CHAPTER 17

MscCa Regulation of Tumor Cell Migration and Metastasis

Rosario Maroto and Owen P. Hamill
Department of Neuroscience and Cell Biology, University of Texas Medical Branch, Galveston, Texas 77555

I. OVERVIEW

The acquisition of cell motility is a required step in order for a cancer cell to migrate from the primary tumor and spread to secondary sites (metastasize). For this reason, blocking tumor cell migration is considered a promising approach for preventing the spread of cancer. However, cancer cells like normal cells can migrate by several different modes referred to as

Current Topics in Membranes, Volume 59 1063-5823/07 $35.00

DOI: 10.1016/S1063-5823(06)59019-2

"amoeboid," "mesenchymal," and "collective cell." Furthermore, under appropriate conditions a single cell can switch between modes. A consequence of this plasticity is that a tumor cell may be able to avoid the effects of an agent that targets only one mode by switching modes. Therefore, a preferred strategy would be to target mechanisms that are shared by all modes. Here we review the evidence that Ca^{2+} influx via the mechanosensitive Ca^{2+}-permeable channel (MscCa) is a critical regulator of all modes of cell migration and therefore represents a very good therapeutic target to block metastasis.

II. INTRODUCTION

Cancer is a multistep process that results in a normal cell, often an epithelial cell lining a gland, duct, or organ surface, undergoing abnormally increased multiplication to produce a localized primary tumor that with time invades and spreads (metastasizes) to surrounding tissues and eventually causes death. However, in order for a tumor to metastasize, the tumor cell must migrate from the primary tumor, pass through blood vessels, penetrate into the secondary tumor site, and migrate through the tissue to establish a metastasis. Therefore, the acquisition of cell motility is a necessary although not a sufficient step for tumor invasion and metastasis, which also require the additional steps of barrier matrix breakdown, tumor cell adherence, growth, and angiogenesis at the secondary sites. Nevertheless, because metastasis will only be achieved if the tumor cell completes every step in the metastatic cascade, identifying the most sensitive and susceptible step that regulates tumor cell migration should provide a promising target to block metastasis (Grimstad, 1987; Stracke *et al.*, 1991; Kassis *et al.*, 2001).

There are currently two models used to explain tumor progression to the metastatic disease. One is the traditional "multi-hit" genetic model that proposes a sequence of mutations that triggers the various stages of cancer (e.g., initiation, promotion) with the final mutation(s) promoting increased tumor cell invasiveness and metastasis (Emmelot and Scherer, 1977; Cahill *et al.*, 2000; Hanahan and Weinberg, 2000; Zhou *et al.*, 2005). Evidence supporting this model includes the existence of several stable human tumor cell lines that demonstrate high invasiveness when implanted in animals (Kaighn *et al.*, 1979; Sung *et al.*, 1998), and the recent discovery that many primary tumor cells already express a genetic signature that predicts their metastatic potential (Ramaswamy *et al.*, 2003; Varambally *et al.*, 2005). The second model is an epigenetic one based on the discovery that growth factors that trigger the epithelial–mesenchymal transition (EMT), in which nonmotile epithelial cells are converted into motile mesenchymal cells (e.g., during

normal embryogenesis and wound healing), are also released by stromal cells surrounding the tumor and promote increased tumor cell invasiveness and metastasis (Thiery, 2002; Thompson and Newgreen, 2005; but see Tarin, 2005). Specific cancers may utilize one or a combination of the two mechanisms since the mechanisms are not exclusive (e.g., one aspect of the metastatic genetic signature may include the potential to undergo EMT). In any case, the regulatory molecules involved in transforming a tumor cell from a nonmotile to a motile phenotype need to be identified. In this chapter we focus on the role of the MscCa, which is identified as a member of the transient receptor potential channel family (Maroto *et al.*, 2005; Saimi *et al.*, 2007) and shown to be essential for prostate tumor cell migration (Maroto *et al.*, 2007). Because MscCa is expressed by both nonmotile and motile cells, we review the evidence for the idea that changes in MscCa properties triggered by events associated with cancer progression may contribute to increased tumor invasiveness and metastasis.

III. DIFFERENT MODES OF MIGRATION

Normal cells and tumor cells move according to one of three major modes of migration referred to as "amoeboid," "mesenchymal," and "collective cell." Furthermore, under specific circumstances a single cell can switch between these modes (Friedl and Wolf, 2003; Sahai and Marshall, 2003; Friedl, 2004; Wolf and Friedl, 2006). Because of this plasticity, a tumor cell may be able to avoid the effects of an agent that blocks only one migratory mode by switching to another mode. Therefore, a preferred strategy would be to identify and target molecular mechanisms that are shared by all modes. With this in mind, we consider the different modes of migration, their similarities and differences, and in particular their possible common dependence on Ca^{2+} influx via MscCa.

A. Amoeboid Migration

Amoeboid movement is expressed by a variety of invertebrate and vertebrate cells, but has been the most intensely studied in the amoeba *Dictyostelium discoideum*. This cell displays an ellipsoidal profile with either a monopodal or polypodal form, and undergoes a rapid (e.g., >20 μm/min) gliding movement that involves repetitive cycles of protrusion and contraction with little adhesiveness to the substrate. This lack of adhesiveness is consistent with the absence of integrin expression by the amoeba (Friedl, 2004). The amoeba uses two mechanically distinct mechanisms to push itself

forward (Yoshida and Soldati, 2006) a filopodia–lamellipodia mechanism that depends on actin polymerization and a bleb mechanism in which a local region of membrane where the cortical-CSK has been disrupted is pushed outward by cytoplasmic pressure generated by myosin II. Both protrusion mechanisms involve significant mechanical distortions of the membrane at the front of the cell that could activate MscCa to provide feedback (via Ca^{2+} influx and/or membrane polarization) between the force-generating mechanisms and resultant membrane distortions.

Neutrophils, eosinophils, lymphocytes, stem cells, and specific tumor cells associated with leukemia, lymphoma, and small cell lung carcinoma also display amoeboid movement. Furthermore, specific cell types that display a mesenchymal mode of migration when crawling on a two-dimensional (2D) substrate can switch to an amoeboid mode when migrating through a 3D substrate (Friedl, 2004). Vertebrate cells undergoing amoeboid migration also display both blebbing and filopodia–lamellipodia mechanisms of forward protrusion (Sahai and Marshall, 2003; Blaser *et al*., 2006). Fish and amphibian keratocyes may represent a hybrid form of amoeboid/mesenchymal locomotion because they normally show a smooth gliding movement but also express a broad flat lamellipodium. Furthermore, when they become stuck on their substrate they tend to pull out a rear tether and display a more discontinuous "mesenchymal-like" locomotion (Lee *et al*., 1999). Interestingly, an amoeba can be induced to develop a broad lamellipodium and undergo keratocyte-like migration by knocking out a gene that regulates the amoeba's aggregation process (Asano *et al*., 2004). However, a double knockout of myosin II and the aggregation gene does not block keratocyte-like migration, indicating that myosin II may be dispensable for this mode of movement.

B. Mesenchymal Migration

Mesenchymal movement is displayed by fibroblasts, neurons, smooth muscle, and endothelial cells, as well as by specific cancer cells from epithelial tumors, gliomas, and sarcomas. In this mode, the cell typically displays a highly polarized morphology with a front lamellipodium, immediately behind which is the lamella, followed by the cell body with the nucleus, and usually ending with a rear tail or tether. Compared with the smooth, gliding amoeboid movement, mesenchymal migration is relatively discontinuous and slower (<1 μm/min) because of its greater adhesiveness and strong dependence on integrin engagement and disengagement from the substrate. Mesenchymal migration can be divided into five steps involving: (1) forward protrusion of the cell's leading edge, (2) formation of adhesions at the front

of the cell with the extracellular matrix (“gripping”), (3) pulling against the ECM via the cell adhesions as the myosin–cytoskeleton (CSK) contracts and exerts traction force against the substrate, (4) progressive stretching of the cell as the traction force develops at the cell front and pulls against the cell rear, and (5) finally, detachment of the rear adhesions from the ECM allowing net cell displacement and relaxation of membrane stretch (Lauffenburger and Horwitz, 1996; Sheetz *et al*., 1999; Ridley *et al*., 2003). The important aspect of this mode of migration in relation to MscCa is that the membrane bilayer of the whole cell will tend to experience a slow ramp of increasing tension for as long as the rate of forward protrusion exceeds the rate or rear retraction (Lee *et al*., 1999; Maroto *et al*., 2007).

C. Collective Cell Migration

In the collective cell mode of migration, the cells are connected by cell junctions formed by cadherins and integrins, and move in a mass with the motile cells at the leading invasive edge generating the adhesion and traction forces (likely via the mesenchymal mode) that tend to pull the rear nonmotile tumor cells along passively. This pattern of migration represents the predominate migration mode for most epithelial cancers *in situ*, and provides the advantage of increased heterogeneity by allowing nonmotile, proliferating cells along with motile path-finding cells to invade the new tissues (Friedl and Wolf, 2003; Wolf and Friedl, 2006).

