Calcium signaling in tissues at criticality: from mathematical modeling to *in vitro* study

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***Abstract*—Calcium ion (Ca^{2+}) is an essential second messenger for intracellular and intercellular signaling, allowing cells to collectively adapt their cellular functions to their external environment. Many models have been developed to explain Ca^{2+} dynamics in cellular signaling observed *in vitro*. These models often assume a restive low Ca^{2+} level. However, live imaging of Ca^{2+} dynamics in epithelial tissues indicates that Ca^{2+} levels "at rest" are hardly stable, exhibiting non-periodic pulse-like blinking. In this work, through mathematical modeling and live imaging of Ca^{2+} dynamics in the epithelial Madin-Darby canine kidney cells, we investigate whether these Ca^{2+} blinks are caused by a hypersensitive Ca^{2+} signaling system responding to intrinsic biochemical noise. We propose that cells operating near criticality drastically impact how Ca^{2+} signals from localized perturbations are processed, relayed from one cell to another, and contribute to the overall response of the tissue.**

I. Introduction

The calcium ion (Ca^{2+}) is a crucial second messenger that enables cells to adjust their functions in response to environmental signals and communication from neighboring cells (Fig. 1A). Numerous models have been developed to explain Ca^{2+} activity at both the single cell [1] and tissue levels [2], [3]. These models often assume a resting low Ca^{2+} concentration. By modulating the feedback from intracellular calcium stores in response to changes in cytoplasmic Ca^{2+} levels (e.g., with Inositol triphosphate (IP3) or membrane voltage), cells can exhibit behaviors like hysteresis (as seen in muscle tissues) or oscillations (as observed in cardiac cells).

From live Ca^{2+} imaging of Madin-Darby canine kidney (MDCK) epithelial cells [4], we observed a lively Ca^{2+} dynamics, showing non-periodic pulse-like blinking, even when cells are "at rest" in the absence of deliberate stimulation. Ca^{2+} blinks persist even when we negate some of the components responsible for mechanosensitivity (with Piezo1 knockout mutants [4]) and intercellular connections (with gap junction blocker 18aGa) (Fig. 1B). No apparent periodicity or synchrony was detected in these blinking events.

We hypothesize that Ca^{2+} dynamics in this epithelial tissue operates at criticality, e.g., near the phase transition between monostable and oscillation regimes. This allows for hypersensitivity in Ca^{2+} activity in response to small intrinsic biochemical noise. Based on a classic mathematical model of Ca^{2+} dynamics [1], we investigate the conditions for the critical system as well as means to validate this conditions from *in vivo* experiments.

II. Mathematical modeling of Calcium dynamics

Ca^{2+} dynamics is modeled as in Atri *et al.* [1] (schematics as in Fig. 1A). Here, we focus on the effects of fluctuations in

