Pflügers Archiv - European Journal of Physiology (2024) 476:639–658
https://doi.org/10.1007/s00424-024-02907-2

REVIEW

# pH-regulated single cell migration

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Received: 22 November 2023 / Revised: 21 December 2023 / Accepted: 2 January 2024 / Published online: 12 January 2024
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**Abstract**

Over the last two decades, extra- and intracellular pH have emerged as fundamental regulators of cell motility. Fundamental physiological and pathological processes relying on appropriate cell migration, such as embryonic development, wound healing, and a proper immune defense on the one hand, and autoimmune diseases, metastatic cancer, and the progression of certain parasitic diseases on the other, depend on surrounding pH. In addition, migrating single cells create their own localized pH nanodomains at their surface and in the cytosol. By this means, the migrating cells locally modulate their adhesion to, and the re-arrangement and digestion of, the extracellular matrix. At the same time, the cytosolic nanodomains tune cytoskeletal dynamics along the direction of movement resulting in concerted lamellipodia protrusion and rear end retraction. Extracellular pH gradients as found in wounds, inflamed tissues, or the periphery of tumors stimulate directed cell migration, and long-term exposure to acidic conditions can engender a more migratory and invasive phenotype persisting for hours up to several generations of cells after they have left the acidic milieu. In the present review, the different variants of pH-dependent single cell migration are described. The underlying pH-dependent molecular mechanisms such as conformational changes of adhesion molecules, matrix protease activity, actin (de-)polymerization, and signaling events are explained, and molecular pH sensors stimulated by $H^+$ signaling are presented.

**Keywords** Acid priming · Acid selection · Cell migration · Molecular pH sensors · pH nanodomains · pH taxis

## Introduction

Cell migration is a central component of embryonic development, wound healing, tissue homeostasis, and a proper immune defense. However, aberrant cell migration contributes to various pathologies, such as metastatic cancer and autoimmune diseases. In addition, a number of parasitic protozoa can migrate through certain tissues of the human body, particularly amoebae like the *Entamoeba histolytica* [79] or the brain-eating *Naegleria fowleri* [58]. The process of lamellipodium-driven single cell migration, whether directed or random, entails a set of requirements. A migrating cell needs to (i) polarize in the direction of movement, (ii) fine-tune its attachment to and detachment from the substrate such as extracellular matrix (ECM) components or surrounding cells, (iii) exert (traction) forces on the substrate, and (iv) reorganize or even remove parts of the surrounding ECM, especially when invading a basement membrane. Each of these mechanisms is one way or another related to, if not even directly dependent on, the pH value. While in the healing skin the migration of both fibroblasts and keratinocytes is most efficient at rather physiological pH values of around 7.5 [78], moderately acidic environments as found in tumor tissue or at inflammatory sites promote motility of tumor cells [121, 155] and neutrophils [111], respectively. Aside from that, extracellular pH gradients direct migrating cells [114, 166], and long-term exposure to relatively acidic pH values can cause cells to adopt a more migratory and invasive phenotype that can persist for hours up to several generations of cells after they have left the acidic milieu [123, 163]. The present review deals with all of these pH-sensitive parameters involved in cell migration. Underlying molecular mechanisms are explained and critically examined.

This article is part of the special issue on Physiology of systemic and cellular pH regulation in Pflügers Archiv—*European Journal of Physiology*.

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## Extracellular $pH_e$ as a clue inducing cell polarity

