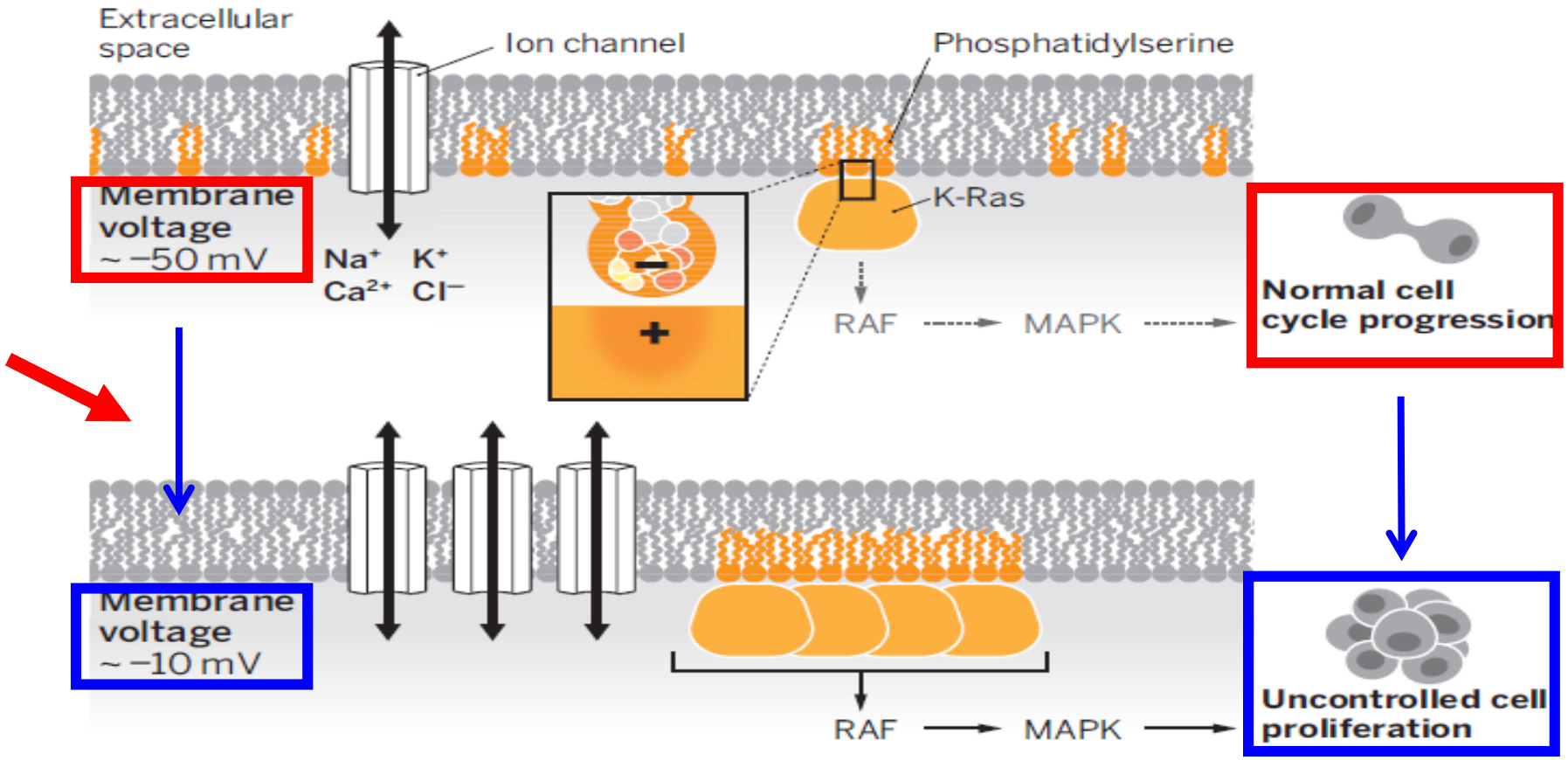
Thus, the *electric potential* regulates the spatial distribution of *signaling molecules* and *ions* over multicellular domains but *biomechanical* effects are also present.



Comm. & Integr. Biol. 2016
10.4161/cib.27155

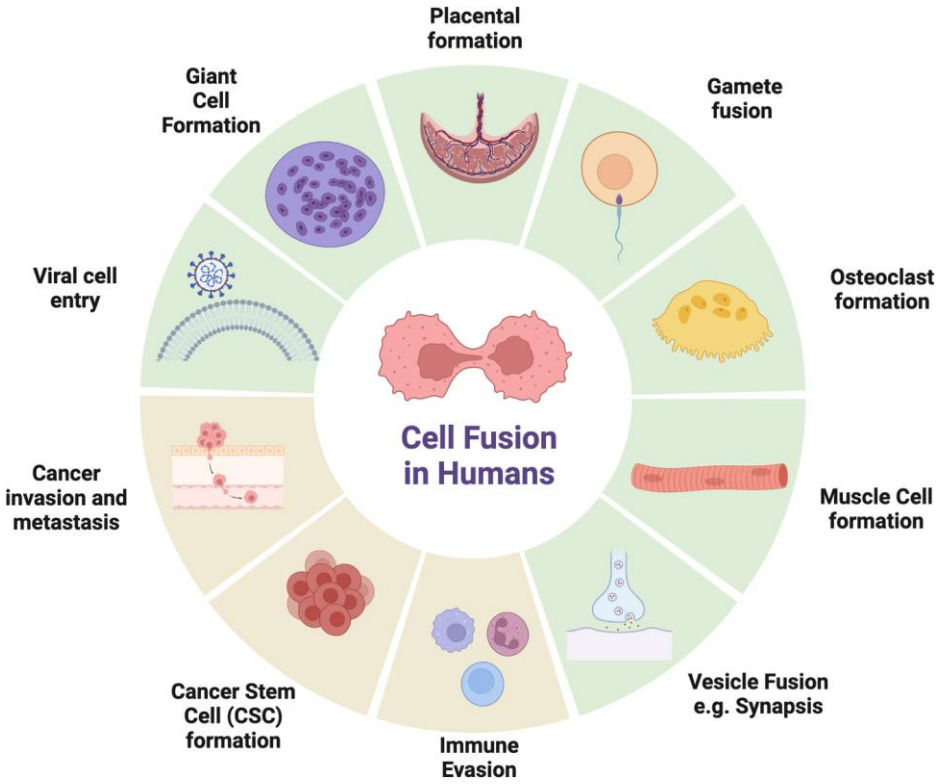
mechano-transduction, bioelectricity, and biochemistry in tumorigenesis