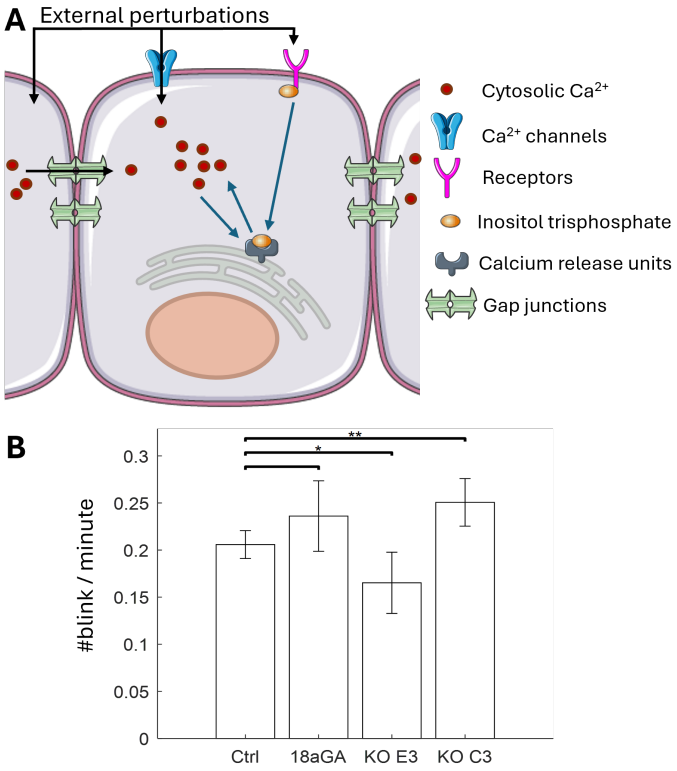

Fig. 1. (A) Schematics of Ca^{2+} dynamics in a single cell responding to external perturbations. (B) Comparison of Ca^{2+} blinking frequency (blinks per minute) when cells are at rest, with gap junction blocker 18aGa, and in Piezo1 knockout strains (KO E3 and KO C3).

the Ca^{2+} influxes $J_\theta(t)$ and the feedback level of the calcium storage $\mu(t)$ on the stability of Ca^{2+} levels at steady-state:

$$dc/dt = \beta + J_\theta\ (t) + J_{pump} + J_{feedback}, \tag{1}$$

in which:

$$J_{pump} = -\gamma\ c/(K_\gamma + c) \tag{2}$$

$$J_{feedback} = \mu(t)\ h\ c/(K_1 + c) \tag{3}$$

$$\tau_h \frac{dh}{dt} = \frac{K_2^2}{K_2^2 + c^2} - h \tag{4}$$

In Eq. 1, β is the basal Ca^{2+} leakage to cell's cytosol from either from cells' or endoplasmic/sarcoplasmic reticulum (ER/SR)'s membrane. $J_\theta(t)$ is the surplus influx of Ca^{2+} through activated calcium channels or from neighbor cells through gap junctions. J_{pump} is the outflux of Ca^{2+} from the cytosol via calcium pumps (e.g. SERCA pumps) (Eq. 2). $J_{feedback}$ is the feedback flux from intracellular calcium

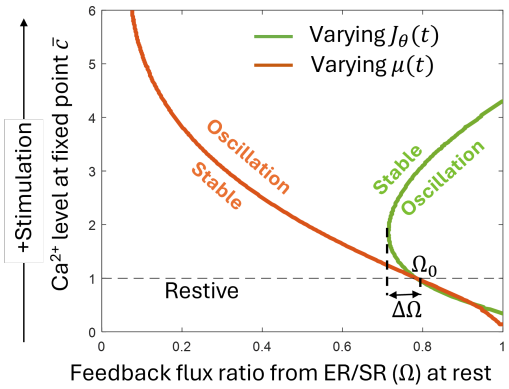

Fig. 2. Stability analysis of Ca^{2+} dynamics: The solid lines correspond to bifurcation line between oscillation and stable regimes under the effect of varying Ca^{2+} influx (green) and varying IP3-controlled feedback level (orange) as a function of the feedback flux ratio at rest Ω.

storage (ER/SR) controlled by calcium-gated IP3 receptors. The degree of feedback $\mu(t)$ can be tuned by varying IP3 concentration (e.g. by adding ATP into the media [5]). $J_{feedback}$ is further controlled by an instantaneous calcium-activation gate and two delayed calcium-inactivation calcium gates (Eq. 3). The fraction of opened calcium-inactivation gate h has a time scale of τ_h (Eq. 4). We assume that the Ca^{2+} concentration at rest c is 1 when $J_\theta(t) = 0$ and $\mu(t) = \mu_0$.