Epithelial cells forming a single-layer epithelium feature a highly pronounced apical-basal polarity with the apical membranes facing lumina of internal cavities and the basolateral membranes being orientated away from these lumina towards the underlying tissue. A functional dichotomy accompanies this morphological polarity. Different sets of membrane proteins such as ion channels and transporters, receptors, and adhesion molecules are expressed in the apical and the basolateral membranes, in order to enable epithelia to fulfill their main physiological functions in addition to being a protective barrier between different compartments, namely secretion and absorption. Tight junctions, strong linkages between adjacent epithelial cells, keep these membrane proteins apart in the different membrane sections. In addition, regulated membrane trafficking directs the proteins to their final destination where a number of them are kept in place by adaptor proteins, such as members of the ezrin, radixin, and moesin (ERM) family that tie membrane proteins to the cortical actin cytoskeleton. Generally, the polarity of epithelial cells is predetermined by the given tissue structure and coordinated by the activity and localization of apical-basolateral polarity regulators, e.g., the PDZ-rich scaffold protein Scribble, the PDZ domain–containing adaptors Par3 and Par 6, the PDZ-containing scaffold PATJ, or the transmembrane protein Crumb 3 [99]. These cell polarity–regulating proteins act through molecular mechanisms such as oligomerization, higher-order complex formation, auto-inhibitory interactions, or electrostatic interactions with the plasma membrane [125]. However, as soon as tightly connected, epithelial cells set out to break out of the epithelial order, e.g., during the process of epithelial-to-mesenchymal transition (EMT), they lose their apical-basolateral polarity due to a fundamental reorganization of the cytoskeleton and a loss of cell–cell junctions. These structural changes come along with a profound redistribution of membrane proteins such as ion channels and transporters and receptors. A new, morphologically and functionally different polarity, a front-back polarity, forms, allowing the cells to acquire a migratory phenotype. Not only epithelial cells undergoing EMT, but also migrating cells of any other origin, including endothelial cells, fibroblasts, mesenchymal cells, immune cells, and osteoblasts/-clasts, regardless of their initial polarization status, establish a front-back polarity when they start to migrate, i.e., a morphological and functional polarity along the direction of movement [116]. As the leading edge of a migrating cell protrudes forward, the trailing end retracts.

Cues that trigger single cell polarization can be chemical gradients, mechanical stimuli, membrane tension, substrate rigidity, and electric fields [120]. The amoeba *Dictyostelium discoideum*, commonly referred to as slime mold, is a rewarding model system for investigating the mechanisms of directed amoeboid movement including the different modes of polarization. Exposure to its adequate chemoattractant cAMP induces a polarized, elongated morphology. In a fluid flow without a chemical stimulus, *Dictyostelium* orientates itself along the current, the leading edge pointing upstream, and the retracting tail downstream [28]; and in an electric field, the leading edge is directed towards the electron-emitting cathode [144]. In contrast to the external, spatial cue–driven polarization, spontaneous polarization is based on the cell's intrinsic ability to break symmetry. Positive feedback loops involving the lipid phosphatidylinositol-3,4,5-trisphosphate and/or the small Rho-type GTPase Cdc42 are sufficient to drive spontaneous polarization [96, 182].

In the end, however, in the absence of any spatial cue or chemical substance, the omnipresent parameter affecting polarity of single cells is the environmental pH value (Fig. 1a). *Amoeba proteus* exhibits the most polarized shape, accompanied by the highest migratory activity, at extracellular pH ($pH_e$) values of between pH 5.0 and pH 6.5 (11). Similarly, fMLP (N-formyl-methionyl-leucyl-phenylalanine)–stimulated neutrophils migrate most efficiently at $pH_e$ 7.4–7.6 [148], and also human melanoma (MV3) cells seeded on, or embedded in, a collagen type I matrix show the highest migration speeds when most perfectly polarized at $pH_e$ values of 7.0–7.2 [155]. Intriguingly, cells equipped with efficient acid/base transporters in their plasma membrane, e.g., NHE1, can countervail the impact of the environmental pH [161] by generating their own pH nanoenvironment right at the cell surface [157], stabilized by the glycocalyx [77].

## Interrelations between cell polarity and pH

Regardless of whether induced or spontaneous, the polarization of migrating cells is accompanied by an accumulation of a multitude of ion transporters and channels at their leading edge [156]. These include acid/base transporters such as $Na^+$-$HCO_3^-$ cotransporters (NBCs) (NBCn1 [9, 143] or NBCe1 [164]), anion exchangers (AEs) [72, 164], NHE1 [157], carbonic anhydrases [164], and water-permeable aquaporins [86, 131]. Regarding the migratory process, this accumulation of ion transporters at the leading edge serves at least two purposes.

First, osmotic water influx through aquaporins adjacent to the ion transporters is concomitant with the movement of osmotically active ions into the cytosol, which then leads to local swelling and outgrowth of the lamellipodium in