Science 2015
10.1126/science.aaa5619
10.1126/science.aad0874

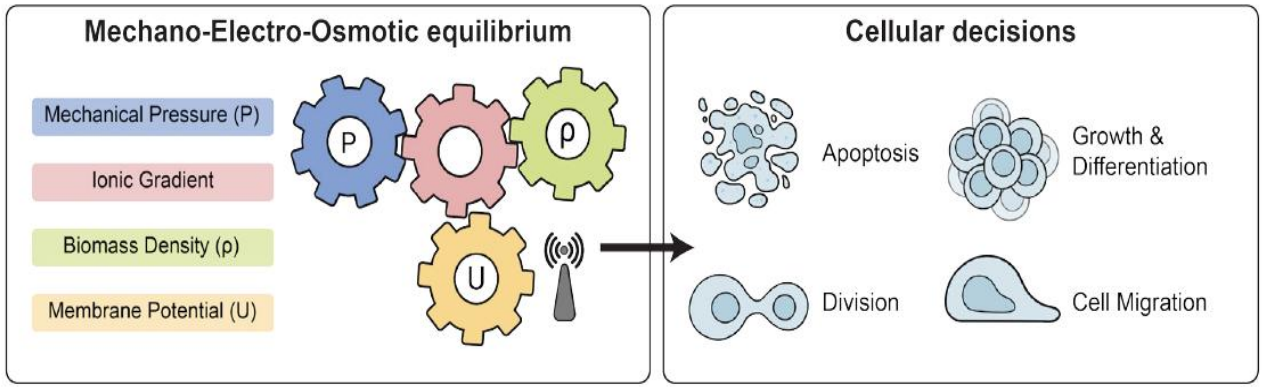


Cell *depolarization* and *tumorigenesis*: the membrane as a *biomechanical field-effect transistor.* The control of mitogenic signaling via specific lipids and proteins suggests *bioelectrical actions*. However, there are significant *clinical risks*: channels regulate *multiple functions* (e.g. cardiac rhythm), being involved in other cell properties (adhesion, volume regulation, apoptosis). *External actions* should be *local* and *tightly controlled*.

Int. J. Biochem. Cell Biol. 2024
10.1016/j.biocel.2024.106649
bioRxiv 2024
10.1101/2023.11.02.565386



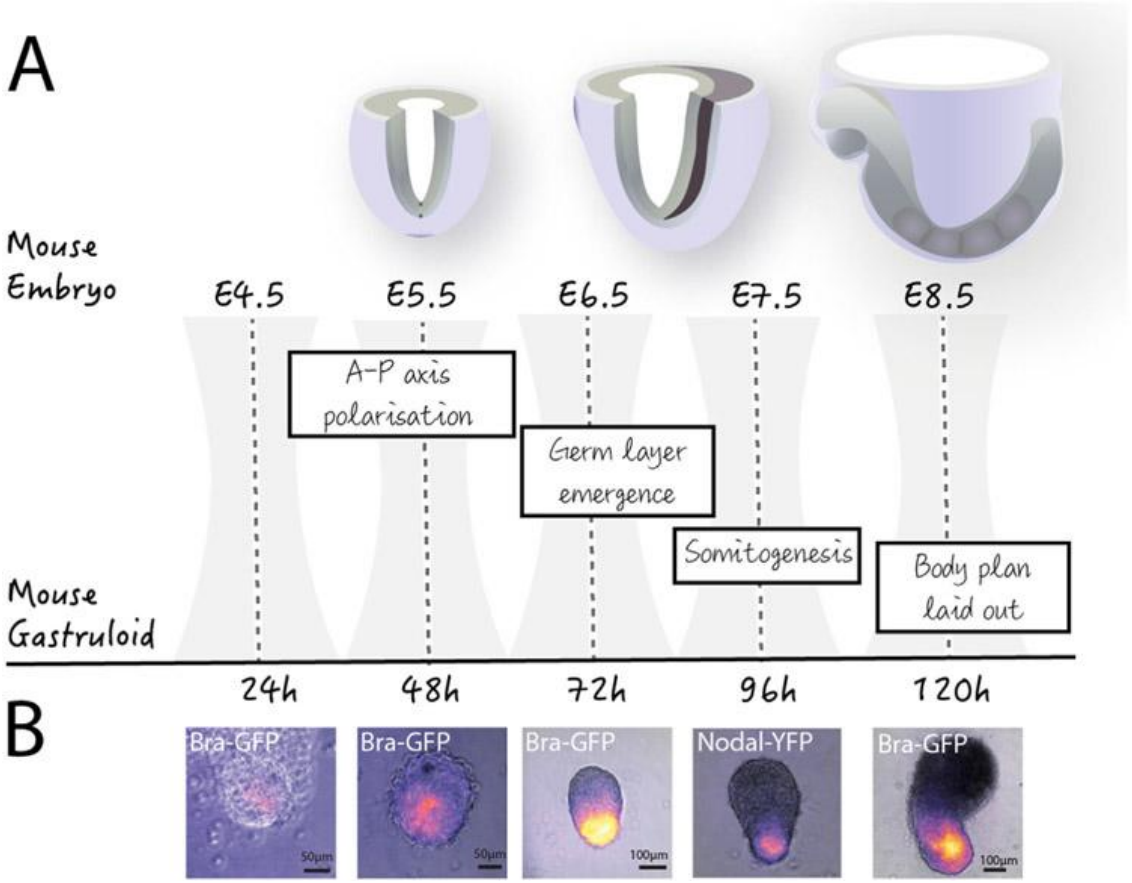
cell-cell fusion in cancer: the next cancer hallmark? Healthy tissues (*green*) and cancerous contexts (*yellow*)



biomechanical pressure, cytoplasmic biomass density and *membrane potential*

gastruloid biomechanics: pluripotent stem cell models of mammalian gastrulation and embryo engineering

