REVIEW / SYNTHÈSE

Flickering calcium microdomains signal turning of migrating cells[1]

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Abstract: It has been well-established that polarized migrating cells exhibit a stable and transient gradient of intracellular calcium concentration ($[Ca^{2+}]_i$), increasing from front-to-rear, that is thought to be responsible for rear retraction. The paradox that arises is how calcium at the front of a cell catalyzes critical high-threshold calcium-dependent processes during cell migration and particularly in decision-making for a cell to turn. In this brief review, we discuss the recent discovery of flickering high-$[Ca^{2+}]_i$ microdomains ("calcium flickers") at the front of migrating fibroblasts and their common role in transducing local membrane mechanical stress (via TRPM7, a stretch-activated calcium-permeating transient receptor potential channel) and chemoattractant-elicited signals (via type 2 inositol 1,4,5-trisphosphate receptor in the endoplasmic reticulum). Furthermore, we present a new model for patterned calcium flicker activity as the mechanism for steering the turning of a migrating cell.

Key words: calcium microdomains, calcium flickers, cell migration, TRPM7, inositol-1,4,5-trisphosphate receptor, confocal microscopy.

Résumé : Il est clairement établi que les cellules migrantes polarisées présentent des gradients de concentrations de calcium intracellulaire ($[Ca^{2+}]_i$ stables et transitoires, lesquels seraient responsables de la rétraction de l'arrière des cellules. Le paradoxe soulevé est lié à la manière dont le calcium situé à l'avant de la cellule catalyse les processus dépendants de fortes concentrations de calcium durant la migration cellulaire et, particulièrement, durant le changement de direction de la cellule. Dans cette brève synthèse, nous discutons de la récente découverte de microdomaines à forte concentration en calcium ($[Ca^{2+}]_i$ (« calcium flickers ») à l'avant des fibroblastes migrants, et de leur rôle en tant que transducteur de la tension mécanique à l'intérieur de la membrane (par le biais du TRPM7, un canal TRP perméable au calcium activé par l'étirement) et des signaux déclenchés par les substances chimiotactiques (par l'intermédiaire du récepteur de l'inositol-1,4,5-trisphosphate de type 2 dans le réticulum endoplasmique). De plus, nous présentons un nouveau modèle d'activité des microdomaines de calcium pour guider le mouvement directionnel d'une cellule migrante.

Mots-clés : microdomaines de calcium, microdomaines à forte concentration en calcium, migration cellulaire, TRPM7, récepteur de l'inositol-1,4,5-trisphosphate, microscopie confocale.

[Traduit par la Rédaction]

Introduction

Directional cell movement at the guidance of environmental cues is fundamental to embryogenesis, organogenesis, and wound healing, as well as the development of atherosclerosis and the metastasis of tumors. The movement involves spatially and temporally coordinated intracellular transduction of chemoattractant signals, traction force generation, dynamic cytoskeletal reorganization, protrusion at the front of the cell, and retraction at the rear. Many calcium signaling proteins, such as calpain, protein kinase C (PKC), and calcium/calmodulin-dependent kinase II, are integrated into intricate signaling cascades (Huttenlocher et al. 1997; Clark and Brugge 1995; Bilato et al. 1997) and play pivotal roles in chemotaxis (Van Haastert and Devreotes 2004) as well as in pathfinding of neuronal processes during the formation and remodeling of neural networks (Bovolenta et al. 2006; Henley and Poo 2004).

In 1991, Fay and colleagues demonstrated a surprisingly stable "front-to-rear" gradient of increasing intracellular calcium concentration ($[Ca^{2+}]_i$) in polarized migrating eosinophils (Brundage et al. 1991), despite the fact that calcium ions are diffusible in the cytosol. Later, $[Ca^{2+}]_i$ transients were also found to arise from the rear of migrating epithelial keratocytes (Lee et al. 1999). The higher $[Ca^{2+}]_i$ at the rear

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Fig. 1. Calcium flickers in a polarized migrating human embryonic lung fibroblast (WI-38 cell). Data are the summation of flickering high-calcium signals (ΣΔF) over 180 s. Enlarged views show individual calcium flickers inside the forefront ruffles. N denotes the nuclear region. Scale bars: upper panel and inset 15 μm, lower panel 5 μm; color bars (in color in the Web version) are shown in arbitrary units (a.u.). (Modified from Wei et al. 2009.)