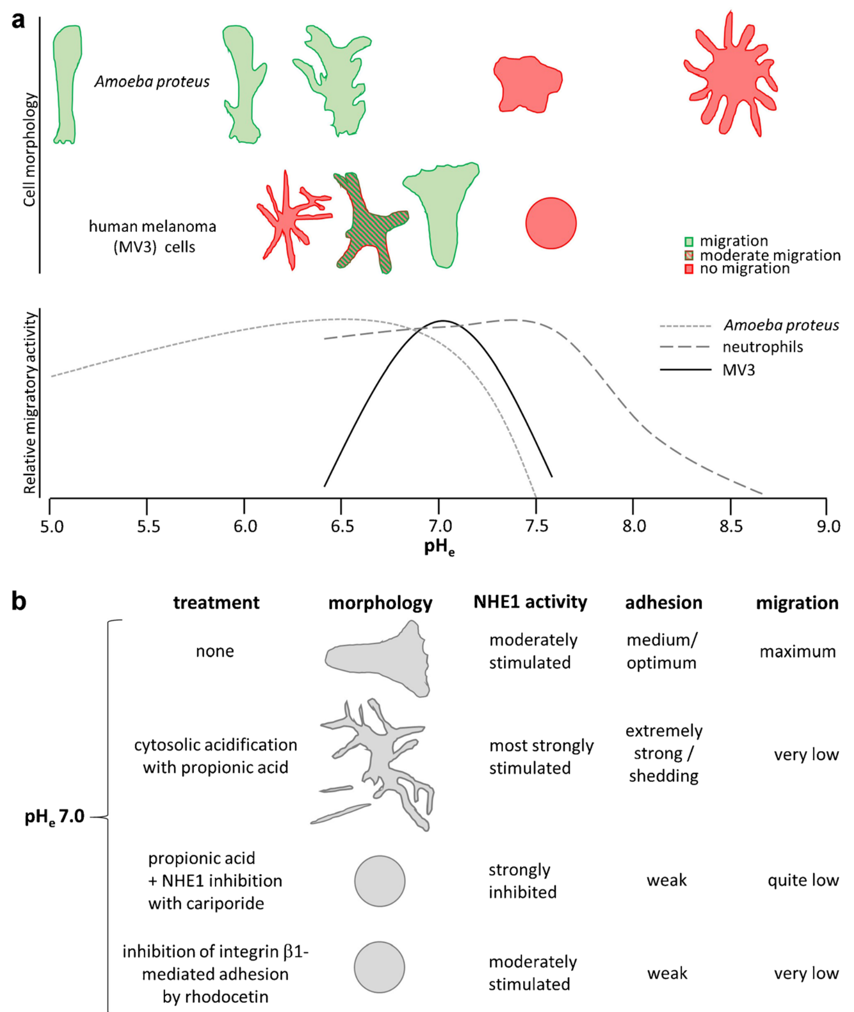


**Fig. 1** Cell polarization and migration depend on extracellular pH ($pH_e$) and acid extrusion. **a** While *Amoeba proteus* shows perfect polarization and high migratory activity in a rather acid $pH_e$ range of between pH 5.0 and 7.0 (11), human melanoma (MV3) cells polarize and migrate in an optimum way at $pH_e$ 6.8–7.2 [155]. Neutrophils can cope with quite a wide $pH_e$ range from values below 6.0 to more than 8.0 [148]. **b** Stimulation of NHE1 by cytosolic acidification with propionic acid at $pH_e$ 7.0 leads to the formation of numerous sticky protrusions accompanied by a strongly inhibited migration. Inhibition of the propionic acid–stimulated NHE1 activity by cariporide causes a drastic change in cell morphology from branched to spherical. Blocking integrin $\alpha_2\beta_1$ with rhodocetin at unaffected NHE1 activity and $pH_e$ 7.0 results in a spherical cell shape as well [155]. These observations point to a role of both NHE1 activity and integrin dimers in cell adhesion and migration

the direction of movement [87, 108, 139, 150]. At the same time, the retraction of the rear part comes along with osmotic shrinkage, triggered by (membrane) stretch-activated, mechanosensitive $Ca^{2+}$-channels [18, 108, 156], and mediated by $Ca^{2+}$-sensitive $K^+$ [137, 138, 142], $Cl^-$ [134], and water efflux, the latter most likely facilitated by aquaporins [129].

