

PERSPECTIVES

Calcium Wave Propagation Underlying Intercellular Signaling and Coordination of Tissue Responses

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A Perspective on “Calcium Signaling in the Photodamaged Skin: In Vivo Experiments and Mathematical Modeling”

Changes in cytosolic Ca^{2+} concentration ($[\text{Ca}^{2+}]_c$) serve as an almost ubiquitous signal in organisms across the four kingdoms of *Eukaryota*, and perhaps in some prokaryotes of *Monera* as well. Much of our understanding of Ca^{2+} signaling has come from studies using isolated cell preparations, refined with studies at the single cell and subcellular level. But studies with isolated cells typically obviate the importance of cell placement and structural organization in higher organisms, where Ca^{2+} signals are often coordinated between cells and even across entire tissues. There are a number of ways in which multicellular systems can respond to an external stimulus with coordinated Ca^{2+} signaling. At its simplest, that stimulus can itself directly impinge on numerous cells simultaneously, as occurs in endocrine regulation. But in many cases a limited number of cells, or even a single cell, may be responsible for the initial detection of an extracellular signal, which is then conveyed to neighboring cells by any one of a number of intercellular Ca^{2+} signaling processes.

In a study by Donati and coworkers published in the previous issue of *Function*,¹ the authors investigated the mechanism by which photodamage of a single keratinocyte results in a radiating wave of $[\text{Ca}^{2+}]_c$ increase in the epidermis of live anesthetized mice. Their study combined direct measurements of $[\text{Ca}^{2+}]_c$ by imaging at the earlobe skin surface with multiphoton microscopy of GCaMP6s-expressing mice. This approach allowed them to follow the three-dimensional distribution of $[\text{Ca}^{2+}]_c$ changes in the epidermis following the laser-induced damage of a single cell. The initial stimulus resulted in a rapidly spreading wave of $[\text{Ca}^{2+}]_c$ increase in the surrounding keratinocytes that progressively engaged adjacent cells to a radius

of about 70 μm in the plane of the epidermis (~100 cells) over a period of 10–20 s. This intercellular Ca^{2+} wave had the property of decaying in $[\text{Ca}^{2+}]_c$ amplitude with distance from the origin, and slowing in velocity from an initial peak rate of 25–30 $\mu\text{m}/\text{s}$, effectively dying out rather than abruptly terminating. Despite the rapid and spatially limited spread of the initial Ca^{2+} wave, the elevated $[\text{Ca}^{2+}]_c$ in the affected cells declined only slowly over tens of minutes. Injection of various pharmacological agents was used to elucidate the components underlying these Ca^{2+} waves. These studies demonstrated a primary role for ATP release from the initiating cell, which acts at P2Y purinergic receptors on adjacent cells to elicit the mobilization of intracellular Ca^{2+} stores. But ATP diffusion from the damaged cell is not the whole story, and as in many multicellular systems there is also a role for connexin channels in propagating the Ca^{2+} waves.

Mathematical modeling is an important tool in understanding the mechanisms underlying $[\text{Ca}^{2+}]_c$ dynamics, and this is especially so for intact tissue studies, where direct experimental manipulations are more limited. Donati and coworkers¹ used a multicellular two-dimensional model, incorporating extracellular ATP diffusion, formation of inositol 1,4,5-trisphosphate (IP_3) in response to ATP-activation of P2Y receptors, and intracellular Ca^{2+} mobilization by IP_3 and $[\text{Ca}^{2+}]_c$. Their model also allows for Ca^{2+} -induced ATP release through connexin gap junction hemichannels in the responsive bystander cells. The combination of experimental studies and mathematical modeling provided evidence that the propagation of intercellular $[\text{Ca}^{2+}]_c$ waves in keratinocytes in vivo following laser photodamage is predominantly due to radial diffusion of the ATP released directly by damage to the hit cell, but they also indicated a role for secondary Ca^{2+} -induced ATP release through hemichannels in distal cells. Nevertheless, the regenerative capacity of this system is insufficient to sustain the Ca^{2+} waves beyond about the eighth order of surrounding cells, which appears to reflect

Submitted: 28 February 2022; Accepted: 28 February 2022

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insufficient purinergic receptor-driven IP₃ formation at this displacement from the origin.

While Ca²⁺ signals can be propagated between cells by a number of mechanisms, including synaptic transmission and direct electrical coupling, the most common mechanisms in nonexcitable cells involve direct propagation of intracellular signals through gap junctions, or extracellular propagation by release of paracrine agonists.² In non-excitable cells, where the latter two mechanisms predominate, propagation distance is largely determined by the ability to regenerate and sustain the initial [Ca²⁺]_c increase.

