SPOTLIGHT

Calling long distance: Cell cycle–dependent Ca²⁺ flows connect stem cells across regeneration tissues

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How adult stem cells signal in vivo over time to coordinate their fate and behavior across self-renewing tissues remains a challenging question. In this issue, Moore et al. (2023. J. Cell Biol. https://doi.org/10.1083/jcb.202302095) combine high-resolution live imaging in mice with machine learning tools to reveal temporally regulated tissue-scale patterns of Ca²⁺ signaling orchestrated by cycling basal stem cells of the skin epidermis.

In self-renewing tissues, the maintenance of functional tissue architecture, known as homeostasis, demands that stem cells communicate with their local neighbors and with cells positioned far away to coordinate the replacement of lost cells. Over the years, we have gained tremendous insight into how adult stem cells and their niches communicate locally to direct stem cell behavior. Yet, understanding how local signaling dynamics integrate into a tissue-wide information network to align large-scale cell behavior in regenerating organs has remained a formidable challenge in mammals. This challenge stems from the complexities in both monitoring and analyzing the signal dynamics from thousands of cells heterogenous in fate and behavior in an uninjured animal at high enough spatial and temporal resolution.

Moore et al. (1) took on both of these challenges (Fig. 1 A). Further advancing their intravital imaging setup previously used to show how the local neighborhood coordinates adult stem cell behavior in the epidermis (2), the authors now capture thousands of signals at single-cell resolution every 2 s, covering a large area of the stem cell compartment. At the same time, they developed a novel unsupervised machine learning tool called geometric scattering trajectory homology (GSTH) to analyze the generated complex intravital imaging

datasets (3). GSTH employs PHATE, a method developed previously by the Krishnaswamy lab, to visualize both local and global structure and patterns within high-dimensional data sets (4). In brief, GSTH applications visualize complex imaging datasets to show how thousands of individual cells signal and, through PHATE time trajectories, whether individual cells are connected in space and time also over large distances (Fig. 1 B). GSTH thus allows the authors to assess whether different settings promote or disturb signal dynamics and signal coordination.

Moore et al. (1) chose Ca²⁺ signaling because this pathway regulates diverse cellular processes that are central for (adult) stem cell function (5, 6). Moreover, sensitive genetic fluorescent sensors, such as GCaMP6s, allow for the imaging of cellular Ca²⁺ signaling dynamics that are well characterized in vivo, e.g., in mammalian neurons (7) and *Drosophila* epithelia (5). To ask how adult stem cells communicate in a regenerating setting, the authors focused on the stem cell compartment of the skin epidermis, the basal layer that also harbors progenitors already committed to differentiation (8).

Initial characterization of in vivo epidermis expressing the GCaMP6s sensor revealed that individual cells comprising both stem cells and committed progenitors

display highly variable Ca²⁺ signal dynamics, and signaling was not spatially restricted to certain domains within the basal layer. This dynamic Ca²⁺ signaling is a unique emerging property of the basal layer as no signal events were observed in the suprabasal compartment within the 30-min time frame. Single cells spiked either in isolation or in clusters with connected Ca²⁺ signals as defined by spikes occurring within 10 s between two neighbors. The majority of these local signaling neighborhoods consisted of 2–10 cells with sporadical waves that spread across hundreds of cells. The larger neighborhoods also showed more persistent single signal events, suggesting that long distance propagation of waves require more robust signals.

This initial analysis indicated that Ca²⁺-signaling cells are connected locally with a few signal-connected neighborhoods spanning hundreds of cells. Do these signaling neighborhoods then function as independent units or are these integrated into larger networks over time across much larger tissue areas? To answer this question, the authors developed GSTH as described above. The resulting PHATE trajectories revealed that cellular Ca²⁺ signaling is consistently connected spatially and over time, as opposed to some other cell types, such as neurons, which are scattered using existing data, and which, under resting conditions,

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A   Live imaging uninjured mouse