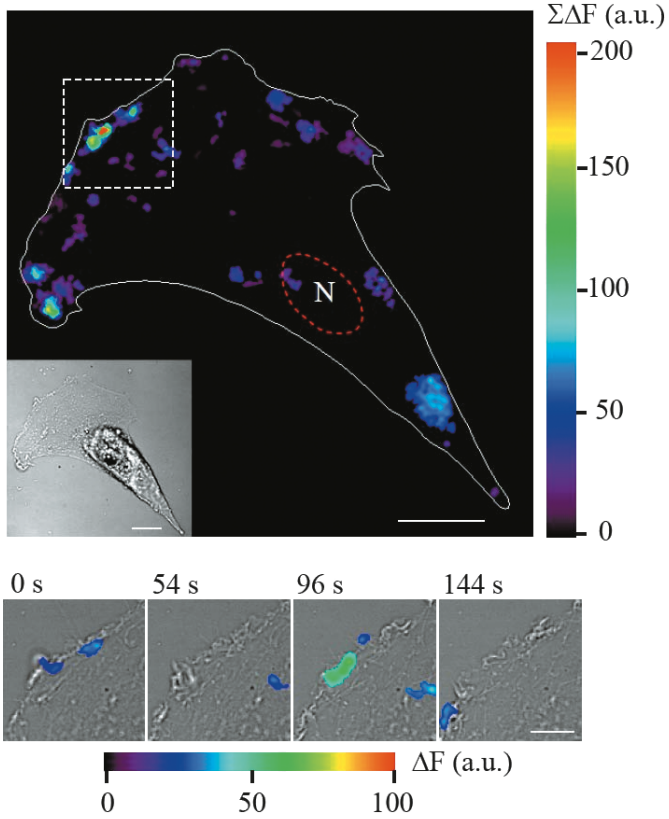
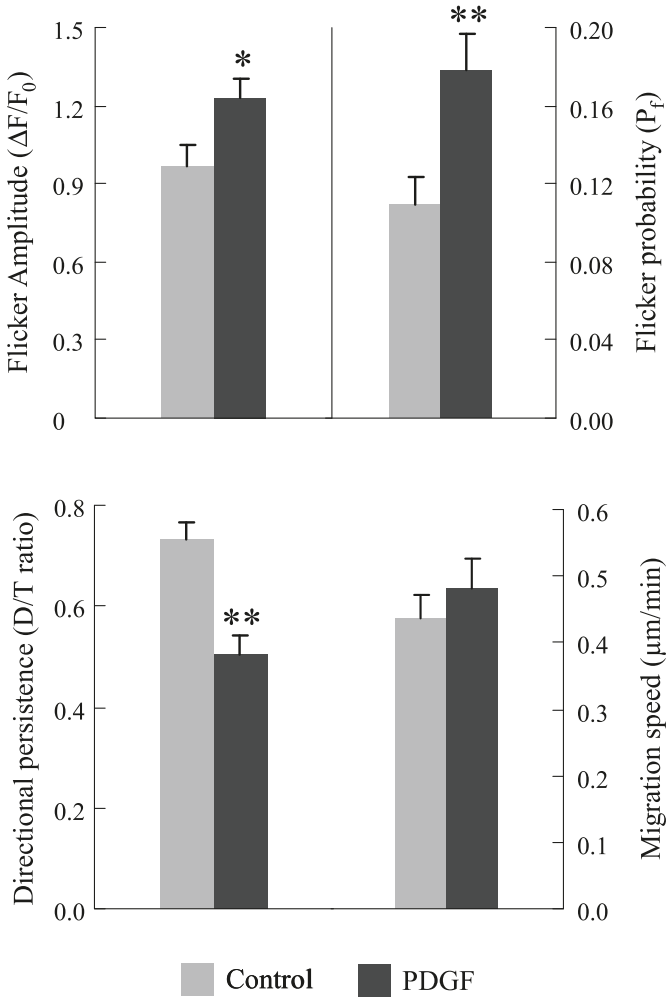


Fig. 2. Effects of uniform PDGF chemoattractant stimulation on characteristics of calcium flickers and directional persistence of migration. Flicker amplitude and probability (P_f) shown in the top graph were calculated from line-scan images (control, $n = 12$; PDGF, $n = 11$). In the graph below, two parameters, migration speed and directional persistence, defined as the distance traveled divided by the number of turns (D/T ratio), were analyzed from 30-minute time-lapse images (control, $n = 27$; PDGF, $n = 20$). Error bars represent SE. *, Significant at $p < 0.05$ and **, $p < 0.01$ vs. control.