Dev Biol. 2022
10.1016/j.ydbio.2022.05.002

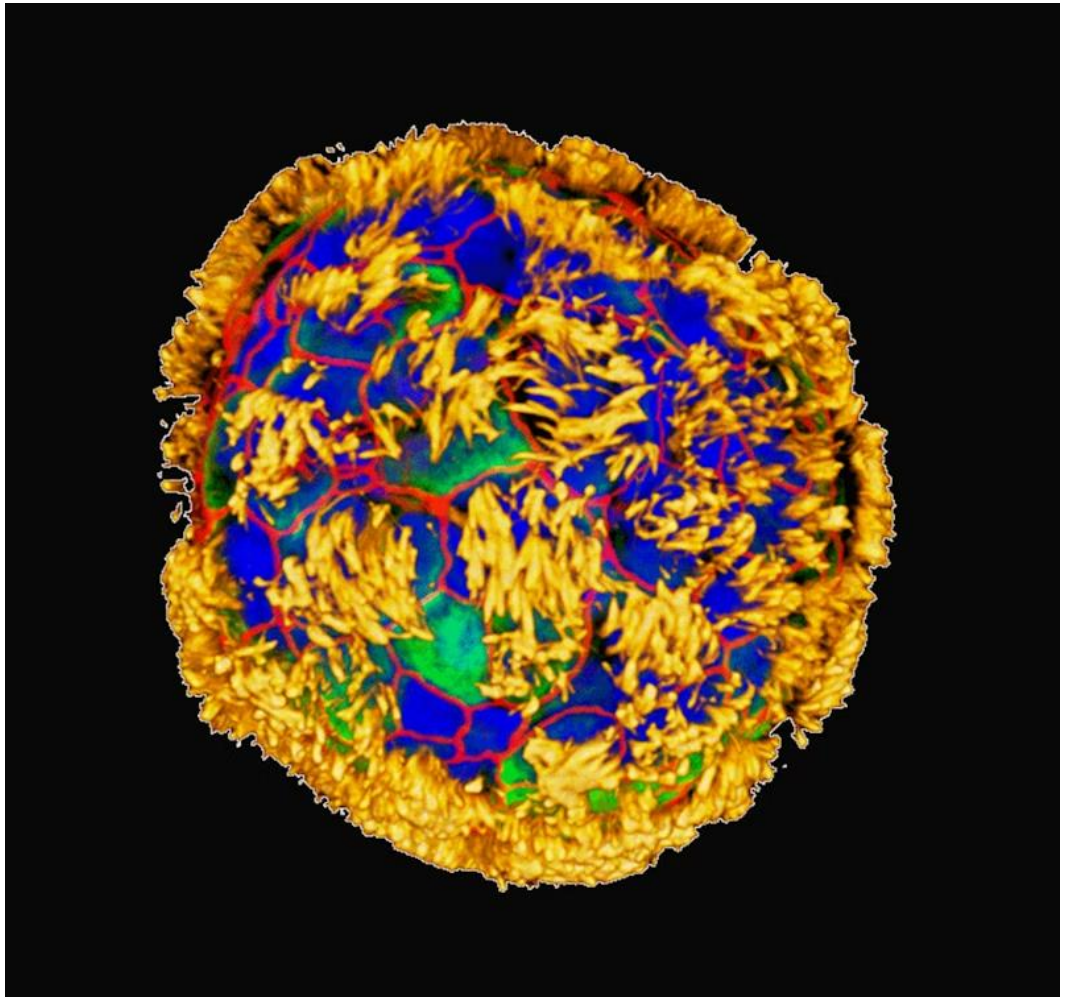


Relationship between mouse embryo (A) and gastruloid (B) development. Gastruloids exhibit the emergence and polarization of *Bra* and *Nodal* gene expression. The regulation of the fate of *stem cells* and *biomechanical field* effects influence the target shape.

anthrobots: what are the multicellular fields that govern what cells can do besides create default features in the body?

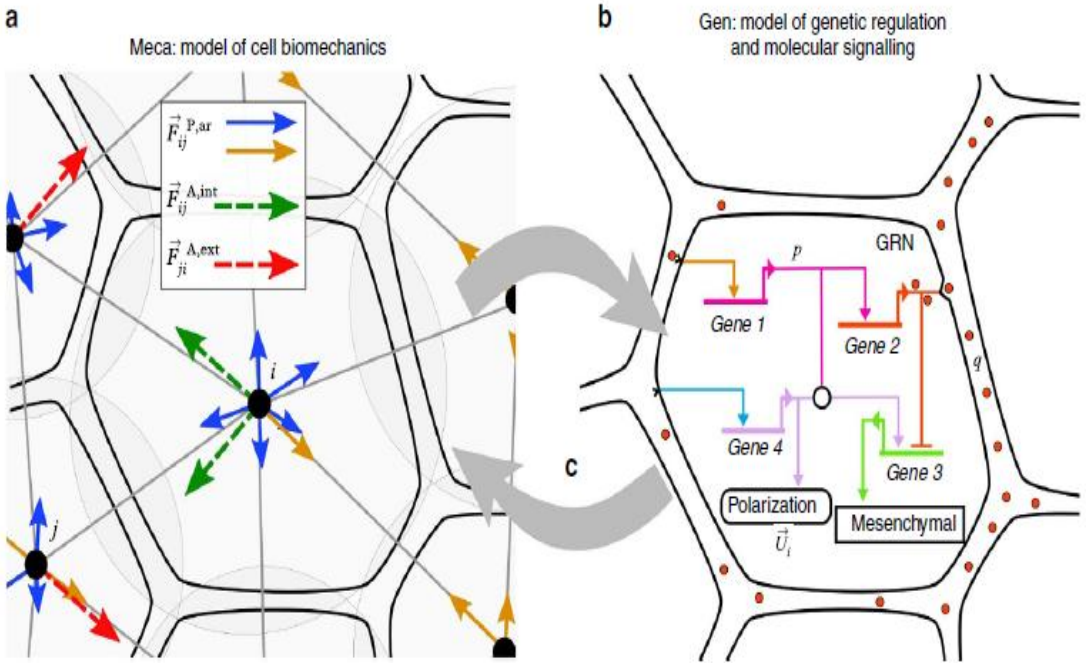
Adv. Sci. 2024
10.1002/advs.20230

Human tracheal skin cells self-assemble into *multi-cellular moving* Anthrobots with cilia on their surface (*yellow*) distributed in different patterns. *Surface patterns* of cilia are correlated with different movement patterns.