B   GSTH: unsupervised machine learning

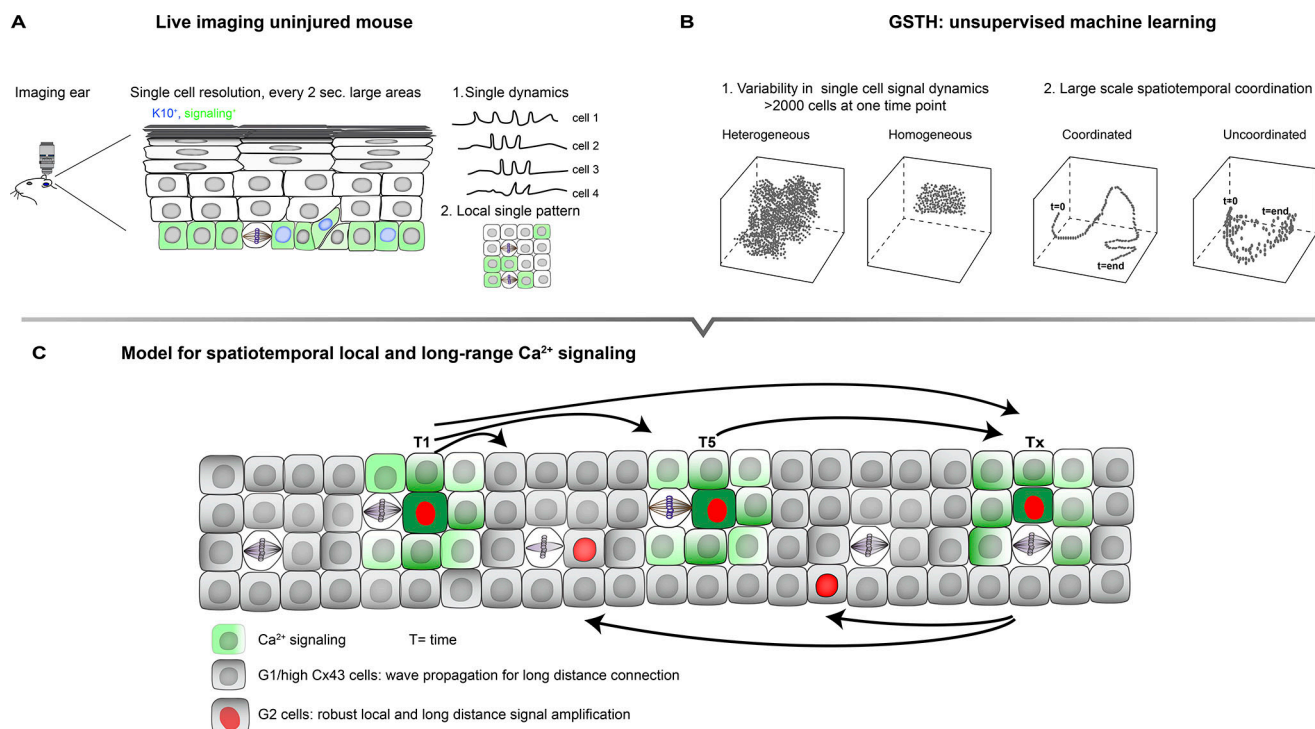**C**   Model for spatiotemporal local and long-range Ca²⁺ signaling

Figure 1. **Cell cycle–controlled short- and long-range Ca²⁺ signal dynamics coordinates stem cell cycle in the epidermis. (A and B)** Moore et al. ([1]) combined high resolution intravital imaging of large areas of the basal epidermis (A) with new unsupervised machine learning called GSTH (B) to assess single cell signaling dynamics of thousands of cells and local and large distance spatiotemporal coordination of signals to monitor Ca²⁺ signaling. **(C)** Model for spatiotemporal coordination of Ca²⁺ signaling across the basal stem cell compartment of the epidermis. Cells in G2 are essential to amplify signals within local signaling neighborhoods to control and reinforce robust long-distance communication, whereas G1 cells, through high expression of Cx43, are essential to propagate signals between local neighborhoods.

are known to spontaneously fire without any topographical or temporal organization. The epidermal basal stem cell compartment thus displays tissue-wide coordination of Ca²⁺ signaling.

Is the cellular state important for this coordination? Basal stem cells exhibit heterogeneity in the cell cycle stage ([9]), which can control cell fate ([10]). The authors found that although both G1/S or G2 propagate Ca²⁺ signals, GSTH revealed that cells in G2 are much more homogenous in signaling dynamics than G1/S cells. Is this difference in cell signal dynamics relevant for the spatiotemporal propagation of local and long-distance signals? Genetic or chemical depletion of G2 cells did not affect the size of the Ca²⁺ signal local neighborhood but strongly impaired the number of events and signal strength, resulting in temporally and spatially uncoordinated signals. Thus, cells in the G2 phase of the cell cycle are essential to initiate robust coordinated long-distance calcium signaling information flows within regenerating tissues. What was most surprising is that an increase in G2 cells did not

seem to affect the Ca²⁺ circuit (e.g., by increasing the number of local neighborhoods that signal at the same time). This result suggests that G2 cells may not initiate Ca²⁺ signals but instead act as signal amplifiers essential to coordinate the propagation of signals.

What then are the molecular mediators that enable the spread of Ca²⁺ signals? Obvious candidates are connexins, proteins that form intercellular channels known as gap junctions (GJs), to exchange small molecules and second messengers (including Ca²⁺) between cells ([11]). Interestingly, Connexin 43 (Cx43)-containing GJs were most prominent in G1 cells and almost absent in G2 cells, suggesting that G2 cells communicate Ca²⁺ through other molecular players. Inactivating Cx43 in the epidermis revealed that Cx43 dampens Ca²⁺-signal intensity and duration, especially in local small signal neighborhoods. Moreover, Cx43 is essential to integrate these local signaling clusters into a tissue-wide temporally connected network. Interestingly, human Cx43 mutations linked to epidermal disease show increased GJ channel activity ([11]). Together,

these results imply that amplification of the long distance Ca²⁺-signaling network may contribute to skin disease.

Finally, the authors explored the functional relevance of the temporally regulated Ca²⁺ signaling network. Manipulating Ca²⁺ signals either chemically or optogenetically changed the number of cycling cells, suggesting a positive feedback loop between cell cycle phase-directed long-distance communication of Ca²⁺ signals and large-scale coordinated stem cell proliferation.

Together, the impressive collection of data presented by Moore et al. ([1]) indicate that cells in different stages of the cell cycle cooperate to coordinate large-scale signaling waves in space and time to direct stem cell behavior. Their data suggest a model ([Fig. 1 C]) in which G2 cells function as amplifiers essential to create robust signals in local neighborhoods, which then, through Cx43-mediated GJ-containing G1 cells that are the most abundant basal population, spread locally and across long distances, perhaps through the very large signaling neighborhoods. G2 cells in the next neighborhood are

then necessary to amplify and reinforce signals again to sustain long distance signaling over larger time periods. In the future, it will be important to examine how different signaling networks integrate over space and time and to explore the physiological relevance of such signaling networks in tissue-wide coordination of stem cell behavior during healthy and pathological regeneration.

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