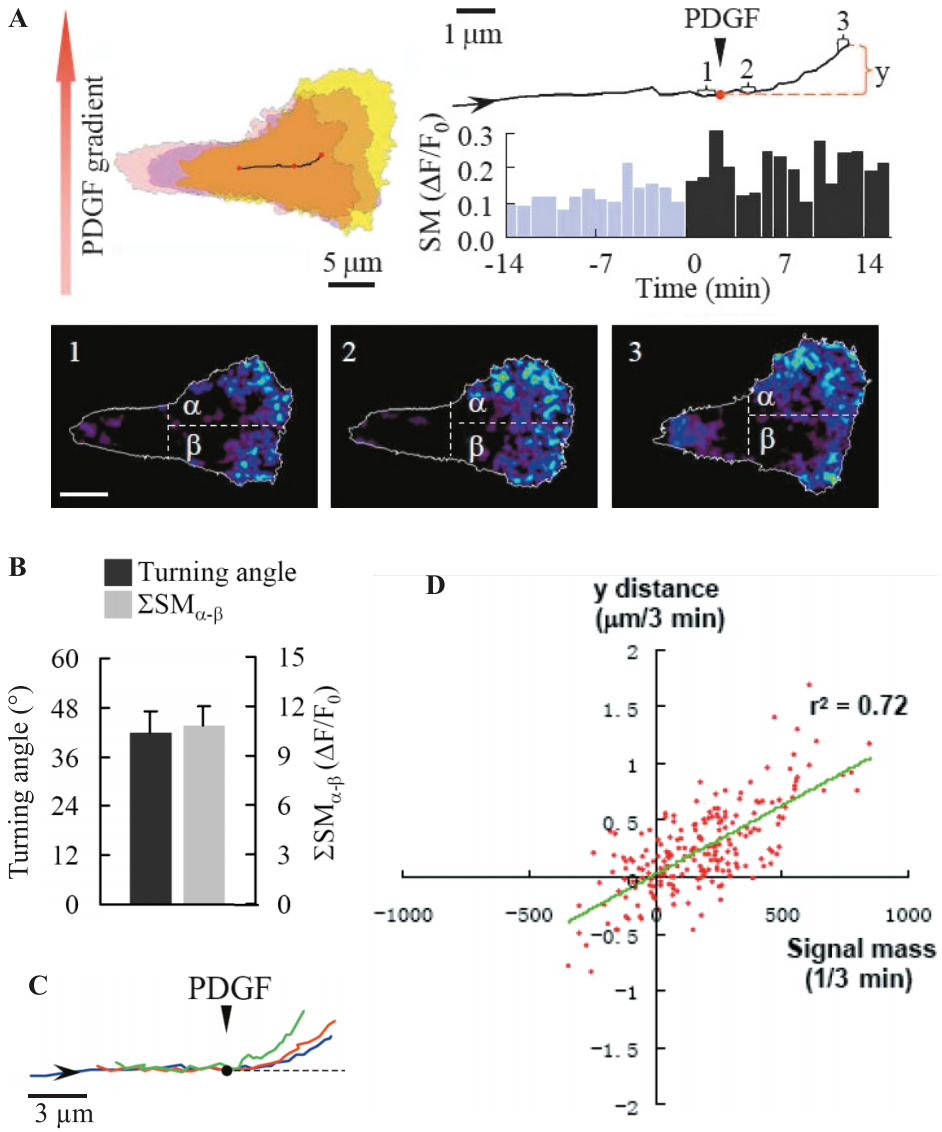
has been thought to be responsible for myosin II contraction and calpain cleavage, resulting in rear detachment and retraction (Ridley et al. 2003). Until most recently (Wei et al. 2009), it remained elusive what is the calcium signal that activates high-threshold calcium-dependent cellular processes (events with a dissociation constant or EC_{50} in the supra-micromolar range) at the front. This signal is of particular importance in decision-making for turning at chemoattractant cues.