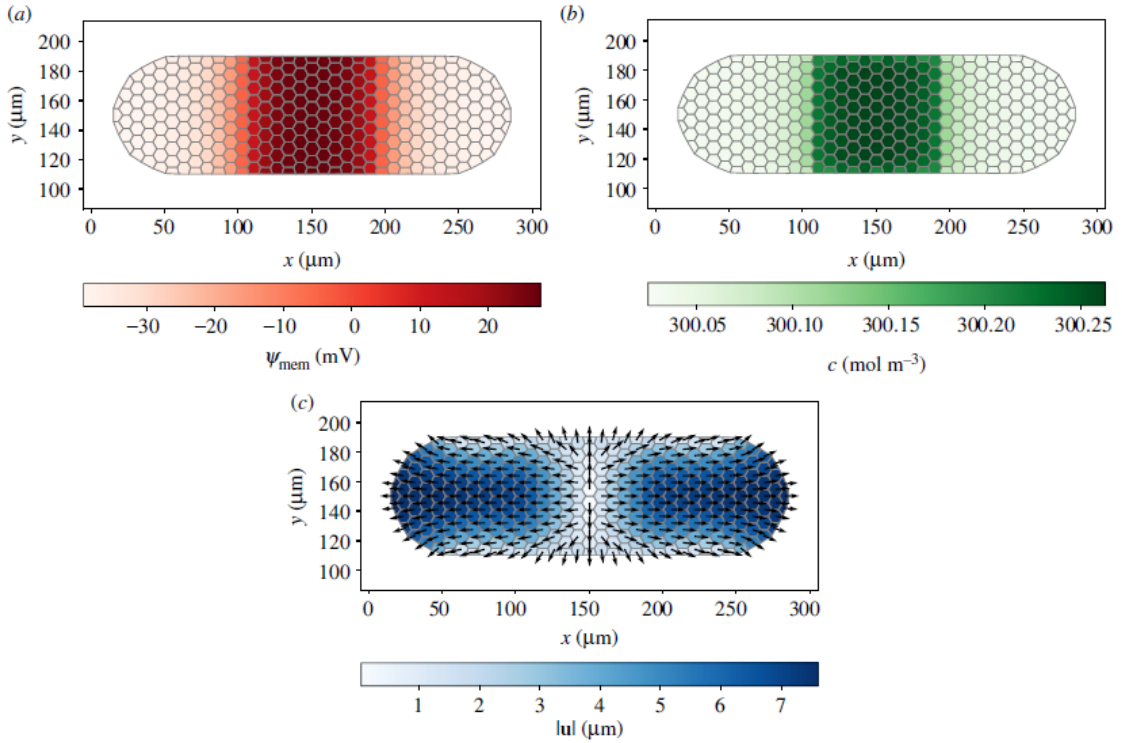


complementary biomechanical models

Nat. Commun. 2017 10.1038/ncomms13929
J. R. Soc. Interface 2020 10.1098/rsif.2020.0177

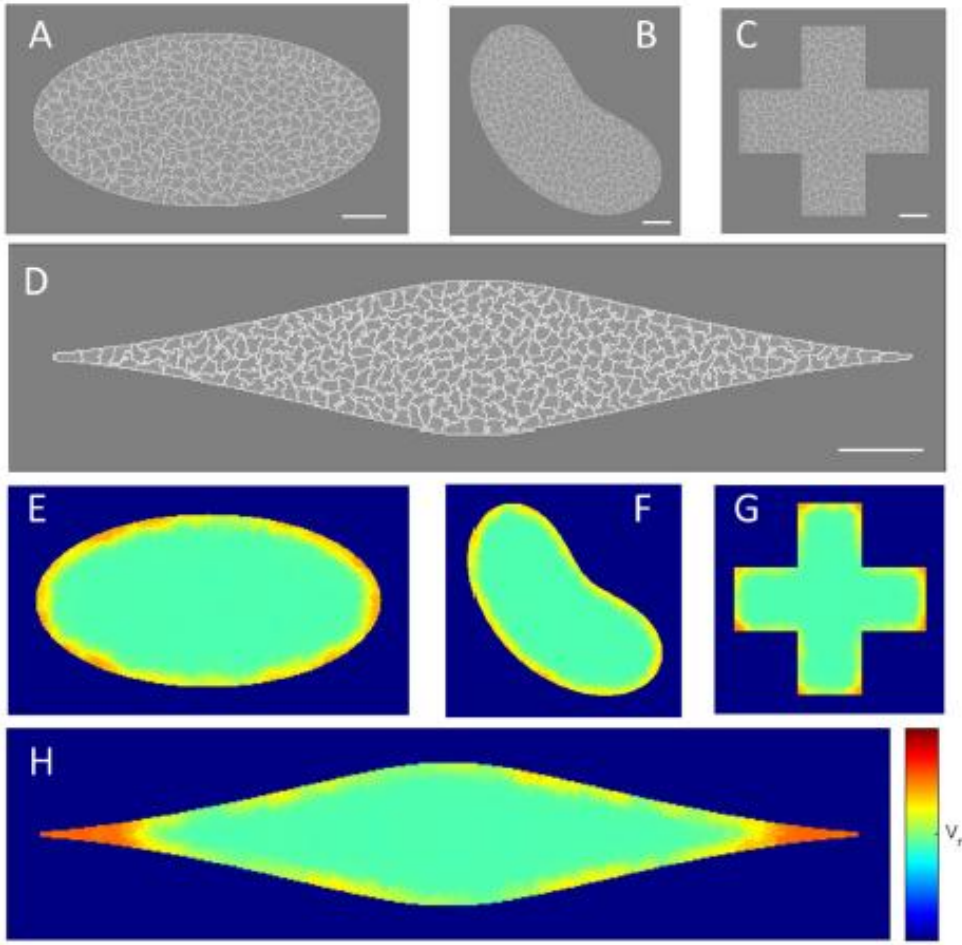


Schematic overview of the *MecaGen* model *coupling* the cell *biomechanical* properties to its *biochemical* activity. Mechanical parameters are specified by the gene expression dynamics and molecular state. Conversely, spatial rearrangements among cells impact protein synthesis via signaling and mechanical stress.

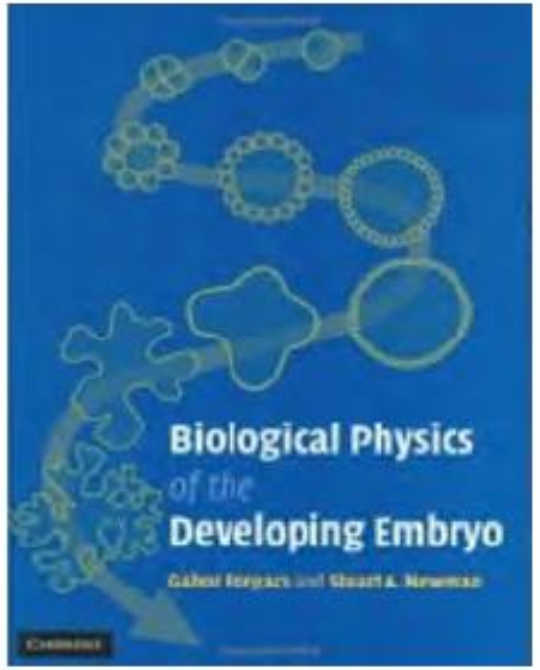


Modeling the *coupling* of *mechanics* with *bioelectricity* and its role in *morphogenesis*: cell membrane potential (a), osmotic concentration (b) and displacement vector (c) at a fixed simulation time. Here, the central depolarized region, in which ions accumulate, determines a symmetric horizontal elongation of the cluster.