D. Mechanisms for Switching Migration Modes

Cells that normally express mesenchymal and/or collective cell migration can be converted to the amoeboid mode by reducing the effectiveness of integrin-ECM adhesion (i.e., with integrin-blocking antibodies or arginine-glycine aspartate (RGD) peptides that compete for integrin-ECM-binding sites), by blocking matrix proteases, or by stimulating the Rho-associated serine/threonine kinase (ROCK) that increases cortical contraction, thereby promoting cell rounding and forward protrusion by membrane blebbing (Friedl, 2004). With this switch, the cell becomes more deformable due to its lack of adhesiveness and can now squeeze between matrix barriers. This lessens the dependence on the actions of matrix-degrading metalloproteinases and increases resistance to metalloproteinase inhibitors. The weakened dependence on integrin adhesion also results in a loss of dependence on calpain proteolytic cleavage important for integrin-linked adhesion turnover (Carragher *et al*., 2005). In neutrophils, rear integrins tend to be endocytosed

rather than dissembled by calpain activity, and in contrast to mesenchymal cells, inhibition of calpain actually promotes, rather than inhibits, migration by enhancing cell protrusion and cell spreading (Lokuta *et al.*, 2003). On the other hand, amoeboid movement retains a strong dependence on myosin II contractility as indicated by increased sensitivity to ROCK inhibition (Sahai and Marshall, 2003). Since that both calpain and myosin II are Ca^{2+} sensitive, one would expect that both modes of migration would display Ca^{2+} dependence. Another mechanism that appears to promote mode switching relates to the relocation of cavoelin-1 (Cav-1), a lipid raft-associated protein that colocalizes with MscCa/TRPC1 (Lockwich *et al.*, 2000; Brazier *et al.*, 2003; Maroto *et al.*, 2005). For example, when endothelial cells switch from migration in a 2D to a 3D matrix there is a redistribution of Cav-1, and possibly MscCa, from the back to the front of the cell (Parat *et al.*, 2003). As described below, this shift would be consistent with intracellular $[Ca^{2+}]$ ($[Ca^{2+}]_i$) transients being initiated in the front of the amoeboid like neutrophils (Kindzelskii *et al.*, 2004) but in the rear of mesenchymal-like cells (Maroto *et al.*, 2007).

IV. Ca^{2+} DEPENDENCE OF CELL MIGRATION

Although a variety of signaling pathways may regulate cell migration, Ca^{2+} signaling has always been considered a significant player because many of the effector molecules that mediate migration are Ca^{2+} sensitive, including myosin light chain kinase (i.e., that regulates myosin II), calpain, gelsolin, α-actinin, and phosphatase (calcineurin) and integrins (Hendey and Maxfield, 1993; Arora and McCulloch, 1996; Eddy *et al.*, 2000; Mamoune *et al.*, 2003; Franco and Huttenlocher, 2005). The Ca^{2+} regulatory role has been reinforced by the finding that a variety of Ca^{2+} transport proteins including pumps, exchangers, and various gated Ca^{2+} channels can modulate cell migration (Dreval *et al.*, 2005).

A. Measuring $[Ca^{2+}]_i$

The most convenient and common method used to measure $[Ca^{2+}]_i$ involves using fluorescent microscopy and Ca^{2+}-sensitive fluorescent dyes like fura-2 and its membrane permeable form fura-2 AM (Grynkiewicz *et al.*, 1985). The main advantage of the approach is that changes in $[Ca^{2+}]_i$ can be monitored while simultaneously measuring cell migration (i.e., by time-lapse videomicrosopy). As a consequence, one can relate specific spatio-temporal changes in $[Ca^{2+}]_i$ to specific events occurring during migration. However,

there are also some practical limitations associated with the method, including the difficulty of detecting local vs global $[Ca^{2+}]_i$ changes and the possibility of compartmentalization of the dyes in organelles. The first limitation has been somewhat overcome by recent technical developments that includes the use of total internal reflectance fluorescence microscopy that offers added spatial resolution to allow detection of single-channel $[Ca^{2+}]_i$ fluctuations at the ventral membrane surface adhering with the glass surface (Demuro and Parker, 2005). In addition, the development of Ca^{2+}-sensor "cameleons" that operate by fluorescence energy transfer and can be targeted to the plasma membrane or the ER can be used to measure $[Ca^{2+}]_i$ changes in these membrane microdomains (Miyawaki *et al.*, 1997; Isshiki *et al.*, 2002). In the case of fura-2 compartmentalization, there are discrepant views on its occurrence and significance. For example, one group has proposed that the apparent $[Ca^{2+}]_i$ gradient seen in T lymphocytes is due to fura-2 accumulation in mitochondria (Quintana and Hoth, 2004), whereas another group found that the $[Ca^{2+}]_i$ gradient seen in fibroblasts was not associated with mitochondria but instead colocalized with the Golgi apparatus in the perinuclear region (Wahl *et al.*, 1992). A further complication is that mitochondria are motile, and their motility varies inversely with $[Ca^{2+}]_i$ so that they move fastest in lower $[Ca^{2+}]_i$ (100-300 nM) but stop movement in higher $[Ca^{2+}]_i$ (i.e., 1 μM) (Yi *et al.*, 2004). As a consequence, one would expect mitochondria to migrate up a $[Ca^{2+}]_i$ gradient and accumulate in regions of highest $[Ca^{2+}]_i$ where they may function as Ca^{2+} buffers and/or prevent the spread of local $[Ca^{2+}]_i$ transients (Tinel *et al.*, 1999; Yi *et al.*, 2004; Levina and Lew, 2006). However, in apparent contradiction of this idea, mitochondria accumulate in the lamellipodium of migrating fibroblasts and prostate tumor cells (DeBiasio *et al.*, 1987; Maroto *et al.*, 2007), and yet these cells develop a global $[Ca^{2+}]_i$ gradient that increases from front to back of the cell (Hahn *et al.*, 1992; Matoto *et al.*, 2007). The stimulus that promotes this accumulation remains unclear but could involve the added requirement for ATP and/or an elevated $[Ca^{2+}]_i$ in membrane subdomains of the lamellipodium. In any case, it would appear that compartmentalization of fura-2 dye cannot alone explain the sustained, and in some cases rapidly reversible, $[Ca^{2+}]_i$ gradients seen in a variety of migrating cells (see Section IV.E.2).

B. Identifying Ca^{2+} Influx Pathways

The simplest method to demonstrate a requirement for Ca^{2+} influx is to show that migration requires the presence of external Ca^{2+} (Strohmeier and Bereiter-Hahn, 1984). Patch clamp recording can then be used to characterize the kinetics, conductance, surface distribution, and pharmacological

properties of the Ca^{2+} channels expressed in the migrating cell (Lee *et al.*, 1999; Maroto *et al.*, 2007). With this knowledge one can then use various treatments to relate particular $[Ca^{2+}]_i$ changes to specific Ca^{2+} channels activities. One perceived practical limitation of patch clamping is that channel current measurements are restricted to the dorsal surface because it is not possible to patch the ventral "adherent" surface, at least with the traditional patch clamp method (Hamill *et al.*, 1981). In this case, one might argue that because CSK-generated mechanical (traction) forces are transmitted to the substrate purely at ventral surface adhesions, then only mechanosensitive processes in these sites will experience mechanical force and become activated (Mobasheri *et al.*, 2002). However, the traction forces that pull on the substrate via the ventral surface adhesions will also tend to stretch the whole cell for as long as the rear of the cell remains firmly attached to the substrate. Apart from causing the cell to become extended, there are other manifestations of these stretching forces including the smoothing out of membrane folds and microvilli in spreading cells (Erickson and Trinkhaus, 1976), an elastic recoil seen occasionally in some migrating cells as presumably stretching forces exceed adhesive forces (Mandeville and Maxfield, 1997), and even cell rupture/fragmentation that can occur when cell retraction is blocked and the pulling forces exceed the elastic limits of the bilayer (Verkhovsky *et al.*, 1989). Galbraith and Sheetz (1999) have elegantly and directly addressed the issue of force distribution on the ventral and dorsal surfaces by using optical tweezers to measure the membrane tension on the dorsal membrane, and a micromachined device to measure tension generated on the ventral membrane. Their measurements indicate that the dorsal matrix is as effectively linked to the force-generating CSK as the ventral adhesions so tension-sensitive channels located in both the dorsal and ventral surfaces should experience the same stretch. In this case, the MscCa properties measured on the dorsal surface (i.e., their gating kinetics and subsurface distribution) should be important in defining the $[Ca^{2+}]_i$ dynamics measured during cell migration (Maroto *et al.*, 2007).

C. Ca^{2+} Dependence of Amoeba Locomotion

One of the earliest observations implicating Ca^{2+} in amoeboid migration was that lanthanum, a known Ca^{2+} channel inhibitor, blocked movement of *Amoeba discoides* (Hawkes and Hoberton, 1973). Subsequently, microinjection of aequorin (a photoprotein that emits light on Ca^{2+} binding) was used to demonstrate a sustained $[Ca^{2+}]_i$ elevation in the tail of the amoeba, as well as transient Ca^{2+} influxes in the tips of advancing pseudopods—lowering external $[Ca^{2+}]_o$ did not immediately reduce rear $[Ca^{2+}]_i$ but it did block

continued migration (Taylor *et al.*, 1980). This was interpreted as indicating that rear $[Ca^{2+}]_i$ can be maintained by Ca^{2+} release from internal stores, but migration is more sensitive to Ca^{2+} influx into the pseudopod tips (Taylor *et al.*, 1980). In another study, direct injection of fura-2 was used to show that monopodal amoebae developed a continuous $[Ca^{2+}]_i$ gradient increasing from front to rear, whereas polypodal amoebae showed a decrease in $[Ca^{2+}]_i$ in extending pseudopodia, and an increase in retracting pseudopodia (Gollnick *et al.*, 1991; Yumura *et al.*, 1996). Subsequently, intracellular BAPTA, a fast Ca^{2+} buffer, was shown to reduce cell spreading, pseudopodia formation, and amoebae locomotion, and these effects could be reversed by raising $[Ca^{2+}]_o$ (Unterweger and Schlatterer, 1995). On the other hand, the same study found that chelation of $[Ca^{2+}]_o$ by the relatively slow Ca^{2+} buffer EGTA did not block pseudopod formation, although it did block the development of any $[Ca^{2+}]_i$ gradient and cell migration. Nebl and Fischer (1997) used recombinant aequorin to demonstrate that chemoattractants induced an increase in $[Ca^{2+}]_i$ that was entirely dependent on Ca^{2+} influx, and speculated that Ca^{2+}-induced actin depolymerization in the rear acted to prevent the formation of stable pseudopod formation in this region of the cell. $[Ca^{2+}]_o$ was shown to be required for shear-flow-induced amoebae motility (but not directionality) and that addition of either EGTA or Gd^{3+} stopped cell movement (Fache *et al.*, 2005). In this case, the effects of external Ca^{2+} were shown to stimulate cell speed by increasing the amplitude, but not the frequency, of both protrusion and retraction events at the cell's leading edge (Fache *et al.*, 2005). Another study based on mutants lacking two major Ca^{2+}-binding proteins in the ER (calreticulum and calnexin) concluded that chemotaxis depended on both Ca^{2+} influx and Ca^{2+}-induced Ca^{2+} release from internal stores (Fisher and Wilczynska, 2006).