Flickering high-calcium microdomains at the front

By using high-resolution confocal calcium imaging, we have visualized dynamic high-$[Ca^{2+}]_i$ microdomains, named calcium flickers, occurring randomly in migrating human embryonic lung fibroblasts (WI-38 cells) and other types of fibroblasts (Wei et al. 2009). Individual calcium flickers display a 1.2-fold local increase of fluo-4 fluorescence, last from 20 ms up to 2000 ms, and spread over a diameter of 5 μm (Fig. 1). This observation immediately suggests that such calcium flickers may represent the long-sought missing calcium signal responsible for the local calcium-dependent events at the front.

There are several lines of evidence substantiating the above idea. First, there is a striking 4:1 front-to-rear asymmetry for calcium flicker frequency, opposite to the shallow increasing resting $[Ca^{2+}]_i$ gradient in the same cells. It should be noted that this sheer calcium flicker gradient does not reverse the front-to-rear increasing resting $[Ca^{2+}]_i$ gradient, because the summation of calcium flickers contributes only a small fraction of the time-averaged $[Ca^{2+}]_i$, even at the front. Second, under the uniform stimulation of a chemoattractant (PDGF, platelet-derived growth factor), both amplitude and probability of calcium flickers increase and these are accompanied by a decrease of directional persistence compared with that of untreated fibroblasts (Fig. 2), supporting the idea that calcium flicker activity endows the fibroblast with the ability of making turns. Third, compared with migrating cells, stationary fibroblasts exhibit much

Fig. 3. Asymmetrical lamellar calcium flicker activity induced by a PDGF gradient and its coupling with the turning of migrating fibroblasts. (A) Representative example of a migrating cell. The initial movement to the right is reoriented toward the direction of the indicated gradient. Top left (colors in the following description refer to the Web version), contours of the cell border at –14 min (pink), 0 min (purple), and 15 min (yellow). Top right, trajectory of the centre of the cell and bar graph (below) showing the time course of calcium flicker production. SM is the signal mass or integral of calcium flickers. (A, bottom row, panels 1, 2, and 3) Overlays of calcium flickers in 1-minute windows (labeled 1–3 in the trajectory above). Scale bar in lower panel represents 5 μm. The α and β denote 2 frontal regions of equal area with the α region facing the higher PDGF gradient. (Modified from Wei et al. 2009.) (B) Statistics on calcium flicker asymmetry and the turning angle from 21 migrating fibroblasts. (C) Examples of cell migrating trajectory. (D) Correlation coefficient between $\Sigma SM_{\alpha-\beta}$ and *y* distance travelled. Each datum was derived from a 3-minute time-lapse image series.



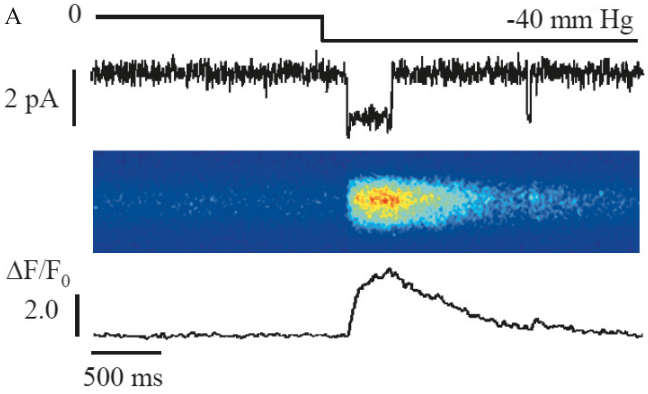
lower calcium flicker activity and show little directional polarization.

More importantly, when an environmental PDGF gradient is established in a direction perpendicular to the migrating path, not only is calcium flicker activity enhanced, but more calcium flickers occur in the side facing the higher PDGF, creating an asymmetric pattern within the leading lamella at the front of the cell (Fig. 3A). This asymmetric activity of calcium flickers correlates with the reorientation of the cell trajectory. The correlation coefficient between the signal mass $\Sigma SM_{\alpha-\beta}$ and the *y* distance travelled is 0.72 on average in 21 migrating fibroblasts (Figs. 3B, 3C, and 3D). In addition, this local asymmetry is maintained during the entire process by which the cell reorients itself along the PDGF gradient (Fig. 3), suggesting that the asymmetry of calcium flicker activity decodes local PDGF gradients.

