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SHORT COMMUNICATION

The cell boundary theorem: a simple law of the control of cytosolic calcium concentration

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Abstract Many molecular biological interventions in current use, as well as inheritable disease conditions, modify the intracellular endowment of molecules that bind Ca^{2+} or channels and pumps that transfer it to and from intracellular storage organelles. A simple law, named the "cell boundary theorem," states that intracellular alterations cannot directly result in changes in the cytosolic concentration, $[Ca^{2+}]_i$, in a true resting state. A demonstration of the validity of this theorem is provided. Several examples are then discussed of interventions or diseases that increase leak of Ca^{2+} from storage organelles and result in greater resting $[Ca^{2+}]_i$. According to the theorem, the increase in $[Ca^{2+}]_i$ cannot be a direct consequence of the greater leak. Its primary cause must be a change of the fluxes at the level of the plasmalemma, caused in turn by the increase in leak through some sort of "store-operated Ca^{2+} entry." While the law is discussed in terms of Ca^{2+} homeostasis, it applies to any solute that may be transported by the plasma membrane.

Keywords Ion transport · Homeostasis · Sarco-endoplasmic reticulum · Plasma membrane · Cellular organelles

The stability of the intracellular milieu is protected by the plasma membrane, with its tightly modulated transport properties for ions and other water-soluble components. Some of these solutes have crucial signaling properties, and the cell has provisions to modify their concentrations, at various rates and under various spatial patterns.

The means that the cell applies toward modifying a solute concentration usually include intracellular membrane-delimited organelles of storage or production, plus other molecules, fixed or mobile, that bind the solute in question. While this note will reach conclusions applicable to any solute, it will be presented in reference to the most studied signaling molecule, the ion Ca^{2+}. The main storage organelle for Ca^{2+} is the sarco-endoplasmic reticulum, SR/ER, but other organelles may store it as well. Organelles and plasma membrane have various Ca^{2+} channels, as well as other transport molecules, and there are many molecules, mostly proteins, that are capable of reversibly binding the ion.

This note deals with the relative relevance of the plasma membrane versus the intracellular organelles and ligands in the long term maintenance of the cytosolic concentration of the solute. Its main goal is to state and demonstrate a simple, basic law of control, and then review some observations on muscle cells that appear to be in conflict with this law.

The basic law can be formulated as a theorem as follows:

An intracellular compartment, be it a soluble buffer, a fixed binding site, or an organelle, cannot determine in any direct way the final steady value of free cytosolic calcium concentration, $[Ca^{2+}]_i$. This value must be determined by exchanges with the outside, through the plasmalemma.

This law, which applies to free cytosolic—rather than total—$[Ca^{2+}]$ has been known for a long time. I first heard it from Tullio Pozzan (Univ. Padova) in a lecture in 1991. As early as the year 1984 Allen et al. [1] wrote: "Although sequestration cannot regulate Ca in the steady state, it may

Dedicated to Professor Makoto Endo, on the 40th anniversary of the discovery of CICR.

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be sufficient for the duration of the present experiments." In a rapid survey of the literature, however, I found many papers where this principle is ignored, probably because it appears to be violated in experimental observations, as described below.

A heuristic justification of the theorem is presented first. Every membrane in the cell separates two aqueous media (say a and b) with different concentrations, $[Ca^{2+}]_a$ and $[Ca^{2+}]_b$. Even though these concentrations may be altered by multiple agonists inside the cell, the relationship between the values at which $[Ca^{2+}]_a$ and $[Ca^{2+}]_b$ stabilize must reflect the properties of the intervening membrane. In other words, the steady $[Ca^{2+}]_b$ is a function of $[Ca^{2+}]_a$ and vice versa, functions that are defined by the membrane in-between. These concentrations are variable; if the intervening membrane properties are modified, both concentrations are expected to change. The exception is the extracellular concentration, $[Ca^{2+}]_e$, which is fixed because the extracellular volume is effectively infinite. Therefore the cytosol, a solution separated from the extracellular medium by the plasma membrane, will have its concentration determined by $[Ca^{2+}]_e$, which is fixed, and by the properties of the plasma membrane alone.