Despite the above results, there are also several studies that seem to discount a critical role for Ca^{2+} in amoeboid migration. For example, based on normal chemotaxis seen in a mutant amoeba lacking an IP_3-like receptor, it was concluded that Ca^{2+} signaling was not required for chemotaxis (Traynor *et al.*, 2000). However, different groups studying the same mutant found that $[Ca^{2+}]_i$ transients dependent on Ca^{2+} influx were not only retained but were required for both chemotaxis and electrotaxis (Schaloske *et al.*, 2005; Shanley *et al.*, 2006). In a different study, it was reported that amoebae can continue their random locomotion with the same speed in the absence of $[Ca^{2+}]_o$ and the presence of 50-mM EGTA or EDTA, apparently ruling out any role for Ca^{2+} influx (Korohoda *et al.*, 2002). However, a more trivial explanation may relate to inadvertent Ca^{2+} leaching from the low profile glass chamber in which both the ventral and dorsal surfaces of the migrating cell make close contact with the glass. Under these conditions, Ca^{2+} may build up in the narrow gaps between the adherent cell and glass

surfaces and reach levels (~1 μM) sufficient to support migration (Fisher and Wilczynska, 2006). A similar phenomenon may also account for the apparent lack of external Ca^{2+} dependence of human leukocyte locomotion when they are "chimneying" between closely apposed glass slide and cover slip (Malawista and Boisfleury-Chevance, 1997).

In summary, while most studies indicate that both Ca^{2+} influx and $[Ca^{2+}]_i$ elevations are required for an amoeba to migrate, the exact role of Ca^{2+} influx in forward protrusion and rear retraction needs to be better defined. There also remains the unresolved issue on whether the reports of amoeba's migration in the absence of $[Ca^{2+}]_o$ are real or artifactual. In particular, it will be interesting to test whether migration by chimneying is retained in the presence of the faster Ca^{2+}-buffering capacity of BAPTA.

D. Ca^{2+} Dependence of Vertebrate Cell Amoeboid Migration

Newt neutrophils, which are relatively large (~100 μm in diameter) and comparable in size to an amoeba, develop a sustained $[Ca^{2+}]_i$ gradient that increases from front to rear of the cell as they migrate. Furthermore, spontaneous changes in $[Ca^{2+}]_i$ gradient direction result in changes in migration direction (Brundage *et al.*, 1991; Gilbert *et al.*, 1994). In contrast, the smaller human neutrophils do not develop a detectable $[Ca^{2+}]_i$ gradient but instead display $[Ca^{2+}]_i$ transients when migrating on adhesive substrates (e.g., polylysine, fibronectin, or vitronectin), but not on nonadhesive substrates (Marks and Maxfield, 1990; Hendey and Maxfield, 1993). These $[Ca^{2+}]_i$ transients can be blocked, along with neutrophil migration, by either removing $[Ca^{2+}]_o$ or buffering $[Ca^{2+}]_i$. The $[Ca^{2+}]_i$-buffered neutrophils apparently become immobilized because they are unable to retract their rear, which remains anchored to the adhesive substrate. However, they are still capable of spreading, assuming a polarized morphology, and extending their plasma membrane. Furthermore, their motility can be restored by using RGD peptides to block specific integrin attachments to the substrate. Since a similar block of motility could be induced by inhibitors of the Ca^{2+}-dependent phosphatase, calcineurin, it was proposed that this enzyme mediated Ca^{2+}-dependent detachment of the integrin–substrate adhesions (Hendey and Maxfield, 1993). However, the same group latter suggested that a more general mechanism for rear detachment may involve Ca^{2+}-increased myosin II contractility (Eddy *et al.*, 2000). A similar Ca^{2+} and RGD sensitivity was seen for neutrophils migrating through a 3D matrigel substrate (Mandeville and Maxfield, 1997), whereas neutrophils migrating on nonadhesive substrates (e.g., glass in the presence of albumin/serum or through cellulose filters) did not display Ca^{2+} transients nor did they require the presence of

external Ca^{2+} or elevations in $[Ca^{2+}]_i$ in order to migrate (Zigmond *et al.*, 1988; Marks and Maxfield, 1990; Hendey and Maxfield, 1993; Laffafian and Hallet, 1995; Alterafi and Zhelev, 1997). A similar phenomena may occur in the normally gliding fish keratocytes that show an increased frequency of $[Ca^{2+}]_i$ transients when their rear becomes transiently stuck on the substrate (Lee *et al.*, 1999). An apparently different role for Ca^{2+} signaling involves Ca^{2+} influx-mediated "priming" of nonmotile eosinophils that enables them to undergo transepithelial migration. However, once the cells are primed, they can migrate in the absence of $[Ca^{2+}]_o$, although they still depend on $[Ca^{2+}]_i$ elevations (Liu *et al.*, 1999, 2003).

In summary, some of the discrepancies in the Ca^{2+} dependence of neutrophil migration may arise through differences in substrate adhesiveness with the strongest Ca^{2+} dependence seen on sticky substrates, but little or no Ca^{2+} dependence on nonadhesive substrates. At least in this respect, vertebrate cells that display the amoeboid mode may differ from the amoeba, which retains Ca^{2+} dependence even though the amoeba does not depend on integrin adhesion. At least for human neutrophils, $[Ca^{2+}]_i$ transients rather than gradients appear to be more important in regulating cell migration by promoting rear retraction possibly by increased adhesion disassembly via increases in calcineurin, MLCK, and/or calpain activity.

E. The Role of $[Ca^{2+}]_i$ Gradients and Transients in Mesenchymal Cell Migration

Cells migrating in the mesenchymal mode can also display sustained $[Ca^{2+}]_i$ gradients and/or fast transients. Since these different spatio-temporal $[Ca^{2+}]_i$ dynamics may regulate different steps associated with the mesenchymal migratory cycle, they will be discussed separately below.

1. A Model for Sustained $[Ca^{2+}]_i$ Gradients

A basic question from the onset is how any cell can maintain a sustained $[Ca^{2+}]_i$ gradient for as long as several hours in a cytoplasm that allows free diffusion of Ca^{2+}. In particular, the existence of any stable regions of different $[Ca^{2+}]_i$ within a continuous aqueous medium would seem to disobey the second law of thermodynamics according to which solutes should passively diffuse down their concentration gradient until they reach equilibrium—in the case of Ca^{2+}, this equilibration should occur in seconds or at most minutes. To explain this apparent paradox, Braiman and Priel (2001) proposed that the cell uses energy to actively take up Ca^{2+} uptake into internal stores that can then be passively allowed to leak out into localized regions of the cytoplasm. By this process, combined with a polarized distribution of Ca^{2+} release

channels on a contiguous ER Ca^{2+} store, the cell could create a sustained $[Ca^{2+}]_i$ elevation in specified subdomains of the cell (Petersen *et al.*, 2001). The interesting aspect of this model is that, one could have uniform Ca^{2+} influx across the cell surface and uniform active uptake by the internal Ca^{2+} stores as long as there was a gradient of Ca^{2+} release from the stores. A further prediction of this model is that if both active uptake and passive leak occur in very close proximity of the membrane, then a subcortical membrane domain of elevated $[Ca^{2+}]_i$ could be maintained that might go undetected by techniques that only measure global $[Ca^{2+}]_i$.

2. $[Ca^{2+}]_i$ Gradients Determine Migrational Directionality

In several cells undergoing mesenchymal migration, $[Ca^{2+}]_i$ gradients have been shown to be important in determining migration directionality. In particular, Xu *et al.* (2004) observed that migrating cerebellar granule cells develop a $[Ca^{2+}]_i$ gradient (low front–high back) according to their migration direction. Furthermore, experimental reversal of the $[Ca^{2+}]_i$ gradient by the application to the front of the cell, an external gradient of various agents that cause $[Ca^{2+}]_i$ elevation (e.g., chemo-repellant slit2, acetylcholine, and ryanodine) was found to be accompanied by a reversal in migration direction. Similarly, if an external gradient of BAPTA-AM was applied to the back of the cell, again the $[Ca^{2+}]_i$ gradient and migration direction was reversed. Although some of the same neurons also displayed occasional $[Ca^{2+}]_i$ transients, no causal relationship was noted between the transients and migration direction (Xu *et al.*, 2004). Similar $[Ca^{2+}]_i$ gradients related to migration direction have been seen in migrating fibroblasts, kidney epithelial tumor cells, vascular endothelial cells, and prostate tumor cells (Hahn *et al.*, 1992; Schwab *et al.*, 1997; Kimura *et al.*, 2001; Maroto *et al.*, 2007). Moreover, Schwab and colleagues have proposed that the relatively high Ca^{2+}-activated K^+ activity evident in the rear of migrating kidney epithelial tumor cells was a direct consequence of a $[Ca^{2+}]_i$ gradient rather than polarized surface expression of the K^+ channels (Schwab *et al.*, 1995, 2006). They also proposed that the underlying basis for the $[Ca^{2+}]_i$ gradient was due to a combination of higher density of Ca^{2+} influx pathways and ER $[Ca^{2+}]_i$ stores in the cell body compared with the lamellipodia (Schwab *et al.*, 1997). Studies of the highly motile prostate tumor cell line, PC-3, have confirmed some of these ideas (Maroto *et al.*, 2007).