Second, in parallel with this “osmotic engine,” driven by the directed movement of ions and water across the cytosol from the leading edge to the trailing end [160], migrating cells utilize the accumulation of pH regulatory transporters at the leading edge to establish pH gradients along the direction of movement [158]. The $Na^+/H^+$ exchanger NHE1 (*SLC9A1*) accumulates at the leading edge of migrating fibroblasts (human and hamster lung [48]; murine embryonic [95]), renal epithelial cells (canine [72]), and melanoma cells (human MV3 [157] and murine B16V cells [95]). In serum-starved, quiescent cervical cancer cells, the epidermal growth factor (EGF) triggers polarization and subsequent migration by inducing a redistribution of randomly distributed NHE1 to the simultaneously developing lamellipodia [20]. There, at the leading edge, the $H^+$-extruding activity of NHE1 causes a local acidification of the cell surface [157] and a complementary cytosolic alkalinization of the lamellipodium [95]. Regardless of whether exposed to a $HCO_3^-/CO_2$ or a HEPES (2-[4-[2-hydroxyethyl]piperazin-1-yl]ethanesulfonic acid)–buffered medium, the cytosolic pH difference between the more alkaline lamellipodium and the more acidic trailing end comes to a ΔpH of 0.15 in human and murine melanoma cells (MV3, B16V). In contrast, it is clearly lower (ΔpH ~ 0.05) in non-malignant cells such as fibroblasts (NIH3T3) and endothelial-like (EA.hy926) cells [95]. This considerable difference in the front-back $\Delta pH_i$ between malignant and non-malignant cells is consistent with the general upregulation of net-acid extruding transporters in metabolically highly active cancer cells compared to normal cells [42]. Accordingly, and consistent with a certain resistance to the absence of $HCO_3^-$, MV3 and B16V cells show significantly higher NHE1 activity at their lamellipodia than NIH3T3 fibroblasts, resulting in a steeper intracellular pH gradient along the direction of movement [95]. In MV3 cells, the intracellular pH gradient is mirrored by a cell surface pH gradient with a ΔpH of up to 0.2 between

the more acidic leading edge and the rear end [157]. The pH gradient at the cell surface is maintained by the cell's intact glycocalyx. Removing N-glycosides from the glycocalyx, either with tunicamycin that suppresses the formation of N-glycosidic linkages in the endoplasmic reticulum or by a mixture of glucosaminidase and PNGaseF (peptide-N4-(N-acetyl-beta-glucosaminyl) asparagine amidase) that remove almost all N-linked oligosaccharides from the cell surface glycoproteins, leads to both a collapse of the cell surface pH gradient and a significant decrease in the migratory activity [77]. Upon stimulating NHE1 activity by cytosolic acidification with propionic acid at unchanged extracellular pH, glycocalyx-deficient cells re-establish their cell surface pH gradient, but with considerably lower pH values, and regain their ability to migrate [77]. These observations suggest that the glycocalyx acts as a diffusion barrier for protons, especially laterally, with the aim of creating locally defined pH domains at the cell surface, which then ensure both the maintenance of cell polarity and a smooth migration.

In fact, in addition to the existence of the cell surface pH gradient, cell migration requires the presence of cell adhesion molecules (CAMs) such as cadherins, selectins, or integrins. In MV3 cells, integrin $\alpha_2\beta_1$ dimers mediate adhesion to, and migration on, a collagen type I substrate [93]. Neither the mere availability of intact $\alpha_2\beta_1$ integrin dimers at the cell surface in the absence of the longitudinal cell surface pH gradient nor the presence of the pH gradient alone, in the absence of intact $\alpha_2\beta_1$ integrins in $\beta_1$-deficient cells, is sufficient to enable MV3 cells to migrate [77]. These findings imply an interdependence between adhesion (forces) and pericellular pH, particularly with regard to the fact that integrins with extended conformation protrude from the surface of the plasma membrane by ~ 20 nm [107].