We denote Ω the ratio of feedback influx $J_{feedback}$ over the total Ca^{2+} influx when cells are at rest (c=1):

$$\Omega = \frac{J_{feedback}}{J_{feedback} + \beta} = \frac{J_{feedback}}{J_{pump}}_{c=1} \quad (5)$$

In the absence of any perturbations, naturally the system stability at $c = 1$ depends on the feedback ratio Ω. We investigate the impact of varying the Ca^{2+} influx $J_\theta(t)$ and the IP3-controlled feedback level $\mu(t)$ on Ca^{2+} dynamics. From the stability analysis of Eq.1-4, we find the analytical constraints of the model parameters for the system to be stable at the resulted Ca^{2+} concentration at stead state $\bar{c}$ during perturbations ($J_\theta(t) > 0$ or $\mu(t) \neq \mu_0$). From Fig. 2, we find that by tuning the feedback strength with IP3 $\mu(t)$, phase transition can occur with almost any feedback flux ratio at rest Ω. On the other hand, phase transition with varying Ca^{2+} influx $J_\theta(t)$ is possible only when the system at rest is close to criticality ($\Omega_0 \geq \Omega \geq \Omega_0 - \Delta\Omega$).

III. Probing for *in vitro* phase transition

We test our hypothesis of Ca^{2+} signaling at criticality in our MDCK culture. This is achieved by adding the drug Yoda1 to the media, which opens mechanosensitive channel Piezo1 and introduces additional Ca^{2+} influx to cell's cytosol $J_\theta(t)$, without affecting the IP3 level $\mu(t)$ (Fig. 3A). We find that not only the Ca^{2+} overall activity increases but also Ca^{2+} blinking is greatly enhanced, with 100% of cells exhibiting Ca^{2+} oscillation-like behavior (Fig. 3B and 3C). This supports our hypothesis of Ca^{2+} signaling operating at criticality in MDCK cells, as suggested by our mathematical model. Interestingly, we did not detect any apparent synchrony in the oscillation, suggesting that gap junctions are closed in response to this non-physiologically high Ca^{2+} cytosolic concentration.

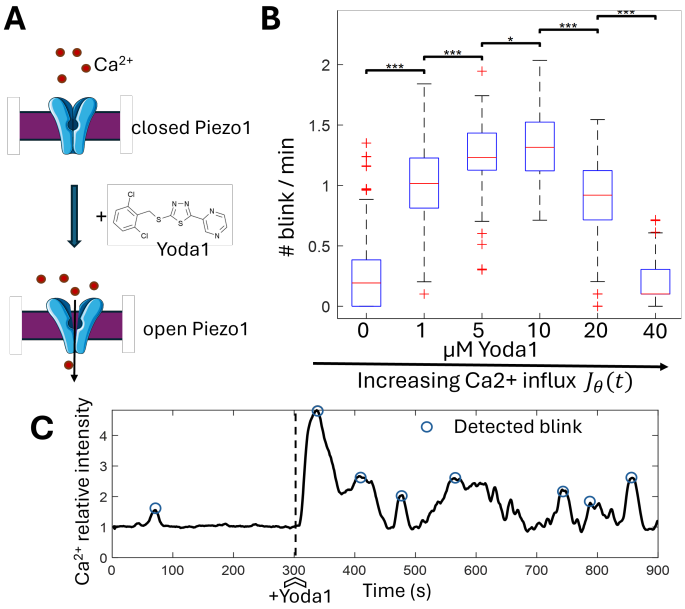

Fig. 3. (A) Yoda1 open Piezo1 channel and introduce Ca^{2+} influx to cell cytosol. (B) Calcium oscillation, indicated by increased Ca^{2+} peaks' frequency, is induced within a specific range of Yoda1 concentration. (C) Example of a Ca^{2+} intensity trace (solid curve) in response to adding Yoda1 at ~300s time.

IV. Conclusions and directions

In epithelial tissues, many cells operate near criticality and exhibit diverse complex behaviors. For example, cells reacting to transient perturbations can exhibit both transient oscillations followed by damped oscillations. Furthermore, critical cells are sensitive to both extrinsic and intrinsic noise. This will have impact at the tissue level where both signal and noise are amplified through the Ca^{2+} signaling cascades, which is not captured with deterministic models [1]–[3]. Thus, a stochastic framework is needed to investigate these impacts and access how distant cells may decode information from about local perturbations from incoming Ca^{2+} signals.

References

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