$[Ca^{2+}]_i$ gradients are seen not only in migrating cells but also in polarized exocrine acinar gland cells where they may regulate unidirectional fluid secretion. In particular, a time-dependent reversal of the $[Ca^{2+}]_i$ gradient from the luminal to blood side of the acinar cell after acetylcholine (ACh) stimulation has been proposed to be the main basis for a push-pull model for unidirectional fluid secretion (Kasai and Augustine, 1990). In this model,

$[Ca^{2+}]_i$ elevation, first on the luminal cytoplasmic side of the cell causes Cl^- and water efflux into the lumen, then $[Ca^{2+}]_i$ elevation on the blood side of the cell causes Cl^- and water influx from the blood side. Although both cell surfaces express the same Ca^{2+}-activated Cl^- channel, the depolarization that follows ACh stimulation shifts the Cl^- driving force from efflux to influx. A somewhat similar mechanism could presumably underlie the role of ion and water movements in coordinating cell locomotion (Schwab *et al.*, 2006). This possibility seems to be reinforced by the demonstration that aquaporins are selectively expressed in the leading edge of migrating cells (Saadoun *et al.*, 2005). A quite different cell function related to a sustained $[Ca^{2+}]_i$ gradient involves tip growth of fungi in which elevated $[Ca^{2+}]_i$ in the growing tip has been proposed to promote increased insertion of new membrane via exocytosis (Silverman-Gavrila and Lew, 2003). This mechanism would seem unlikely to account for migration directionally since exocytosis predominates at the cell front while endocytosis occurs mainly at the cell rear (Bretscher and Aguado-Velasco, 1998). A more plausible effect of the $[Ca^{2+}]_i$ gradient in promoting cell migration would be to induce polarization of the activities of enzymes regulating actin polymerization/depolymerization, integrin activation/assembly/disassembly, and myosin II contractility (Lauffenburger and Horwitz, 1996; Sheetz *et al.*, 1999; Ridley *et al.*, 2003).

3. $[Ca^{2+}]_i$ Transients

$[Ca^{2+}]_i$ transients have been associated with an even wider variety of other processes including fertilization, cell differentiation, exocytosis, muscle contraction, phagocytosis, and neuronal outgrowth and migration (Berridge *et al.*, 2003). This may be because a $[Ca^{2+}]_i$ transient provides a more efficient and safe way to achieve high levels of $[Ca^{2+}]_i$ compared with steady-state elevations. Furthermore, the temporal component of the signal provides an added dimension in terms of encoding information. $[Ca^{2+}]_i$ transients can take a number of forms in motile cells—they can be highly localized and associated with pseudopod (or bleb) protrusion or retraction, they can spread throughout the cell as a regenerative $[Ca^{2+}]_i$ wave, or they can circumnavigate the perimeter of a cell in a clockwise or anticlockwise direction (Kindzelskii *et al.*, 2004). $[Ca^{2+}]_i$ transients can be generated spontaneously or can be induced experimentally by electrical, chemical, and mechanical stimuli. In particular, it has been shown that direct mechanical stretch of fibroblasts and keratocytes, and osmotic swelling of endothelial cells can induce $[Ca^{2+}]_i$ transients (Arora *et al.*, 1994; Oike *et al.*, 1994; Lee *et al.*, 1999; Wu *et al.*, 1999). $[Ca^{2+}]_i$ transients may also have different initiation sites on different cells and these site may vary within a single cell during the course of the migratory cycle. In particular, the initiation sites of

$[Ca^{2+}]$ transients have been related to the distribution of membrane rafts and caveolae (i.e., invaginated membrane structures), which contain the molecular signaling machinery required for Ca^{2+} signaling, and can undergo redistribution during migration and specific forms of stimulation. Membrane raft- and caveolae-dependent Ca^{2+} signaling has been observed in cells undergoing both mesenchymal migration (Manes *et al.*, 1999; Isshiki *et al.*, 2002; Parat *et al.*, 2003; Rizzo *et al.*, 2003) and amoeboid migration (Gomez-Mouton *et al.*, 2001; Pierini *et al.*, 2003; Kindzelskii *et al.*, 2004). For example, Isshiki *et al.* (2002) found that the caveolae in quiescent endothelial cells are clustered around the edge of the cell but when stimulated to migrate, either by wounding a cell monolayer or by exposing the cells to laminar shear stress, the caveolae move to the trailing edge of the cell, concomitant with this relocation the sites of Ca^{2+} waves initiation move to the same location (see also Rizzo *et al.*, 2003; Beardsley *et al.*, 2005). In contrast, in human neutrophils lipid rafts and $[Ca^{2+}]_i$ transient initiation sites have been localized to the leading edge of the migrating cells, and cholesterol depletion, which disrupts raft structure, was found to block both $[Ca^{2+}]_i$ transient initiation and cell migration (Manes *et al.*, 1999; Kindzelskii *et al.*, 2004). Some insight into the different results may be related to the demonstration that both the leading edge and rear of lymphocytes are enriched in lipid components that partition into different raft-like domains (Gomez-Mouton *et al.*, 2001) and that Cav-1, a raft maker, shows a different polarized distribution in endothelial cells depending on whether the cells were migrating on 2D substrate or through a 3D matrix (Parat *et al.*, 2003). In particular, Cave-1 moves from the cell's rear to the cell's front during the switch from the 2D/mesenchymal to the 3D/amoeboid migration modes. These findings are highly intriguing giving that TRPC1, a structural subunit of MscCa (Maroto *et al.*, 2005), colocalizes with Cave-1-associated membrane lipid rafts (Lockwich *et al.*, 2000; Brazier *et al.*, 2003) and has been localized at the leading edge of migrating neutrophils (Kindzelskii *et al.*, 2004) and the rear of migrating prostate tumor cells (Maroto *et al.*, 2007). Together these results indicate that MscCa may redistribute to different regions of the cell surface and perform different, yet critical functions depending on the mode of migration. In this case, MscCa seems to meet the critical criterion of modulating all modes of migration, and unlike integrins, myosin II, calpain, and metalloproteases should not become dispensable following a switch in migration mode.

4. $[Ca^{2+}]_i$ Transients Promote Cell Migration but Inhibit Neurite Outgrowth

$[Ca^{2+}]_i$ transients have been positively correlated with cell migration in cerebellar granular cells, neutrophils, vascular smooth muscle, keratocytes and astrocytoma cells (Komuro and Rakic, 1996; Lee *et al.*, 1999; Ronde

et al., 2000; Scherberich *et al.*, 2000; Giannone *et al.*, 2002). Furthermore, the cessation of $[Ca^{2+}]_i$ transients has been correlated with the termination of granule cell migration (Kumuda and Komuro, 2004). In contrast, high-frequency $[Ca^{2+}]_i$ transients cause nerve growth cone stalling and axon retraction, while the inhibition of $[Ca^{2+}]_i$ transients stimulates the extension of axonal growth cones and the outgrowth of axonal and dendritic filipodia (Gomez and Spitzer, 1999; Gomez *et al.*, 2001; Robles *et al.*, 2003; Lohmann *et al.*, 2005). The $[Ca^{2+}]_i$ transients in all cases appear to depend on MscCa-mediated Ca^{2+} influx because they are blocked by anti-MscCa agents (Lee *et al.*, 1999; Jacques-Fricke *et al.*, 2006). Furthermore, the opposite effects both appear to depend on calpain activity (Huttenlocher *et al.*, 1997; Robles *et al.*, 2003). However, whereas calpain activity in the cell rear acts to cleave integrin–CSK linkages and in this way promotes rear retraction and cell migration (Huttenlocher *et al.*, 1997), calpain activity in the nerve growth cone and filopodia acts by promoting actin–integrin disengagement at the front of the process, thereby reducing the traction forces required for lamellar protrusion and growth cone translocation (Robles *et al.*, 2003). Interestingly, calpain inhibition in resting neutrophils promotes polarization and random migration whereas it reduces the neutrophil's capacity for directional migration toward chemotactic stimuli (Lokuta *et al.*, 2003). This may occur because constitutive calpain activity in resting neutrophils acts as a negative regulator of polarization and migration, whereas the polarized calpain activity in chemotaxing neutrophils promotes directional persistence in a chemo-attractant gradient.

V. THE ROLE OF MscCa IN CELL MIGRATION

A key issue for all modes of cell migration is the nature of the mechanosensitive molecules that act to coordinate forward cell protrusion with rear cell retraction. An attractive candidate is MscCa that because of its unique ability to transduce membrane stretch/cell extension and transduce this into a Ca^{2+} influx (Guharay and Sachs, 1984; Sachs and Morris, 1998; Hamill and Martinac, 2001; Hamill, 2006) can provide feedback between mechanical forces that tend to extend the cell and the Ca^{2+}-sensitive regulators of force generation and cell–substrate adhesion. The first indirect evidence for a role of MscCa in cell migration was provided by the demonstration that the nonspecific MscCa blocker Gd^{3+} (Yang and Sachs, 1989; Hamill and McBride, 1996) blocked fish keratocyte migration (Lee *et al.*, 1999; Doyle and Lee, 2004; Doyle *et al.*, 2004). Subsequent studies, also using Gd^{3+}, further implicated MscCa in migration of a mouse fibroblast cell line, NIH3T3 (Munevar *et al.*, 2004), and the human fibrosarcoma cell line, HT1080 (Huang *et al.*, 2004).

However, these studies indicated different sites (i.e., front or back) and different actions (i.e., rear retraction, development of tractions forces, and disassembly of focal adhesions) for MscCa mediated Ca^{2+} influx, which may partly depend upon different modes of cell migration. Significant limitations in these early studies were the lack of protein identity of MscCa and the absence of MscCa-specific reagents, which have been overcome by the recent identification of the canonical transient receptor potential (TRPC1) (Wes *et al.*, 1995) as an MscCa subunit (Maroto *et al.*, 2005), and the discovery of a highly selective MS channel blocker, GsMTx4 a peptide isolated from the tarantula (*Grammostola spatulata*) venom (Suchyna *et al.*, 2004). Several studies have already implicated TRPC1 in regulating cell migration. For example, Huang *et al.* (2003) showed immunohistologically that TRPC1 was expressed in a punctuate pattern around the cell periphery, and based on Gd^{3+} block proposed that TRPC1 supported $[Ca^{2+}]_i$ transients and cell migration. Rao *et al.* (2006) while studying an intestinal epithelial cell line demonstrated that suppression of TRPC1 inhibited cell migration, whereas TRPC1 overexpression of TRPC1 enhanced cell migration as measured by an *in vitro* wound closure assay. Maroto *et al.* (2007) characterized MscCa in both motile (PC-3) and nonmotile (LNCaP) human prostate tumor cell lines and found that MscCa displayed the same single-channel conductance, Mg^{2+} and Gd^{3+} sensitivity as the MscCa endogenously expressed in *Xenopus* oocytes identified as formed by TRPC1 (Maroto *et al.*, 2005). Furthermore, MscCa activity was shown to be required for cell migration based on the block by anti-MscCa/TRPC1 agents including GsMTx4, an anti-TRPC1 antibody raised against the external pore region of the channel, siRNA suppression, and overexpression of TRPC1.