Interplay among TRPM7, type 2 IP_3 receptors, and cytoskeletal elements

In search of the molecular nature of calcium flickers, we have shown that individual calcium flickers are triggered by

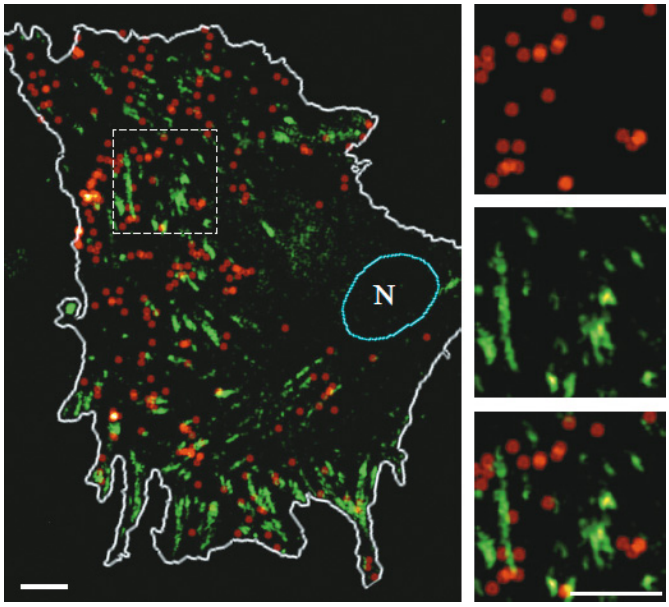
Fig. 4. Molecular nature of calcium flickers. (A) Visualization of the calcium flicker triggered through SACCs during single-channel electrophysiological recording. (Modified from Wei et al. 2009.) From top to bottom: suction through the patch pipette, single-channel currents, line-scan image (20 μm), and line plot of local calcium transients. (B) Diagram shows the coupling between TRPM7 and IP_3R2 in the genesis of a calcium flicker. TRPM7 is both a channel and an α-kinase (Runnels et al. 2001). SACC, stretch-activated cation channel; TRPM7, transient receptor potential melastatin 7 channel; IP_3R2, inositol 1,4,5-trisphosphate receptor type 2; PLC, phospholipase C; PIP_2, phosphatidylinositol 4,5-bisphosphate; PI3K, phosphatidylinositol 3-kinase; GR, growth factor receptor; ER, endoplasmic reticulum; SERCA, sarcoplasmic/endoplasmic reticulum Ca^{2+}-ATPase.



the opening of a single calcium-permeating stretch-activated cation channel (SACC) (Fig. 4A). We then took advantage of the small RNA interference technology to pinpoint the channel as TRPM7, a member of the transient receptor potential (TRP) channel superfamily (Nilius et al. 2007). That TRPM7 is a critical molecular player in cell migration is in general agreement with previous work by Clark et al. (2006) and Su et al. (2006). Similarly, other members of the TRP channels, TRPC3 or TRPC6, are implicated in pathfinding of neuronal processes (Li et al. 2005).

Detailed mechanistic study has further revealed two important features of the genesis of calcium flickers: the involvement of intracellular calcium release channels and the feedback regulation by cytoskeletal elements. It has been shown that individual TRPM7 calcium influx is tightly coupled to local calcium release from type 2 inositol 1,4,5-trisphosphate receptor (IP_3R2) in the endoplasmic reticulum (Fig. 4B). As such, blockade of IP_3R2 halves the amplitude of calcium flickers without significantly altering the frequency (Wei et al. 2009). In contrast, blockade of TRPM7 virtually inhibits the calcium flicker activity completely.

Fig. 5. Matrices of focal integrin-containing adhesion sites (green in the Web version; white in print version) seen in the large image (left) and in the middle and bottom images of the enlargements (right). Calcium flickers (red in the Web version; grey in print version) are seen in the large image (left) and in the top and bottom images (right). N denotes the nuclear region. Scale bars represent 8 μm. (Modified from Wei et al. 2009.)