J. Theoret. Biol. 2023
10.1016/j.jtbi.2022.111338
Biological Physics of the Developing Embryo 2005
10.1017/CBO9780511755576



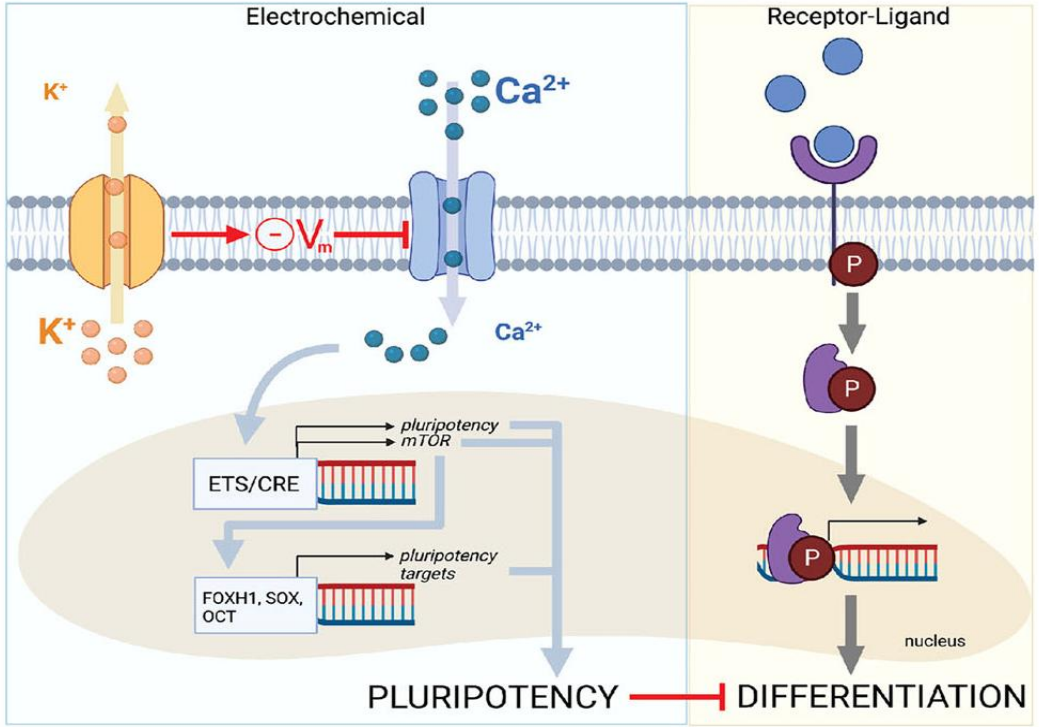
Shape effects matter: simulated average *multicellular potentials* V_m in *different geometries*: elliptical, bean-like, cross, and eye-like (A to H).



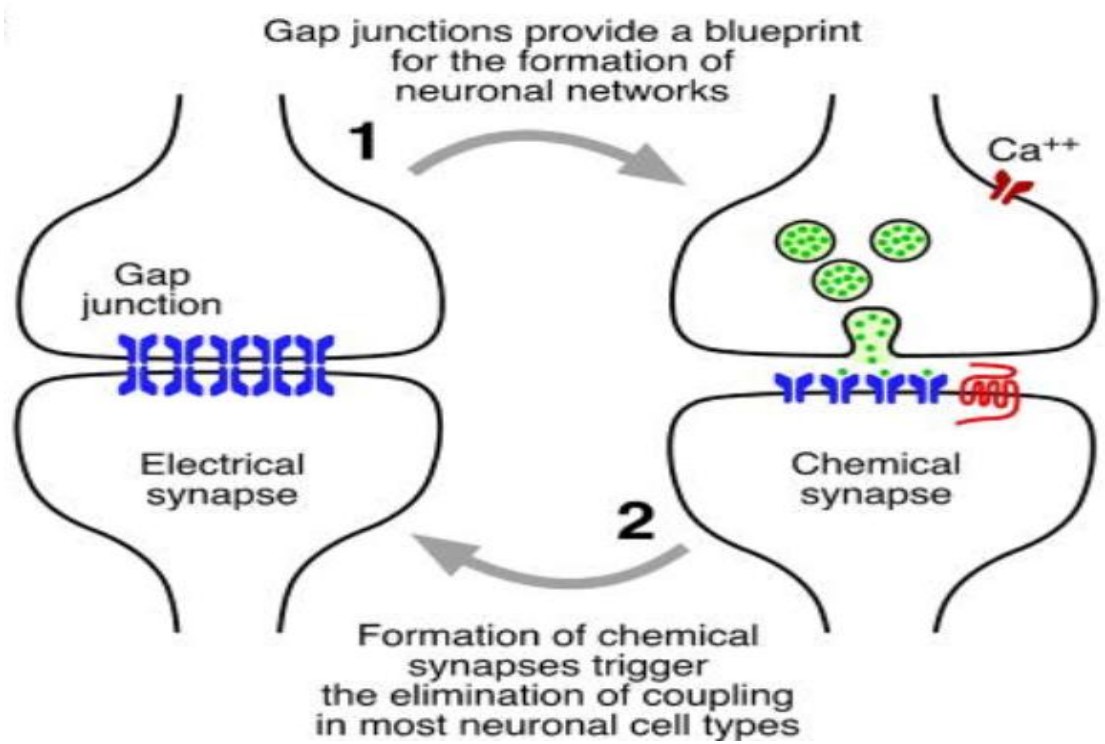
Biological Physics of the Developing Embryo
Gabor Forgacs and Stuart A. Newman. Cambridge University Press, 2005. *Biomechanical* compartment formation and gastrulation are central in *morphogenetic* processes.

bioelectrical and biochemical feedbacks are ubiquitous in development

Nat. Commun. 2022
10.1038/s41467-022-34363-w
Nat Rev Neurosci. 2014
10.1038/nrn3708