Apart from MscCa, there are other Ca^{2+} channels that have been implicated in regulating cell migration including both the T-type (Huang *et al.*, 2004) and L-type voltage-gated Ca^{2+} channels (Yang and Huang, 2005) that may also display mechanosensitivity (Morris and Juranka, Chapter 11, this volume). Also in addition to the TRPCs, which have been implicated in forming MscCa, other TRP subfamily members are expressed in tumor cells and have been implicated in different steps associated with cancer (Peng *et al.*, 2001; Wissenbach *et al.*, 2001; Nilius *et al.*, 2005; Sánchez *et al.*, 2005). Of particular interest is TRPM7 that has been shown to regulate cell adhesion by regulating calpain via Ca^{2+} influx through the channel (Su *et al.*, 2006) and actomyosin contractility via intrinsic kinase activity of TRPM7 (Clark *et al.*, 2006). Although TRPM7 stretch sensitivity has not been directly demonstrated, it has been shown that fluid shear stress-applied human kidney epithelial cells promote membrane trafficking of TRPM7 to the cell surface (Oancea *et al.*, 2006). Given that fluid shear stress can also trigger cell migration (Isshiki *et al.*, 2002), this may provide an additional MS mechanism to regulate cell motility. In this case, it will be interesting to

determine whether the shear-induced increase in TRPM7 surface expression is also dependent on specific integrin engagement (Maroto and Hamill, 2001) and/or related to the flow-induced recruitment of caveolae to specific regions of the migrating cell (Rizzo *et al*., 2003; Navarro *et al*., 2004).

There are other classes of gated channels that have been implicated in regulating cell migration including voltage-gated Na^+ (Grimes *et al*., 1995; Bennett *et al*., 2004; Onganer and Djamgoz, 2005) and K^+ channels (Laniado *et al*., 2001) and Ca^{2+}-activated K^+ channels (Schwab *et al*., 1994). These different channels may participate in a variety of processes to modulate the pattern of cell migration in the same way as different channels act to produce specific patterns of firing and synaptic release in excitable cells. One would expect that MscCa plays a central role in orchestrating the other channels because of its unique ability to transduce internally and externally generated forces into both depolarization and Ca^{2+} influx.

VI. CAN EXTRINSIC MECHANICAL FORCES ACTING ON MscCa SWITCH ON CELL MIGRATION?

A key question is what causes a cell to switch from a nonmotile to a motile phenotype and vice versa? Although there are numerous studies indicating that growth factors including tumor necrosis factor-α and transforming growth factor-β can increase cell motility by promoting the EMT (Bates and Mercurio, 2003; Masszi *et al*., 2004; Montesano *et al*., 2005; Nawshad *et al*., 2005), less well studied is the potential role of extrinsic mechanical forces in turning on cell motility. However, there are at least two key observations that support such a role. In the first place, it has been demonstrated that stationary cell fragments formed from fish keratocytes and lacking a cell nucleus or a microtubular CSK can be stimulated to polarize and undergo persistent locomotion by the application of fluid shear stress or direct mechanical poking (Verkhovsky *et al*., 1989). Similarly, the application of shear stress to quiescent *Dictyostelium* can cause CSK reorganization and stimulate cell migration (Décavé *et al*., 2003; Fache *et al*., 2005). Furthermore, these latter mechanical effects were shown to be critically dependent on the presence of external Ca^{2+} (Fache *et al*., 2005). One possible explanation is that mechanical forces alter the membrane trafficking (Maroto and Hamill, 2001; Isshiki *et al*., 2002; Rizzo *et al*., 2003) and/or the MscCa-gating properties (Hamill and McBride, 1992, 1997; McBride and Hamill, 1992), which in turn alters the $[Ca^{2+}]_i$ dynamics generated by intrinsic mechanical forces and contributes to further polarization of the cell and directional migration. Several previous studies have already discussed the possible role of the changing mechanical environment in terms of

promoting tumor malignancy, including the possible role of increasing interstitial stress and fluid pressure within a growing tumor (Sarntinoranont *et al*., 2003) and the increased tumor stiffness due to perturbed vasculature and fibrosis (Paszek *et al*., 2005) of stimulating increased cell motility and escape from the encapsulated tumor. In this case, MscCa may serve as both a trigger and mediator of tumor progression to malignancy.

Note Added in Proof

Numata, T., Shimizu, T., and Okada, Y. (*Am. J. Physiol.* **292,** C460–C467, 2007) have recently reported that TRPM7 is a stretch- and swelling-activated cation channel expressed in human epithelial cells and is blocked by Gd^{3+}. These results are consistent with the notion that several classes of mechanosensitive channels may regulate different aspects of tumor cell migration (i.e., forward protrusion and rear retraction) depending upon their differential surface distribution and interaction with downstream Ca^{2+}-sensitive effectors.

Acknowledgments

We thank the Department of Defense, Prostate Cancer Research Program and the National Cancer Institute for their funding support.

References

Alterafi, A., and Zhelev, D. (1997). Transient increase of cytosolic calcium during neutrophil motility responses. *J. Cell. Sci.* **110,** 1967–1977.

Arora, P. D., Bibby, K. J., and McCulloch, C. A. G. (1994). Slow oscillations of free Intracellular calcium ion concentration in human fibroblasts responding to mechanical stretch. *J. Cell. Physiol.* **161,** 187–200.

Arora, P. D., and McCulloch, C. A. (1996). Dependence of fibroblast migration on actin severing activity of gelsolin. *J. Biol. Chem.* **271,** 20516–20523.

Asano, Y., Mizuno, T., Kon, T., Nagasaki, A., Sutoh, K., and Uyeda, T. Q. P. (2004). Keratocyte-like locomotion of *amiB*-null *Dictyostelium* cells. *Cell Motil. Cytoskel.* **59,** 17–27.

Bates, R. C., and Mercurio, A. M. (2003). Tumor necrosis factor-α stimulates the epithelial-to-mesenchymal transition of human colonic organoids. *Mol. Biol. Cell* **14,** 1790–1800.

Beardsley, A., Fang, K., Mertz, H., Castranova, V., Friedn, S., and Liu, J. (2005). Loss of caveolin-1 polarity impedes endothelial cells polarization and directional movement. *J. Biol. Chem.* **280,** 3541–3547.

Bennett, E. S., Smith, B. A., and Harper, J. M. (2004). Voltage-gated Na^{+} channels confer invasive properties on human prostate cancer cells. *Pflügers Arch.* **447,** 908–914.

Berridge, M. J., Bootman, M. D., and Roderick, H. L. (2003). Calcium signaling: Dynamics homeostasis and remodeling. *Nature Revs. Mol. Cell. Biol.* **4,** 517–529.

Blaser, H., Reichman-Fried, M., Castanon, I., Dumstrel, K., Marlow, F. L., Kawakami, K., Soinica-Krezel, L., Heisenberg, C. P., and Raz, E. (2006). Migration of Zebrafish primordial germ cells: A role for myosin contraction and cytoplasmic flow. *Develop. Cell* **11,** 613–627.

Braiman, A., and Priel, Z. (2001). Intracellular stores maintain stable cytosolic Ca^{2+} gradients in epithelial cells by active Ca^{2+} redistribution. *Cell Cal.* **30**(6), 361–371.

Brazier, S. C., Singh, B. B., Liu, X., Swaim, W., and Ambudkar, I. S. (2003). Caveolin-1 contributes to assembly of store-operated Ca^{2+} influx channels by regulating plasma membrane localization of TRPC1. *J. Biol. Chem.* **278,** 27208–27215.

Bretscher, M. S., and Aguado-Velasco, C. (1998). Membrane traffic during cell locomotion. *Curr. Opin. Cell Biol.* **10,** 537–541.

Brundage, R. A., Fogarty, K. E., Tuft, R. A., and Fay, F. S. (1991). Calcium gradients underlying polarization and chemotaxis of eosinophils. *Science* **254,** 703–706.

Cahill, D. P., Kinzler, K. W., Vogelstein, B., and Lengauer, C. (2000). Genetic instability and Darwinian selection in tumors. *Trends Genet.* **15,** M57–M60.

Carragher, N. O., Walker, S. M., Scott, L. A., Harris, F., Sawyer, T. K., Brunton, V. G., Ozanne, B. W., and Frame, M. C. (2005). Calpain 2 and Src dependence distinguishes mesenchymal and amoeboid modes of tumor cell invasion linking to integrin function. *Oncogene* **25,** 5726–5740.

Clark, K., Langeslag, M., Van Leeuwen, B., Ran, L., Ryazanov, A. G., Figdor, C. G., Moolenaar, W. H., Jalink, K., and Van Leeuwen, F. N. (2006). TRPM7, a novel regulator of actomyosin contractility and cell adhesion. *EMBO J.* **25,** 290–301.

DeBiasio, R., Bright, G. R., Ernst, L. A., Waggoner, A. S., and Taylor, D. L. (1987). Five parameter fluorescent imaging: Wound healing of living Swiss 3T3 cells. *J. Cell Biol.* **105,** 1613–1622.