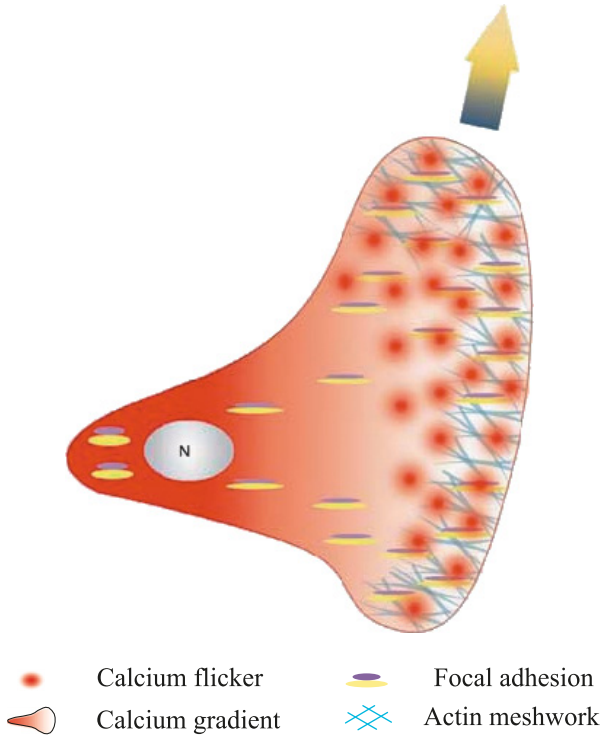


Furthermore, there appears to be a positive feedback between membrane stress and calcium flicker production. Being a SACC, TRPM7 senses local mechanical stress and transduces it into frequency-modulated calcium flicker activity. As shown in Fig. 5, the frontal region of high calcium flicker activity thus generally overlaps with the matrix of integrin-containing adhesion sites (although individual calcium flickers are usually not colocalized to individual adhesion sites) where the greatest membrane stress has been found (Beningo et al. 2001). Disruption of cytoskeletal elements or inhibition of force-producing contractile filaments diminishes the calcium flicker activity (Wei et al. 2009). Conversely, removal of extracellular calcium relaxes the membrane stress (Munevar et al. 2004).

How does a cell make a turn: the calcium flicker model

These recent advances suggest a new model of how flickering calcium microdomains steer directional cell movement (Fig. 6). In this model, individual calcium flickers at the leading edge act as a miniature steering wheel to drive the cell to turn. Decision-making for turning is likely a democratic poll of all calcium flickers as the cell turns to the direction with higher calcium flicker activity. Experimental evidence bolstering this model includes a near-linear correlation between the asymmetry of frontal calcium flicker ac-

Fig. 6. A calcium flicker model for turning in a migrating fibroblast (see text for details). N denotes the nuclear region.



tivity and the turning angle of the cell. Pharmacological and genetic manipulations that enhance or subdue the asymmetric signal of calcium flickers proportionally increase or decrease the cell's turning ability. Administration of a membrane-permeable IP_3 analogue enhances both the calcium flicker activity and the turning process. Conversely, intracellular loading of EGTA to chelate intracellular calcium slows the turning in a dose-dependent manner. Interestingly, after complete abolition of the calcium flicker activity, cells can still migrate, at even faster velocity along a smoother trajectory; however, they are no longer able to make turns when exposed to a PDGF gradient perpendicular to their path. In this scenario, the low background $[Ca^{2+}]_i$ at the front might be considered as a means to enhance the signal-to-noise for calcium flicker signaling.

Perspectives

As the newest addition to the calcium spark family (Cheng et al. 1993; Cheng and Lederer 2008), calcium flickers illustrate how exquisite the spatiotemporal organization of calcium microdomains is in functioning cells and how such patterned calcium microdomains can orchestrate complex cellular processes such as turning of a migrating cell. Nevertheless, how is the asymmetry in calcium flicker activity developed at the front when exposed to a PDGF gradient? What are the local events immediately downstream of a calcium flicker event? Can we target TRPM7 to correct aberrant migration in diseases? These remain open questions. We speculate that when facing higher concentrations of chemoattractant, pseudopod protrusion will be enhanced, and so will traction force. The increased membrane stress will directly augment calcium flicker activity, which in turn further increases local membrane stress. In addition, growth factor receptors that respond to chemoattractants can increase IP_3-mediated signal transduction, enhancing calcium flicker activity via IP_3R2. Given the positive feedback loop mentioned earlier, any slight initial asymmetric calcium flicker activity will be amplified, resulting in significantly higher calcium flicker activity within the migrating cell on the side facing the chemoattractant. Nevertheless, future investigation is warranted to test this hypothesis. Moreover, it is equally important to investigate what the immediate signaling events activated locally by a calcium flicker may be. For instance, is there local polymerization and de-polymerization of cytoskeletal filaments or activation of calcium signaling proteins such as PKC (Evans and Falke 2007) in these flickering calcium microdomains? Understanding of these molecular mechanisms will allow us to devise strategies to enhance or suppress directional cell movement in the treatment of a variety of diseases with abnormalities in cell migration.

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