The formal demonstration starts from the differential equation that determines cytosolic $[Ca^{2+}]$ and assumes spatial homogeneity (an assumption removed later):

$$\begin{aligned} d[Ca]_i/dt = {} & \text{influx}\left([Ca^{2+}]_e, [Ca^{2+}]_i\right) \\ & - \text{efflux}\left([Ca^{2+}]_e, [Ca^{2+}]_i\right) \\ & - \text{removal}\left([Ca^{2+}]_i, [Ca^{2+}]_{org}\right) \\ & + \text{release}\left([Ca^{2+}]_i, [Ca^{2+}]_{org}\right), \end{aligned} \quad (1)$$

where influx and efflux are unidirectional fluxes across the plasmalemma, while removal and release are fluxes to or from internal organelles and binding sites (as represented in Fig. 1). Concentrations $[Ca^{2+}]_e$, $[Ca^{2+}]_i$, and $[Ca^{2+}]_{org}$ are respectively outside the cell, in cytosol, and inside the organelles. Analogously:

$$\begin{aligned} d[Ca]_{org}/dt = {} & \text{removal}\left([Ca^{2+}]_i, [Ca^{2+}]_{org}\right) \\ & - \text{release}\left([Ca^{2+}]_i, [Ca^{2+}]_{org}\right) \end{aligned} \quad (2)$$

The equations state explicitly that these unidirectional fluxes are a function of the concentration in the compartments of origin and destination only (there is no action at a distance). The theorem will apply regardless of the functional form of the dependences, which can be complex. When $[Ca^{2+}]_i$ reaches steady value the time derivatives are null in Eqs. 1 and 2; then release − removal is zero and can be taken out of the equation. The other terms

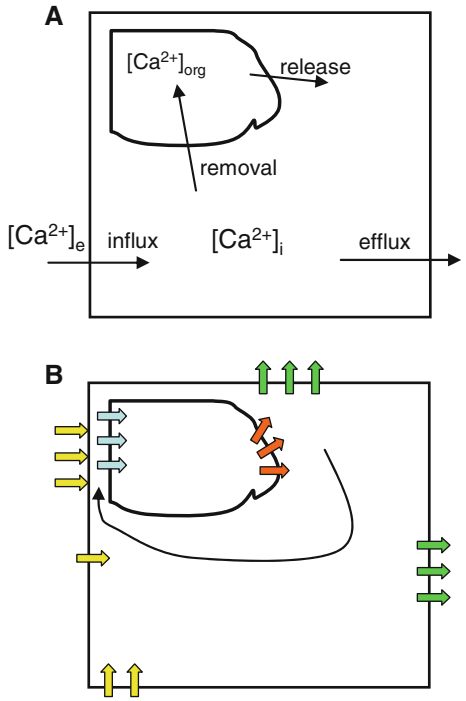

Fig. 1 **a** Definition of unidirectional fluxes, which depend on $[Ca^{2+}]$ in the compartments of origin. **b** Example with spatially inhomogeneous ion concentrations. See text for details

(plasmalemmal fluxes) are the ones to consider. In the final state, $[Ca^{2+}]_i$ is determined by the plasmalemmal fluxes only, through the equality:

$$\text{influx}\left([Ca^{2+}]_e, [Ca^{2+}]_i\right) = \text{efflux}\left([Ca^{2+}]_e, [Ca^{2+}]_i\right), \quad (3)$$

while the organellar/binding site fluxes will separately determine $[Ca^{2+}]_{org}$ through

$$\text{removal}\left([Ca^{2+}]_i, [Ca^{2+}]_{org}\right) = \text{release}\left([Ca^{2+}]_i, [Ca^{2+}]_{org}\right) \quad (4)$$

Obviously, a change in properties of the organelles, say, channels opening in their membrane, will change $[Ca^{2+}]_i$, as prescribed by Eq. 1. However, the change in $[Ca^{2+}]_i$ cannot last forever. Eventually Eq. 3, which only involves membrane properties and the extra- and intracellular concentrations, will be satisfied, and $[Ca^{2+}]_i$ will be determined by it.