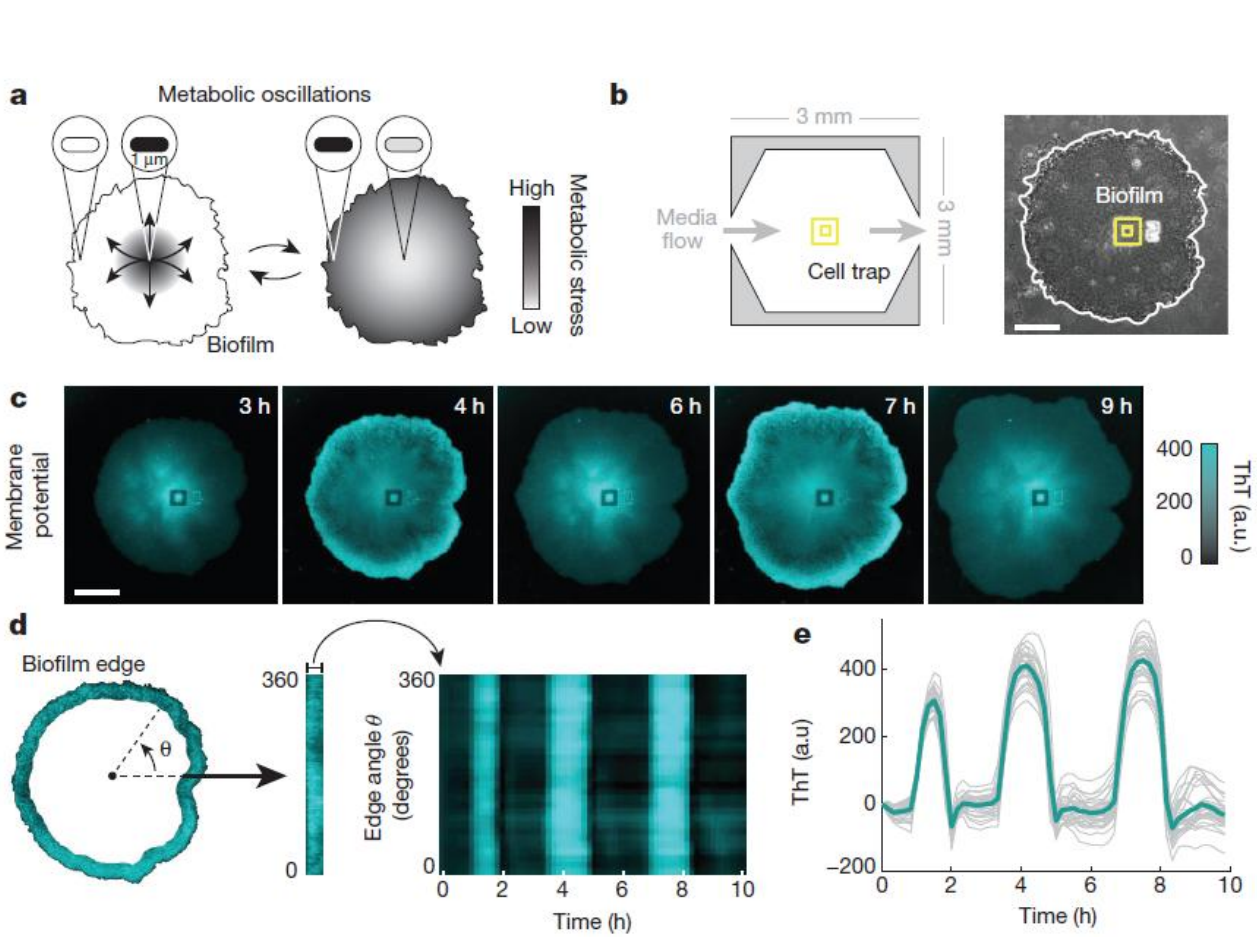
Membrane potential (*left*) and *receptor-ligand* (*right*) *signaling* in the onset of *embryonic differentiation*. While gene expression is also mediated by intracellular signal transducers and biochemical pathways, *bioelectricity* influence also the timing of pluripotency genes.

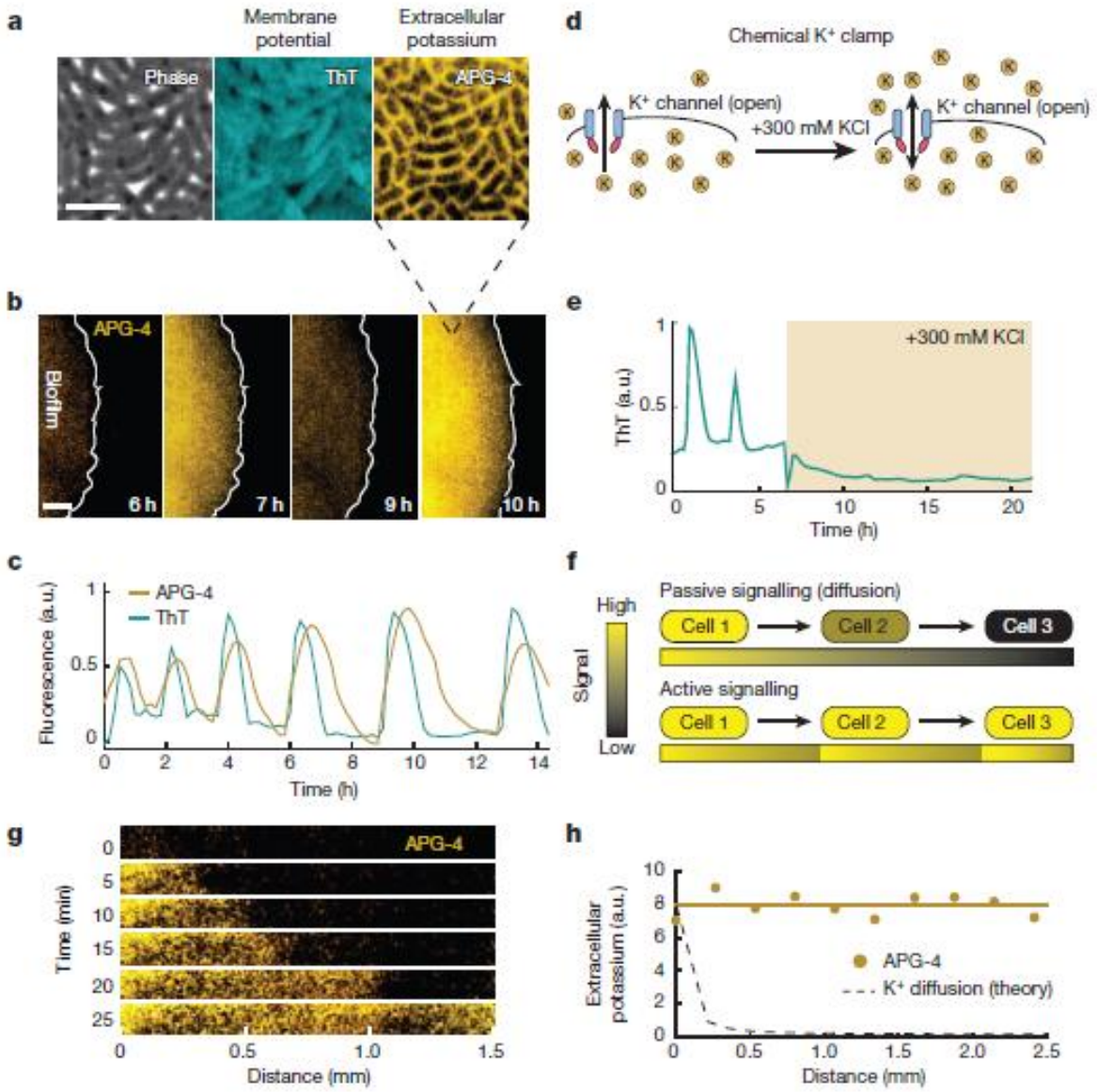


Synaptic transmission is both *chemical* and *electrical.* The interplay between these two forms of interneuronal communication may be required for normal brain *development* and *function.*