Décavé, E., Rieu, D., Dalous, J., Fache, S., Brechet, Y., Fourcade, B., Satre, M., and Bruckert, F. (2003). Shear flow-induced motility of *Dictyostelium discoideum* cells on solid substrate. *J. Cell Sci.* **116,** 4331–4343.

Demuro, A., and Parker, I. (2005). ''Optical Patch-clamping'': Single-channel recording by imaging Ca^{2+} flux through individual muscle acetylcholine receptor channels. *J. Gen. Physiol.* **126,** 179–192.

Doyle, A., Marganski, W., and Lee, J. (2004). Calcium transients induce spatially coordinated increases in traction force during the movement of fish keratocytes. *J. Cell Sci.* **117,** 2203–2214.

Doyle, A. D., and Lee, J. (2004). Cyclic changes in keratocyte speed and traction stress arise from Ca^{2+}-dependent regulation of cell adhesiveness. *J. Cell Sci.* **118,** 369–379.

Dreval, V., Dieterrich, P., Stock, C., and Schwab, A. (2005). The role of Ca^{2+} transport across the plasma membrane for cell migration. *Cell Physiol. Biochem.* **16,** 119–128.

Eddy, R. J., Pierini, L. M., Matsumura, F., and Maxfield, F. R. (2000). Ca^{2+}-dependent myosin II activation is required for uropod retraction during neutrophil migration. *J. Cell Sci.* **113,** 1287–1298.

Emmelot, P., and Scherer, E. (1977). Multi-hit kinetics of tumor formation with special reference to experimental liver and human lung carcinogenesis and some general conclusions. *Cancer Res.* **37,** 1702–1708.

Erickson, C. A., and Trinkhaus, J. P. (1976). Microvilli and blebs as sources of reserve surface membrane during cell spreading. *Exp. Cell Res.* **99,** 375–384.

Fache, S., Dalous, J., Engelund, M., Hansen, C., Chamaraux, F., Fourcade, B., Satre, M., Devreotes, P., and Bruckert, F. (2005). Calcium mobilization stimulates *Dictyostelium discoideum* shear-flow-induced cell motility. *J. Cell Sci.* **118,** 3445–3457.

Fisher, P. R., and Wilczynska, Z. (2006). Contribution of endoplasmic reticulum to Ca^{2+} signals in *Dictyostelium* depends on extracellular Ca^{2+}. *FEMS Microbial. Lett.* **257,** 268–277.

Franco, S. J., and Huttenlocher, A. (2005). Regulating cell migration: Calpains make the cut. *J. Cell Sci.* **118,** 3829–3838.

Friedl, P. (2004). Prespecification and plasticity: Shifting mechanisms of cell migration. *Curr. Opin. Cell Biol.* **16,** 14–23.

Friedl, P., and Wolf, K. (2003). Tumor cell invasion: Diversity and escape mechanisms. *Nature Rev. Cancer* **3,** 362–374.

Galbraith, C. G., and Sheetz, M. P. (1999). Keratocytes pull with similar forces on their dorsal and ventral surfaces. *J. Cell. Biol.* **147,** 1313–1323.

Giannone, G., Ronde, P., Gaire, M., Haiech, J., and Takeda, K. (2002). Calcium oscillations trigger focal adhesion disassembly in human U87 astrocytoma cells. *J. Biol. Chem.* **277,** 26364–26371.

Gilbert, S. H., Perry, K., and Fay, F. S. (1994). Mediation of chemoattractant-induced changes in $[Ca^{2+}]_i$ and cell shape, polarity and locomotion by $InsP_3$, DAG, and protein kinase C in Newt Eosinophils. *J. Cell Biol.* **127,** 489–503.

Gollnick, F., Meyer, R., and Stockem, W. (1991). Visualization and measurement of calcium transients in *Amoeboid proetus* by fura-2 fluorescence. *Eur. J. Cell Biol.* **55,** 262–271.

Gomez, T. M., and Spitzer, N. C. (1999). *In vivo* regulation of axon extension and pathfinding by growth cone calcium transients. *Nature* **397,** 350–355.

Gomez, T. M., Robles, E., Poo, M., and Spitzer, N. C. (2001). Filopoidal calcium transients promote substrate-dependent growth cone turning. *Science* **291,** 1983–1987.

Gomez-Mouton, C., Abad, J. L., Mira, E., Lacalle, R. A., Gallardo, E., Jimenez-Baranda, S., Illa, I., Bernad, A., Manes, S., and Martinez-A, C. (2001). Segregation of leading-edge and uropod components into specific lipid rafts during T cell polarization. *Proc. Natl. Acad. Sci. USA* **98,** 9642–9647.

Grimes, J. A., Fraser, S. P., Stephens, G. J., Downing, J. E. G., Laniado, M. E., Foster, C. S., Abel, P. D., and Djamgoz, M. B. A. (1995). Differential expression of voltage-activated Na^+ currents in two prostatic tumor cell lines: Contribution to invasiveness *in vitro*. *FEBS Letts.* **369,** 290–294.

Grimstad, I. A. (1987). Direct evidence that cancer cell locomotion contributes importantly to invasion. *Exp. Cell Res.* **173,** 515–523.

Grynkiewicz, G., Poenie, M., and Tsien, R. (1985). A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* **260,** 3440–3450.

Guharay, F., and Sachs, F. (1984). Stretch activated single ion channel currents in tissue cultured embryonic chick skeletal muscle. *J. Physiol.* **352,** 685–701.

Hahn, K., Debiasio, R., and Taylor, D. L. (1992). Patterns of elevated free calcium and calmodulin in living cells. *Nature* **359,** 736–738.

Hamill, O. P. (2006). Twenty odd years of stretch activated channels. *Pflügers Arch.* **453,** 333–351.

Hamill, O. P., and Martinac, B. (2001). Molecular basis of mechanotransduction in living cells. *Physiol. Rev.* **81,** 685–740.

Hamill, O. P., and McBride, D. W., Jr. (1992). Rapid adaptation of single mechanosensitive channels in *Xenopus* oocytes. *Proc. Natl. Acad. Sci. USA* **89,** 7462–7466.

Hamill, O. P., and McBride, D. W., Jr. (1996). The pharmacology of mechanogated membrane ion channels. *Pharmacol. Rev.* **48,** 231–252.

Hamill, O. P., and McBride, D. W., Jr. (1997). Induced membrane hypo/hyper-mechanosensitivity: A limitation of patch-clamp recording. *Ann. Rev. Physiol.* **59,** 621–631.

Hamill, O. P., Marty, A., Neher, E., Sakmann, B., and Sigworth, F. J. (1981). Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. *Pflügers Arch.* **391,** 85–100.

Hanahan, D., and Weinberg, R. A. (2000). The hallmarks of cancer. *Cell* **100,** 57–70.

Hawkes, R. B., and Hoberton, D. V. (1973). A calcium-sensitive lanthanum inhibition of amoeboid movement. *J. Cell. Physiol.* **81**(3), 365–370.

Hendey, B., and Maxfield, F. R. (1993). Regulation of neutrophil motility and adhesion by intracellular calcium transients. *Blood Cells* **19,** 143–164.

Huang, J. B., Kindzelskii, A. L., Clark, A. J., and Petty, H. R. (2004). Identification of channels promoting calcium spikes and waves in HT1080 Tumor cells: Their apparent roles in cell motility and invasion. *Cancer Res.* **64,** 2482–2489.

Huttenlocher, A., Palecek, S. P., Lu, Q., Zhang, W., Mellgren, R. L., Lauffenburger, D. A., Ginsberg, M., and Horowitz, A. (1997). Regulation of cell migration by calcium-dependent protease calpain. *J. Biol. Chem.* **272,** 32719–32722.

Isshiki, M., Ando, J., Yamamoto, K., Fujta, T., and Ying, Y. (2002). Sites of Ca^{2+} wave initiation move with caveola to the trailing edge of migrating cells. *J. Cell Sci.* **115,** 475–484.

Jacques-Fricke, B. T., Seow, Y., Gottlieb, P. A., Sachs, F., and Gomez, T. M. (2006). Ca^{2+} influx through mechanosensitive channels inhibits neurite outgrowth in opposition to other influx pathways and release of intracellular stores. *J. Neurosci.* **26,** 5656–5664.

Kaighn, M. E., Narayan, K. S., Ohnuki, Y., Lechner, J. F., and Jones, L. W. (1979). Establishment and characterization of a human prostatic carcinoma cell line (PC-3). *Invest. Urol.* **17,** 16–23.

Kasai, H., and Augustine, G. J. (1990). Cytosolic Ca^{2+} gradients triggering unidirectional fluid secretion from exocrine pancreas. *Nature* **348,** 735–738.

Kassis, J., Lauffenburger, D. A., Turner, T., and Wells, A. (2001). Tumor invasion as dysregulated cell motility. *Semin. Cancer Biol.* **11,** 105–117.

Kimura, C., Oike, M., Koyama, T., and Ito, Y. (2001). Alterations of Ca^{2+} mobilizing properties in migrating endothelial cells. *Am. J. Physiol.* **281,** H745–H754.

Kindzelskii, A. L., Sitrin, R. G., and Petty, H. R. (2004). Cutting edge: Optical microspectrophotometry supports the existence of gel phase lipid rafts at the lamellipodium of neutrophils: Apparent role in calcium signaling. *J. Immunol.* **172,** 4681–4685.

Komuro, H., and Rakic, P. (1996). Intracellular Ca^{2+} fluctuations modulate the rate of neuronal migration. *Neuron* **17,** 275–285.

Korohoda, W., Madeja, Z., and Sroka, J. (2002). Diverse chemotactic responses of *Dictystelium discoideum* Amoeba in the developing (temporal) and stationary (spatial) concentration gradients of folic acid, cAMP, Ca^{2+} and Mg^{2+}. *Cell Motil. Cytoskel.* **53,** 1–25.

Kumuda, T., and Komuro, H. (2004). Completion of neuronal migration regulated by loss of Ca^{2+} transients. *Proc. Natl. Acad. Sci. USA* **101,** 8479–8484.