## Extracellular pH nanodomains locally modulate essential components of the migratory machinery

### Cell-matrix adhesion

The interaction forces between integrin dimers at the cell surface of osteoclasts and their Arg-Gly-Asp peptide (RGD) sequence containing ligands, such as fibronectin and vitronectin, are strongly pH-dependent, with the highest binding force at pH 6.5 [81]. As shown for integrin $\alpha_v\beta_3$, extracellular pH modulates the conformation of integrin dimers and thus regulates their activity, including the avidity between the integrin dimer and an ECM molecule [113]. In migrating MV3 cells, $\alpha_2\beta_1$ integrins and NHE1 colocalize at focal adhesion sites of the outgrowing lamellipodium, where NHE1 activity creates a locally acidic nanoenvironment at the cell surface (Fig. 2). Pericellular pH, including the more acidic pH nanodomains at focal contacts identified by DsRed2-paxillin, was determined by ratiometric fluorescence measurements using total internal reflection fluorescence (TIRF) microscopy, after either the N-glycosidic linkages of the glycocalyx or the outer leaflet of the plasma membrane had been labeled with the pH-sensitive fluorescein-conjugates WGA (wheat germ agglutinin) and DHPE (1,2-dihexadecanoyl-sn-glycero-3-phosphoethanolamine), respectively [77, 90, 157]. This local acidification then supports the formation and possibly the maturation of focal adhesions, most likely by unfolding the headpieces of the $\alpha_2\beta_1$ integrin dimers, allowing a strong interaction with ECM proteins [90]. The accumulation of hypoxia-induced, cell surface-bound carbonic anhydrase IX (CAIX) at both nascent and maturing focal adhesions strongly suggests its assisting role in the generation of acidic nanodomains at the cell surface and possibly alkaline nanodomains in the cytosol [26]. Becker and Deitmer [6] advanced the more than 20-year-old concept of carbonic anhydrase–associated transport metabolons [122, 153]. Transport metabolons are structural and functional complexes that reside in the plasma membrane and consist of a carbonic anhydrase such as CAIX and a $HCO_3^-$ or $H^+$ transporter, e.g., AEs, NBCs, NHEs, or monocarboxylate transporters (MCTs). Especially in metabolically highly active tumor cells under hypoxic conditions, glycolytic metabolites and $H^+$ ions accumulate. Becker and Deitmer [6] propose that intracellular CAII directly bound to the C-terminal tail of MCT and CAIX bound to the MCT chaperone CD147 both function as "proton antennae," thus facilitating the rapid exchange of $H^+$ between the transporter pore and surrounding protonable residues near the cell membrane. This efficient mechanism would not only drive the export of $H^+$ and lactate to allow a high glycolytic rate but could also modulate proton-sensitive interactions between cell surface and ECM.

Quite recently, the presence of acidic nanodomains around focal adhesions was verified by a different experimental approach: single cells were seeded on coverslips coated with chemically immobilized pH-sensitive fluorescein isothiocyanate (FITC), and the fluorescence intensity of the substrate underneath the cells was detected by utilizing classical fluorescence microscopy. Here, focal adhesions were identified by immune-labeling vinculin, a major cytosolic component of focal contacts [98]. No matter whether identified by DsRed2-paxillin or immune-labeled vinculin, single, typically oval focal adhesions cover an area of ~ 3 $\mu m^2$, measuring 4 µm in length and nearly 1.4 µm at their widest [69]. The areal extent of focal adhesions therefore does not allow pH domains with a radial expansion of less than ~ 1.4 µm to be measured. However, since (i) in the applied TIRF microscopy [90] the evanescent wave penetration depth at an excitation wave length of 488 nm is 80–150 nm [36], (ii) the distance between ventral cell