bioelectrical and biochemical communication in bacterial communities

Nature 2015
10.1038/nature15709

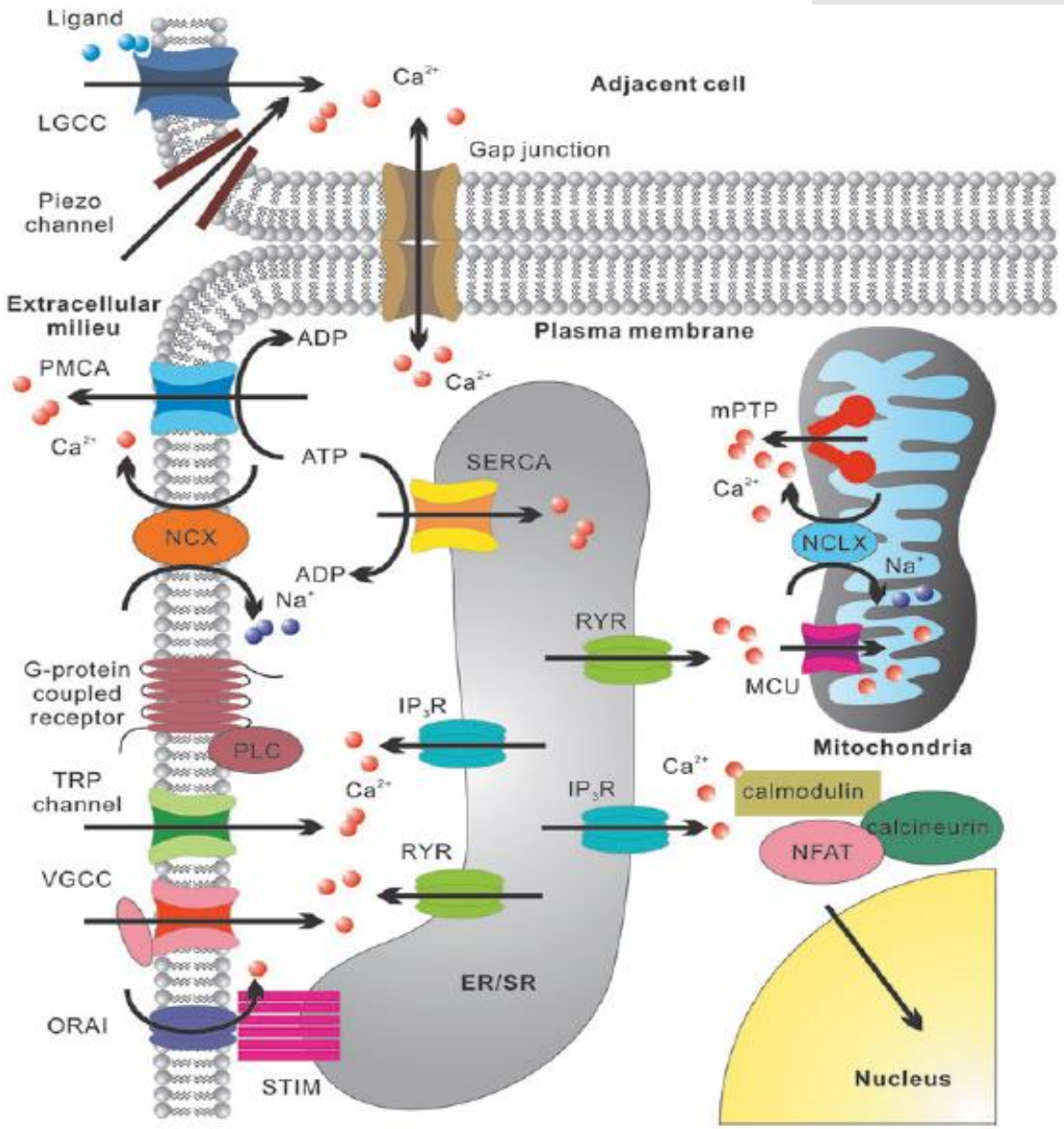




Biofilms produce *synchronized* oscillations in membrane potential. *Ion channels* and *potassium* release are involved in active signal propagation within the biofilm.

Bioelectricity 2020
10.1089/bioe.2020.0001

multitude of ion transporters, not only voltage-gated channels, can establish bioelectrical and biochemical feedbacks



Schematic representation of key regulators of cytosolic Ca^{2+}: *extensions* of the bioelectrical model must be considered in real cases.