Equations 3 and 4 are formally isomorphic; therefore, it might be tempting to conclude that both the plasma and organellar membranes will determine the final balance. This conclusion is wrong because the equations differ in a crucial aspect. As stated above, $[Ca^{2+}]_e$ in Eq. 3 is actually constant because in the usual experimental conditions the extracellular volume is much greater than the volume of the cell. Eventually $[Ca^{2+}]_i$ has to adopt the value prescribed by Eq. 3, regardless of what happens at the level of the organelle.

In conclusion changes exclusively limited to the SR/ER can only change $[Ca^{2+}]_i$ transiently. And it is mathematically true that a change in SR/ER properties cannot alter the steady $[Ca^{2+}]_i$ unless it causes changes at the plasma membrane. In the end, steady changes in $[Ca^{2+}]_i$ can only be determined at the boundary of the cell.

As should be clear from the demonstration, the theorem only applies to the truly steady condition, which is seldom approximated during function in living animals. Instead, the condition is routinely satisfied in the periods of rest that occur in experiments on single cells (as during dissection, mounting, and initial equilibration of a skeletal muscle fiber, the establishment of the "rested" condition in a cardiomyocyte, or the times that follow initial whole-cell patching, when the experimenter waits for internal perfusion of the cell).

The true steady state becomes especially difficult to reach upon severe slowing of plasmalemmal transport, passive and active. In this case the consequences of changes in intracellular membranes or organelles will be especially pronounced and long-lasting. The theorem will eventually apply, but the characteristic times to reach steady concentration values may be much longer.

The theorem is applicable in general to every transported molecule in all tissues. Examples of applications are easily found in the fields of skeletal and cardiac muscle, where inheritable mutations of the SR Ca release channel, RyR, or the main SR Ca buffer, calsequestrin, result in diseases that in turn may course with alterations in $[Ca^{2+}]_i$. As stated initially, the theorem is often ignored when interpreting the pathogenesis of disease or results of experimental manipulations that change the transport properties of storage organelles. In part, this is due to the existence of paradoxical observations, which seem to imply direct control of steady $[Ca^{2+}]_i$ by the SR.

Two examples, which I call "Lopez's paradoxes," stem from an influential series of studies by José López and colleagues (IVIC, Caracas, and Brigham and Women's Hospital, Boston). The first is the observation that the inheritable susceptibility to malignant hyperthermia (MH), linked (in humans and animal models) to mutations in the RyR of skeletal muscle, is accompanied by an increase in $[Ca^{2+}]_i$ [2]. The second is the observation that increases in the abundance of isoform 3 of this Ca^{2+} channel are associated with an increase in $[Ca^{2+}]_i$ in skeletal myotubes [3]. Both the mutated MH channel and the RyR3 isoform are known to be "leakier," i.e., more easily and frequently opened than the WT RyR1 isoform. Both of these changes cause extra leak of Ca^{2+} from the SR of the affected cells, which appears to directly determine the increased $[Ca^{2+}]_i$ in apparent violation of the cell boundary theorem.

An additional example is the recent discovery that a strain of mice engineered to lack calsequestrin 1, which is the main Ca^{2+}-binding protein inside skeletal muscle SR, exhibits both an MH phenotype and increased $[Ca^{2+}]_i$ [4]. Similar to Lopez's paradoxes, it is tempting to interpret the association of the primary defect (absence of a Ca buffer) and an increase in free ion concentration as a simple consequence of a shift of bound to free Ca^{2+}. The theorem indicates that this interpretation is wrong; an indirect mechanism, involving the plasma membrane, should be at work in every case. (Put another way, neither $[Ca^{2+}]_{org}$ nor the organellar fluxes are present in Eq. 3, which determines steady $[Ca^{2+}]_i$, hence, the effect must be secondary to changes in plasmalemmal properties.)