Laffafian, I., and Hallet, M. B. (1995). Does cytosolic free Ca^{2+} signal neutrophil chemotaxis in response to formylated chemotactic peptide? *J. Cell Sci.* **108,** 3199–3205.

Laniado, M. E., Fraser, S. P., and Djamgoz, M. B. (2001). Voltage-gated K^+ channel activity in human prostate cancer cell lines of markedly different metastatic potential: Distinguishing characteristics of PC-3 and LNCaP cells. *Prostate* **46,** 262–274.

Lauffenburger, D. A., and Horwitz, A. F. (1996). Cell migration: A physically integrated molecular process. *Cell* **84,** 359–369.

Lee, J., Ishihara, A., Oxford, G., Johnson, B., and Jacobson, K. (1999). Regulation of cell movement is mediated by stretch-activated calcium channels. *Nature* **400,** 382–386.

Levina, N. N., and Lew, R. R. (2006). The role of tip localized mitochondria in hyphal growth. *Fungal Genet. Biol.* **43,** 65–74.

Liu, L., Ridefelt, P., Håkansson, L., and Venge, P. (1999). Regulation of human eosinophil migration across lung epithelial monolayers by distinct calcium signaling mechanisms in the two cells types. *J. Immunol.* **163,** 5649–5655.

Liu, L., Håkansson, L., Ridefelt, P., Garcia, R. C., and Venge, P. (2003). Priming of eosinophil migration across lung epithelial cell monolayers and upregulation of CD11b/CD18 are elicited by extracellular Ca^{2+}. *Am. J. Respir. Cell Mol. Biol.* **28,** 713–721.

Lockwich, T. P., Liu, X., Singh, B. B., Jadlowiec, J., Weiland, S., and Ambudkar, I. S. (2000). Assembly of Trp1 in a signaling complex associated with caveolin-scaffolding lipid raft domains. *J. Biol. Chem.* **275,** 11934–11942.

Lohmann, C., Finski, A., and Bonhoeffer, T. (2005). Local calcium transients regulate the spontaneous motility of dendritic filopodia. *Nature Neurosci.* **8,** 305–312.

Lokuta, M. A., Nuzzi, P. A., and Huttnelocher, A. (2003). Calpain regulates neutrophils chemotaxis. *Proc. Natl. Acad. Sci. USA* **100,** 4006–4011.

Malawista, S. E., and Boisfleury-Chevance, A. (1997). Random locomotion and chemotaxis of human blood polymorphonuclear leukocytes (PMN) in the presence of EDTA: PMN in close quarters require neither leukocyte integrins nor external divalent cations. *Proc. Natl. Acad. Sci. USA* **94,** 11577–11582.

Mamoune, A., Luo, J. H., Lauffenburger, D. A., and Wells, A. (2003). Calpain-2 as a target for limiting prostate cancer invasion. *Cancer Res.* **63,** 4632–4640.

Mandeville, J. T. H., and Maxfield, F. R. (1997). Effects of buffering intracellular free calcium on neutrophil migration through three-dimensional matrices. *J. Cell. Physiol.* **171,** 168–178.

Manes, S., Mira, E., Gomez-Mouton, C., Lacalle, R. A., Keller, P., Labrador, J. P., and Martinez-A, C. (1999). Membrane raft domains mediate front-rear polarity in migrating cells. *EMBO J.* **18,** 6211–6220.

Marks, P. W., and Maxfield, F. R. (1990). Transient increases in cytosolic free calcium appear to be required for the migration of adherent human neutrophils. *J. Cell Biol.* **110,** 43–52.

Maroto, R., and Hamill, O. P. (2001). Brefeldin A block of integrin-dependent mechanosensitive ATP release from *Xenopus* oocytes reveals a novel mechanism of mechanotransduction. *J. Biol. Chem.* **276,** 23867–23872.

Maroto, R., Raso, A., Wood, T. G., Kurosky, A., Martinac, B., and Hamill, O. P. (2005). TRPC1 forms the stretch-activated cation channel in vertebrate cells. *Nature Cell Biol.* **7,** 179–185.

Maroto, R., Kurosky, A., and Hamill, O. P. (2007). The role of MscCa in prostate tumor cell migration. (submitted for publication.)

Masszi, A., Fan, L., Rosivall, L., McCulloch, C. A., Rotstein, O. D., Mucsi, I., and Kapus, A. (2004). Integrity of cell-cell contacts is a critical regulator of TGF-beta 1-induced epithelial-to-myofibroblast transition: Role for beta-catenin. *Am. J. Pathol.* **165,** 1955–1967.

McBride, D. W., Jr., and Hamill, O. P. (1992). Pressure-clamp: A method for rapid step perturbation of mechanosensitive channels. *Pflügers Arch.* **421,** 606–612.

Miyawaki, A., Llopis, J., Heim, R., McCaffery, J. M., Adams, J. A., Ikura, M., and Tsien, R. Y. (1997). Fluorescent indicators for Ca^{2+} based on green fluorescent proteins and calmodulin. *Nature* **388,** 882–887.

Mobasheri, A., Carter, S. D., Martin-Vasallo, P., and Shakibaei, M. (2002). Integrins and stretch-activated ion channels; putative components of functional cell surface mechano-receptors in articular chondrocytes. *Cell Biol. Int.* **26,** 1–18.

Montesano, R., Soulie, P., Eble, J. A., and Carrozzino, F. (2005). Tumor necrosis factor-α confers an invasive transformed phenotype on mammary epithelial cells. *J. Cell Sci.* **118,** 3487–3500.

Munevar, S., Wang, Y. L., and Dembo, M. (2004). Regulation of mechanical interactions between fibroblasts and the substratum by stretch-activated Ca^{2+} entry. *J. Cell Sci.* **117,** 85–92.

Navarro, A., Anand-Apte, A. B., and Parat, M. O. (2004). A role for caveola in cell migration. *FASEB J.* **18,** 1801–1811.

Nawshad, A., Lagamba, D., Polad, A., and Hay, E. D. (2005). Transforming growth factor-β-signaling during epithelial-mesenchymal transformation: Implications for embryogenesis and tumor metastasis. *Cells Tissues Organs* **179,** 11–23.

Nebl, T., and Fisher, P. R. (1997). Intracellular Ca^{2+} signals in *Dictyostelium* chemotaxis are mediated exclusively by Ca^{2+} influx. *J. Cell Sci.* **110,** 2845–2853.

Nilius, B., Voets, T., and Peters, J. (2005). TRP channels in disease. *Sci. STRKE,* re8.

Oancea, E., Wolfe, J. T., and Clapham, D. E. (2006). Functional TRPM7 channels accumulate at the plasma membrane in response to fluid flow. *Circ. Res.* **98,** 245–253.

Oike, M., Droogmans, G., and Nilius, B. (1994). Mechanosensitive Ca^{2+} transients in endothelial cells from human umbilical vein. *Proc. Natl. Acad. Sci. USA* **91,** 2940–2944.

Onganer, P. U., and Djamgoz, M. B. A. (2005). Small-cell lung cancer (human); potentiation of endocytoc membrane activity by voltage-gated Na^{+} channel expression *in vitro*. *J. Membr. Biol.* **204,** 67–75.

Parat, M. O., Anand-Apte, B., and Fox, P. L. (2003). Differential caveolin-1 polarization in endothelial cells during migration in two and three dimensions. *Mol. Biol. Cell* **14,** 3156–3168.

Paszek, M. J., Zahir, N., Johnson, K. R., Lakins, J. N., Rozenberg, G. I., Gefen, A., Reinhardt-King, C. A., Margulies, S. S., Dembo, M., Boettiger, D., Weaver, D. A., and hammer, V. A. (2005). Tensional homeostasis and the malignant phenotype. *Cancer Cell* **8,** 241–254.

Peng, J. B., Zhuang, L., Berger, U. V., Adam, R. M., Williams, B. J., Brown, E. M., Hediger, M. A., and Freeman, M. R. (2001). CaT1 expression correlates with tumor grade in prostate cancer. *Biochem. Biophys. Res. Commun.* **282,** 729–734.

Petersen, O. H., Tepikin, A., and Park, M. K. (2001). The endoplasmic reticulum: One continuous or several separate Ca^{2+} stores? *Trends Neurosci.* **24,** 271–276.

Pierini, L.M, Eddy, R. J., Fuortes, M., Seveau, S., Casulo, C., and Maxfield, F. R. (2003). Membrane lipid organization is critical for human neutrophil polarization. *J. Biol. Chem.* **278,** 10831–10841.

Quintana, A., and Hoth, M. (2004). Apparent cystosolic calcium gradients in T-lymphocytes due to fura-2 accumulation in mitochondria. *Cell Calc.* **36,** 99–109.

Ramaswamy, S., Ross, K. N., Lander, E. S., and Golub, T. R. (2003). A molecular signature in primary solid tumors. *Nature Genet.* **33,** 49–54.

Rao, J. N., Platoshyn, O., Golovina, V. A., Liu, L., Zou, T., Marasa, B. S., Turner, D. J., Yuan, J. X. L., and Wang, J. Y. (2006). TRPC1 functions as a store-operated Ca^{2+} channel in intestinal epithelial cells and regulates early mucosal restitution after wounding. *Am. J. Physiol.* **290,** G782–G792.

Ridley, A. J., Schwartz, M. A., Burridge, K., Firtel, R. A., Ginsberg, M. H., Borisy, G., Parsons, J. T., and Horwitz, A. R. (2003). Cell migration: Integrating signals from front to back. *Science* **302,** 1704–1709.

Rizzo, V., Morton, C., Depaola, N., Schnitzer, J. E., and Davies, P. F. (2003). Recruitment of endothelial caveola into mechanotransduction pathways in flow conditioning *in vitro*. *Am. J. Physiol.* **285,** H1720–H1729.

Robles, E., Huttenlocher, A., and Gomez, T. M. (2003). Filopodial calcium transients regulate growth cone motility and guidance through local activation of calpain. *Neuron* **38,** 597–609.