Many studies have shown intracellular Ca²⁺ signals can propagate as intercellular Ca²⁺ waves, but these are typically with cell monolayers in culture, or sometimes partially dissociated small clumps of cells that retain only some of their original structure. Relatively few studies have investigated these Ca²⁺ waves in intact tissue preparations, where the individual cell geometry, multicellular architecture and the restricted extracellular milieu are all maintained. Amongst the many relevant components, the subcellular location of connexin channels and their relative distribution between functional gap junction structures and plasma membrane hemichannels is a key determinant of intercellular Ca²⁺ signaling. In addition to the Donati work in skin keratinocytes,¹ this can be seen in a number of intact tissue studies, including Mike Berridge's favorite, the blow fly salivary gland,³ our favorite organ preparation, the intact perfused liver,^{4,5} airway and blood vessels of the lung,^{6,7} astrocyte networks in the brain,^{2,8} and returning to the work of Mammano and coworkers, the cochlea.⁹

What determines how far intercellular Ca²⁺ waves will spread, and what leads to their termination? The key to robust long-distance propagation is regeneration of the initiating stimulus by the increase of [Ca²⁺]_c in successive cells. In the skin keratinocyte system regeneration is relatively weak, with only a minor contribution from secondary ATP release via Ca²⁺-activated hemichannels in the propagating cells. However, in other multicellular systems longer distance Ca²⁺ waves can be sustained by more robust paracrine ATP signaling. These may be driven by Ca²⁺-activated plasma membrane channels (eg. connexin hemichannels and pannexin channels) or by Ca²⁺-stimulated vesicular ATP release. Of course, Ca²⁺-stimulated vesicular secretion is not limited to purinergic signals, but can also include Ca²⁺-induced secretion of other paracrine agonists that act to elevate [Ca²⁺]_c and hence sustain a propagating Ca²⁺ wave. Paracrine-dependent Ca²⁺ waves can be limited by the extracellular milieu, including dilution and degradation of the paracrine signal, diffusional barriers and fluid flow.

Intercellular signal propagation through functional gap junctions is shielded from most extracellular constraints, and thus has the capacity to generate longer distance Ca²⁺ waves. Nevertheless, a regenerative mechanism is still required. Ca²⁺ itself is a poor intercellular propagating signal because of its limited intracellular diffusion. Large IP₃-dependent [Ca²⁺]_c increases in an initiating cell can spread to surrounding cells by IP₃ diffusion through gap junctions, but without IP₃ regeneration these tend to be of limited distance. Although IP₃ can be regenerated in a Ca²⁺-dependent manner, stimulation with a phospholipase C-activating agonist is usually required to achieve robust Ca²⁺-induced IP₃ formation. Thus, systems that rely on gap junction communication to propagate IP₃-dependent intercellular Ca²⁺ waves typically also require subthreshold systemic stimulation of the tissue. Taking the liver as an example, local norepinephrine release from sympathetic nerve terminals leads to [Ca²⁺]_c increases in only a small number of periportal

hepatocytes, but these propagate to the entire liver lobule (thousands of cells) when the liver is perfused with a subthreshold IP₃-linked hormone.⁵ This provides a mechanism to generate a tissue-wide activation of hepatic glucose metabolism from a local neuroendocrine stimulation. Referring to another recent paper in *Function*,¹⁰ one of the acute effects of ethanol on the liver is to disrupt the coordination of lobular Ca²⁺ signaling by interfering with gap junction permeability. In a broader context, alterations in signaling through gap junctions and connexin hemichannels are involved in a wide array of pathologies, and are an important target for potential therapeutics.

In conclusion, intercellular Ca²⁺ waves provide a means to amplify and coordinate tissue responses to a focal external stimulus, and the scale of that response is a function of the underlying mechanisms of Ca²⁺ signal propagation and regeneration.

Funding

The authors acknowledge funding from the Thomas P. Infusino Endowment and NIH grants R01DK078019, R21DK082954, and R01AI099277.

Conflict of Interest Statement

A.P.T. holds the position of Editorial Board Member for *FUNCTION* and is blinded from reviewing or making decisions for the manuscript. A.P.T. and J.C.V. declare no competing interests.

Data Availability Statement

This paper does not report original data. Any additional information regarding the previously published data cited in this paper is available from the lead contact upon request.

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