Changes in Ca^{2+} flux at the plasma membrane secondary to changes in the SR are by definition alterations of SOCE (store-operated calcium entry [5]). SOCE must be altered in the examples above. Indeed, a steady depletion of the SR is expected to provide a strong stimulus for activation of the Ca^{2+} entry pathway, which would result, as observed, in steady increase in $[Ca^{2+}]_i$ in the absence of other changes. In general, adjustments at the level of the plasma membrane should be expected whenever a change in ion buffering or storage is found to be associated with steady changes in cytosolic ion concentration. In fact, López and colleagues have now found such adjustments in both MH and models with altered RyR channels (P.D. Allen, personal communication), while J. Ma's laboratory has recently shown that silencing of calsequestrin in skeletal muscle is accompanied by an increase in SOCE [6].

That SOCE must be invoked to explain long term changes in $[Ca^{2+}]_i$ does not necessarily implicate the plasmalemmal channels involving Orai1 and activated by STIM1, which were recently identified within the classic store-operated pathway (e.g. [7]). One alternative that does not require a separate class of channels is illustrated in panel B of Fig. 1. A large clustering of Ca release channels may cause locally intense release ("leak") flux (orange arrows) in the steady situation. If this leak is balanced by removal that is more intense in a subsarcolemmal region (blue arrows), the result may include standing gradients of $[Ca^{2+}]_i$, indicated by the curved arrow, which lead to an increased Ca^{2+} entry near the depleted subsarcolemmal region (yellow arrows). This unspecific form of SOCE still must comply with Eq. 4, although now the balance prevails only in the global sense (i.e., after integration over the entire plasmalemma). In fact, preferential localization of the reporter in regions of low $[Ca^{2+}]_i$ is the likely explanation of other apparent violations of this theorem, including the observation of an unexpectedly low force response to a solution with elevated $[Ca^{2+}]$ in fibers with plasmalemma removed manually [8].

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References

1. Allen DG, Eisner DA, Orchard CH (1984) Characterization of oscillations of intracellular calcium concentration in ferret ventricular muscle. J Physiol 352:113–128
2. López JR, Alamo LA, Jones DE, Papp L, Allen PD, Gergely J, Sréter FA (1986) $[Ca^{2+}]_i$ in muscles of malignant hyperthermia susceptible pigs determined in vivo with Ca^{2+}-selective microelectrodes. Muscle Nerve 9:85–86
3. Pérez CF, López JR, Allen PD (2005) Expression levels of RyR1 and RyR3 control resting free Ca^{2+} in skeletal muscle. Am J Physiol Cell Physiol 288:C640–C649
4. Dainese M, Quarta M, Lyfenko AD, Paolini C, Canato M, Reggiani C, Dirksen RT, Protasi F (2009) Anesthetic- and heat-induced sudden death in calsequestrin-1-knockout mice. FASEB J 23:1710–1720
5. Putney JW (1986) A model for receptor-regulated calcium entry. Cell Calcium 7:1–12
6. Kee Min C, Zhao X, J-K KO, Pan Z, Parness J, Kim DH, Weisleder N, Ma J (2009) Increased store-operated Ca entry in skeletal muscle with knockdown of calsequestrin. Biophys J 96:A115
7. Lewis RS (2007) The molecular choreography of a store-operated calcium channel. Nature 446:284–287
8. Lamb GD, Cellini MA (1999) High intracellular $[Ca^{2+}]$ alters sarcoplasmic reticulum function in skinned skeletal muscle fibres of the rat. J Physiol 519:815–827