Ronde, P., Giannone, G., Gerasymova, I., Stoeckel, H., Takeda, K., and Haiech, J. (2000). Mechanism of Calcium oscillations in human astrocytoma cells. *Biochim. Biophys. Acta* **1498,** 273–280.

Saadoun, S., Papadopoulos, M. C., Hara-Chikuma, M., and Verkman, A. S. (2005). Impairmant of angiogeneisis and cell migration by targeted aquaporin-1 gene disruption. *Nature* **434,** 786–792.

Sachs, F., and Morris, C. E. (1998). Mechanosensitive ion channels in nonspecialized cells. *Revs. Physiol. Biochem. Pharmacol.* **132,** 1–77.

Sahai, E., and Marshall, C. J. (2003). Different modes of tumor cell invasion have distinct requirements for Rho/ROCK signaling and extracellular proteolysis. *Nat. Cell Biol.* **5,** 711–719.

Saimi, Y., Zhou, X., Loukin, S. H., Haynes, W. J., and Kung, C. (2007). Microbial TRP channels and their mechanosensitivity. *Curr. Top. Memb.* **58,** 311–327.

Sánchez, M. G., Sanchez, A. M., Collado, B., Malagarie-Cazenave, S., Olea, N., Carmena, M. J., Prieto, J. C., and Diaz-Laviada, I. I. (2005). Expression of the transient receptor potential vanilliod 1 (TRPV1) in LNCaP and PC-3 prostate cancer cells and in prostate tissue. *Eur. J. Pharmacol.* **515,** 20–27.

Sarntinoranont, M., Rooney, F., and Ferrari, M. (2003). Interstitial stress and fluid pressure within a growing tumor. *Ann. Biomed. Eng.* **31,** 327–335.

Schaloske, R. H., Lusche, D. F., Bezares-Roder, K., Happle, K., Malchow, D., and Schlatterer, C. (2005). Ca^{2+} regulation in the absence of the *iplA* gene product in *Dictyostelium discoideum*. *BMC Cell Biol.* **6,** 13–30.

Shanley, L. J., Walczysko, P., Bain, M., MacEwan, D. J., and Zhao, M. (2006). Influx of extracellular Ca^{2+} is necessary for electrotaxis in *Dictyostelium*. *J. Cell Sci.* **119,** 4741–4748.

Scherberich, A., Campos-Toimil, M., Ronde, P., Takeda, K., and Baretz, A. (2000). Migration of human vascular smooth muscle cells involves serum-dependent repeated cytosolic calcium transients. *J. Cell Sci.* **113,** 653–662.

Schwab, A., Wojnowski, L., Gabriel, K, and Oberleither, H. (1994). Oscillating activity of a Ca^{2+}-sensitive K^+ channel: A prerequisite for migration of transformed Mardin-Darby canine kidney focus cells. *J. Clin. Invest.* **93,** 1631–1636.

Schwab, A., Finsterwalder, F., Kersting, U., Danker, T., and Oberleithner, H. (1997). Intracellular Ca^{2+} distribution in migrating transformed renal epithelial cells. *Pflügers Arch.* **434,** 70–76.

Schwab, A., Nechyporuk-Zloy, V., Fabian, A., and Stock, C. (2006). Cells move when ions and water flow. *Pflügers Arch.* **453,** 421.

Sheetz, M. P., Felsenfeld, D., Galbraith, C. G., and Choquet, D. (1999). Cell migration as a five-step cycle. *Biochem. Soc. Sympos.* **65,** 233–243.

Silverman-Gavrila, L. B., and Lew, R. R. (2003). Calcium gradient dependence of *Neurospora crassa* hyphal growth. *Microbiology* **149,** 2475–2485.

Stracke, M. L., Aznavoorian, S. A., Beckner, M. E., Liotta, L. A., and Schiffmann, E. (1991). Cell motility, a principal requirement for metastasis. *EXS* **59,** 147–162.

Strohmeier, R., and Bereiter-Hahn, J. (1984). Control of cell shape and locomotion by external calcium. *Exp. Cell Res.* **154,** 412–420.

Su, L. T., Agapito, M. A., Li, M., Simonson, W. T. N., Huttenlocher, A., Habas, R., Yue, L., and Runnels, L. W. (2006). TRPM7 regulates cell adhesion by controlling the calcium-dependent protease calpain. *J. Biol. Chem.* **281,** 11260–11270.

Suchyna, T. M., Tape, S. E., Koeppe, R. E., II, Andersen, O. S., Sachs, F., and Gottlieb, P. A. (2004). Bilayer-dependent inhibition of mechanosensitive channels by neuroactive peptide enantiomers. *Nature* **430,** 235–240.

Sung, V., Gilles, C., Clarke, M. R., Aaron, A. D., Azumi, N., and Thompson, E. W. (1998). The LCC15-MB human breast cancer cell line expresses osteopontin and exhibits an invasive and metastatic phenotype. *Exp. Cell Res.* **241,** 273–284.

Tarin, D. (2005). The fallacy of epithelial mesenchymal transition in neoplasia. *Cancer Res.* **65,** 5996–6001.

Taylor, D. L., Blinks, J. R., and Reynolds, G. (1980). Contractile basis of amoeboid movement VIII. Aequorin Luminescence during amoeboid movement, endocytosis and capping. *J. Cell Biol.* **86,** 599–607.

Thiery, J. P. (2002). Epithelial-mesenchymal transitions in tumor progression. *Nature Rev. Cancer* **2,** 442–454.

Thompson, E. W., and Newgreen, D. F. (2005). Carcinoma invasion and metastasis: A role for epithelial-mesenchymal transition? *Cancer Res.* **65,** 5991–5995.

Tinel, H., Cancela, J. M., Mogami, H., Geraimenko, J. V., Gerasimenko, O. V., Tepikin, A. V., and Petersen, O. H. (1999). Active mitochondria surrounding the pancreatic acinar granule region prevent spreading of inositol trisphosphate-evoked local cytosolic Ca^{2+} signals. *EMBO J.* **18,** 4999–5008.

Traynor, D., Milne, J. L. S., Insall, R. H., and Kay, R. R. (2000). Ca^{2+} signaling is not required for chemotaxis in. *Dictyostelium. EMBO J.* **19,** 4846–4854.

Unterweger, N., and Schlatterer, C. (1995). Introduction of calcium buffers into the cytosol of *Dictyostelium discoideum* amoeba alters cell morphology and inhibits chemotaxis. *Cell Cal.* **17,** 97–110.

Varambally, S., Yu, J., Laxman, B., Rhodes, D. R., Mehra, R., Tomlins, S. A., Shah, R. B., Chandran, U., Monzon, F. A., Becich, M. J., Wei, J. T., Pienta, K. J., *et al.* (2005). Integrative genomic and proteomic analysis of prostate cancer reveals signatures of metastatic progression. *Cancer Cell* **8,** 393–406.

Verkhovsky, A. B., Svitkina, T. M., and Borisy, G. G. (1989). Self-polarization and directional motility of cytoplasm. *Curr. Biol.* **9,** 11–20.

Wahl, M., Sleight, R. G., and Gurenstein, E. (1992). Association of cytoplasmic free Ca^{2+} gradients with subcellular organelles. *J. Cell Physiol.* **150,** 593–609.

Wes, P. D., Chevesich, J., Jeromin, A., Rosenberg, C., Stetten, G., and Montell, C. (1995). TRPC1, a human homolog of a *Drosophila* store-operated channel. *Proc. Natl. Acad. Sci. USA* **92,** 9652–9656.

Wissenbach, U., Niemeyer, B. A., Fixemer, T., Schneidewind, A., Trost, C., Cavalié, A., Reus, K., Mee se, E., Bonkhoff, H., and Flockerzi, V. (2001). Expression of CaT-like, a novel calcium-selective channel, correlates with the malignancy of prostate cancer. *J. Biol. Chem.* **276,** 19461–19468.

Wolf, K., and Friedl, P. (2006). Molecular mechanisms of cancer cell invasion and plasticity. *Brit. J. Dermatol.* **154,** 11–15.

Wu, Z., Wong, K., Glogauer, M., Ellen, R. P., and McCulloch, C. A. G. (1999). Regulation of stretch-activated intracellular calcium transients by actin filaments. *Biochem. Biophys. Res. Commun.* **261,** 419–425.

Xu, H., Yuan, X., Guan, C., Duan, S., Wu, C., and Feng, L. (2004). Calcium signaling in chemorepellant Slit2-dependnet regulation of neuronal migration. *Proc. Natl. Acad. Sci. USA* **101,** 4296–4301.

Yang, S., and Huang, X. Y. (2005). Ca^{2+} influx through L-type Ca^{2+} channels controls the trailing tail contraction in growth factor-induced fibroblast cell migration. *J. Biol. Chem.* **280,** 27130–27137.

Yang, X. C., and Sachs, F. (1989). Block of stretch-activated ion channels in *Xenopus* oocytes by gadolinium and calcium ions. *Science* **243,** 1068–1071.

Yi, M., Weaver, D., and Hajnoczky, G. (2004). Control of mitochondrial motility and distribution by the calcium signal: A homeostatic circuit. *J. Cell Biol.* **167,** 661–672.

Yoshida, K., and Soldati, T. (2006). Dissection of amoeboid movement into two mechanically distinct modes. *J. Cell Sci.* **119,** 3833–3844.

Yumura, S., Furuya, K., and Takeuchi, I. (1996). Intracellular free calcium responses during chemotaxis of *Dictyostelium* cells. *J. Cell Sci.* **109,** 2673–2678.

Zhou, X., Rao, N. P., Cole, S. W., Mok, S. C., Chen, Z., and Wong, D. T. (2005). Progress in concurrent analysis of loss of heterozygosity and comparative genomic hybridization utilizing high density nucleotide polymorphism arrays. *Cancer Genet. Cytogenet.* **159,** 53–57.

Zigmond, S. H., Slonczewski, J. L. M., Wilde, M. W., and Carson, M. (1988). Polynuclear leukocyte locomotion is insensitive to lowered cytoplasmic calcium levels. *Cell Motil. Cytoskel.* **9,** 184–189.