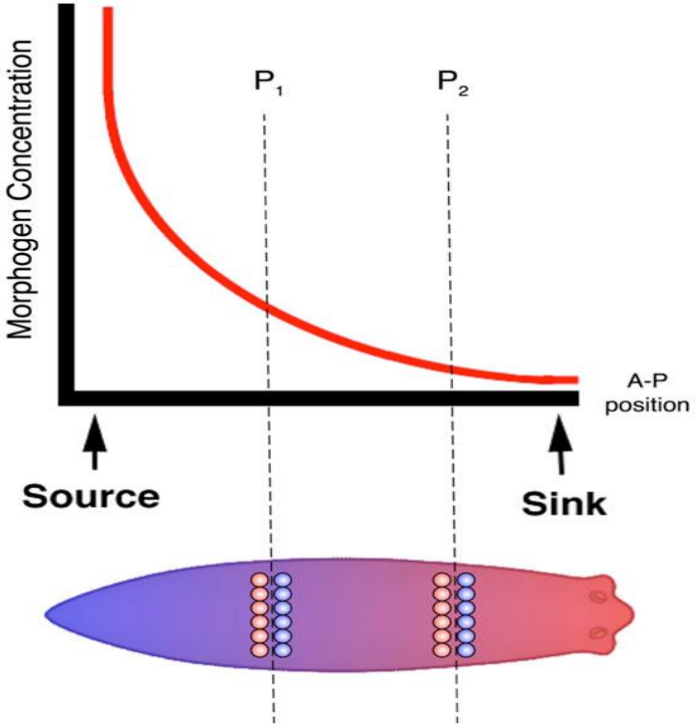
6.b Bioelectrical opportunities

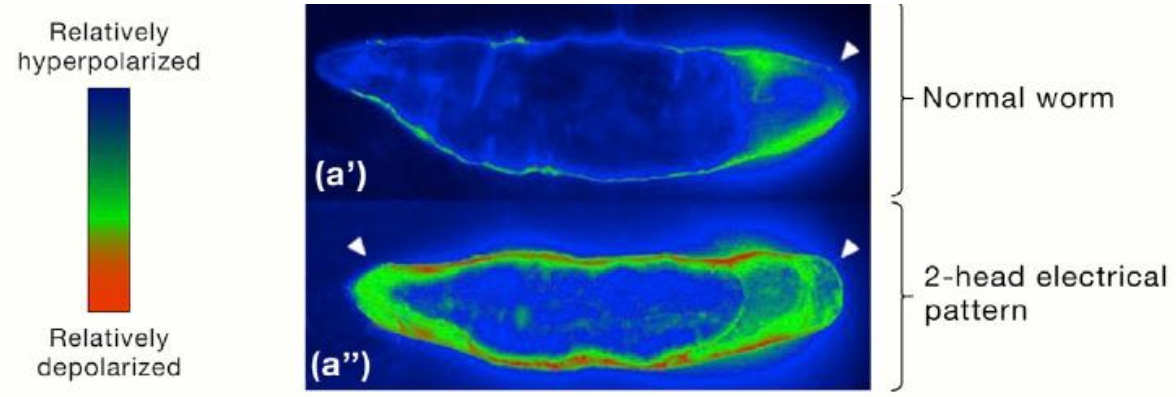
1) The progress from an *individual cell* to *multicellularity* allows the emergence of *average* biophysical magnitudes. They may be regarded as different *levels of abstraction* that influence *development* and *regeneration*, providing an actuation level *complementary* to cell-level management.

2) The differences between *multicellular* regions influence *gene expression*: cells are sensitive to *spatio-temporal patterns* to develop specific programs by *collective* decision. Not only *spatial regions* but also the *time windows* characteristic of the different (developmental or quiescent) *biological stages* can influence the resulting outcomes.

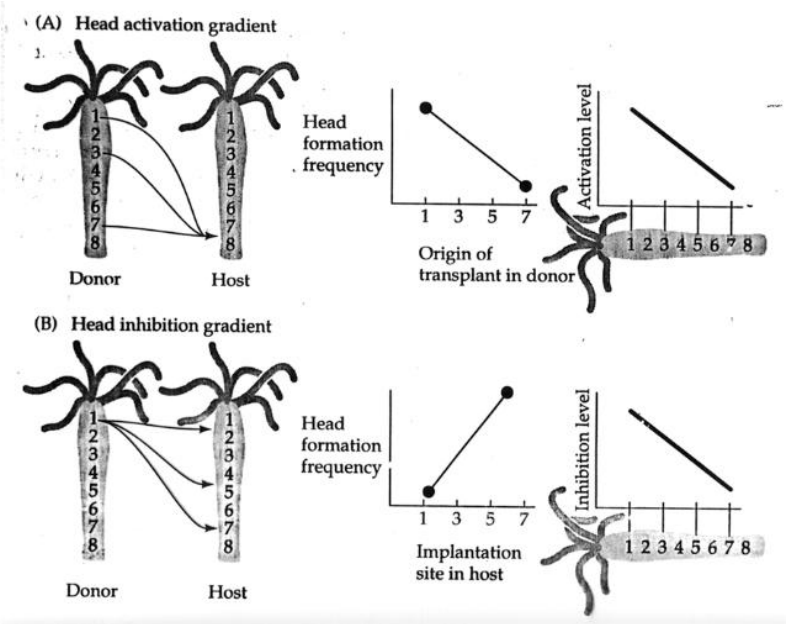
3) A *data-driven bioelectrical view*, complementary to traditional biochemical and biomechanical models, can provide *qualitative insights* into the interplay between bioelectricity and transcription. Here, we attempt to reduce complexity by identifying a small number of observable magnitudes that may control key steps. To this end, a model is proposed to explore *operational actions* based on *average multicellular potentials*.

biochemical (*top*) and *bioelectrical* (*bottom*) views:





two *morphogen gradients* needed: a head activator and a head inhibitor



could a *bioelectrical/biomechanical field* contribute also to polarity?

biofield level strong + (head)

biofield level weak – (no head)

4) *What are the main features of multicellular fields? –a Statistical Physics view*

Phys. Rep. 2023
10.1016/j.physrep.2022.12.003

- basic *units* need *not* be *equal* for a *robust multicellular response*: individual heterogeneity, which allows the *diversity* needed to respond to uncontrollable environmental changes, can be compensated by intercellular *connectivity* and high-level *redundancy* mechanisms;

- *multicellular fields* can manage individual *variability* and *environmental noise*, thus alleviating the requirement of tight regulatory mechanisms at the single-cell level. Also, multicellular *average responses,* described here by *low-dimension* models, demand *small system resources* compared with an exhaustive control of every unit in the whole system;

- it is the *difference* between the *multicellular potentials* that can give *distinct* downstream *gene expression patterns*: cells should be sensitive to spatio-temporal bioelectrical patterns to develop specific programs;

- a rich palette of *transitions* between *instructive multicellular patterns* can be obtained by *local remodeling* of the *intercellular connectivity*; while this characteristic makes the whole system more sensitive to externally-induced *vulnerabilities*, it can also be exploited in controlled *correcting actions* based on networks of multiple units (*cells*); and

- if *morphogenetic processes* are exceedingly *complex to micromanage*, identifying and manipulating instructive set of signals such as *average multicellular fields* may offer new opportunities.

5) In principle, the *bioelectric state* of non-neural tissues may be modified by molecular-genetics, pharmacological, and opto-genetical techniques to *open* and *close* ion channels and gap junctions, thus modulating *instructive patterns*. Hopefully, these options, complementary to actions at the subcellular level, might be developed further in the *future*. Here, we have addressed a limited number of bioelectrical mechanisms, with an emphasis on *modeling*. However, the *bioelectric concepts* introduced immediately suggest particular hypotheses that can be tested by relatively simple *reductionist procedures* in each specific case.

Note that a *significant limitation*, inherent to biosystems, must be taken into account: *specific actions* are *system-dependent* because multiple single-cell and multicellular feedbacks between channels and junctions may polarize or depolarize the cell in a *context-sensitive* way. In addition, *localized* and *time* limited actions must be essential, because ion channels and intercellular junctions control cardiac rhythm, muscle contraction, and neurological functions at *different levels* of biological organization. Thus, a detailed knowledge of the *target* and *anti-target* channels and mechanisms is required to implement any bioelectrical action, with the *evident risk* of interrelated and non-canonical channel functions that can lead to *unexpected adverse events*.

Cell 2021
10.1016/j.cell.2021.02.034
Biochim. Biophys. Acta 2023
10.1016/j.bbagen.2023.130440
Bioelectricity 2022
10.1089/bioe.2022.0014
Annu. Rev. Cell Dev. Biol. 2015
10.1146/annurev-cellbio-100814-125338
Development 2021
10.1242/dev.180794

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