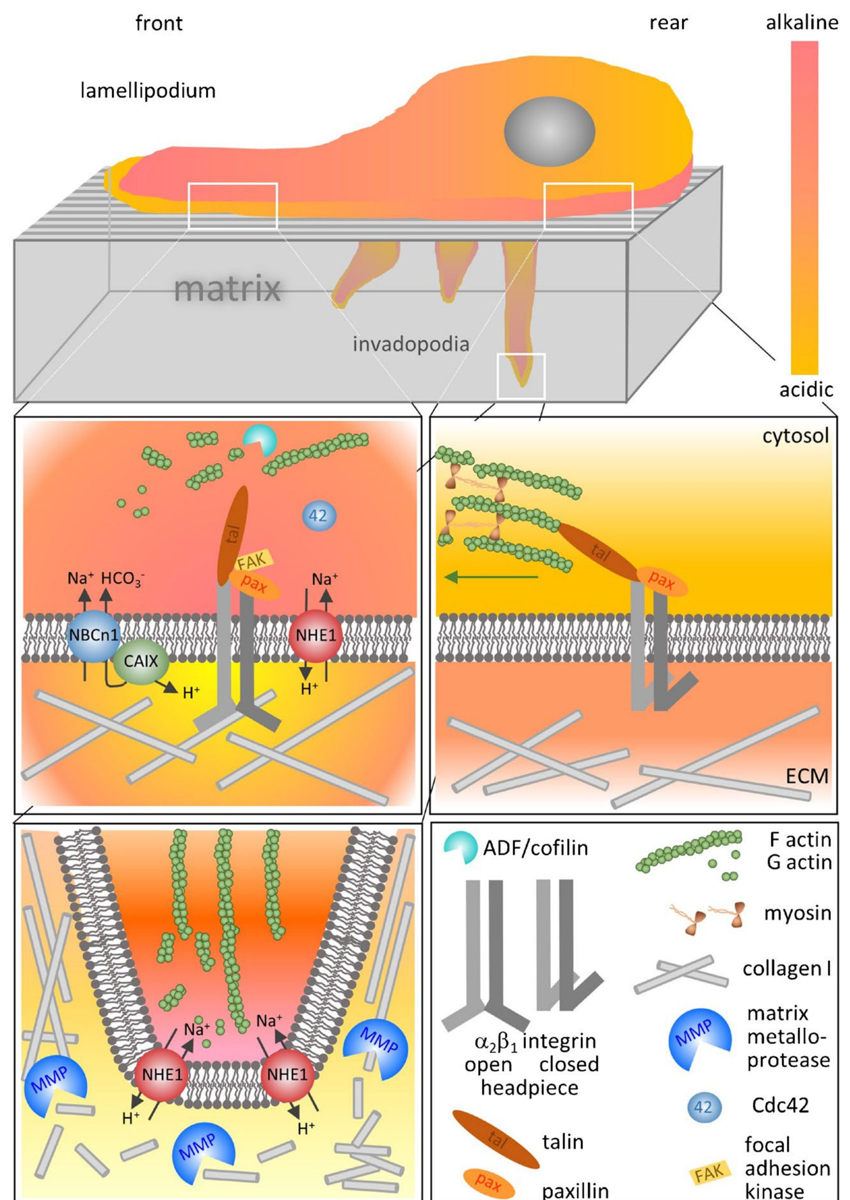


**Fig. 2** pH gradients and pH nanodomains in the cytosol and at the cell surface modulate focal adhesion dynamics, migration, and invasion. NHE1 and other acid/base regulators such as NBCn1 accumulate around focal adhesions at the leading edge where they locally (i) alkalinize the submembranous zone of the cytosol and (ii) acidify the cell surface. Membrane-bound carbonic anhydrases such as CAIX physically interact with NBCn1 and supply the $HCO_3^-$ to be transported into the cell. Alkaline $pH_i$ values reduce actin binding by talin and stimulate the activities of FAK, Cdc42, and the actin-severing protein cofilin, leading to increased actin and focal adhesion dynamics. Acidic $pH_e$ values increase integrin avidity and promote the formation of integrin-matrix bonds. At the cell rear, an alkaline $pH_e$ weakens adhesion, while an acidic $pH_i$ (i) slows down actin and focal adhesion dynamics due to reduced cofilin and FAK activity, (ii) strengthens actin-talin binding, and (iii) stimulates myosin activity, which then jointly promotes the retraction process. At the tips of invadopodial structures, NHE1 activity acidifies the surface and thus provides optimum pH conditions for ECM-degrading MMPs that clear the way. Please see text for further details and references

surface and the substrate at the focal contacts is approximately 10–20 nm [15], and (iii) removal of the glycocalyx leads to a disappearance of the pH domains [77], which have different pH values directly at the plasma membrane than in the glycocalyx [157], the axial expansion of the pH nanodomains is actually in the range of 10 to several 100 nm.

Once strong focal contacts are established, the cell can move over the substrate utilizing it as an opposite force to exert its own traction and compressive forces, mainly generated by actomyosin contractility [49]. Towards the trailing end, both the density and the activity of NHE1 decrease. The resulting relative alkalization around the integrins weakens the adhesion forces between cell and substrate, and eventually facilitates the disengagement of focal adhesions so that the cell can smoothly retract its rear end and move forward.

This is consistent with the observation that stimulation of NHE1 activity by cytosolic acidification with propionic acid at unchanging physiological bulk $pH_e$ values stimulates the formation of sticky lamellipodia-like protrusions (Fig. 1b). These protrusions are sticky to such an extent that they cannot be detached from the matrix anymore in order to be retracted. They need to be shed off the cell body instead. Conversely, specific inhibition of NHE1 with cariporide (HOE642) at physiological $pH_e$ drastically reduces the cells' adhesiveness resulting in a nearly perfectly spherical cell shape, like that found after trypsinization of cultured cells when passaging them [155, 157, 161]. Similarly, at $pH_e$ 7.0 and regular NHE1 activity, the inhibition of $\alpha_2\beta_1$ integrin dimers by rhodocetin, a C-type lectin–related protein isolated form the venom of the Malayan pit viper [31], causes

the cells to become spherical [155], and blocks the residual, pH-dependent migratory activity in $\beta_1$-deficient MV3 cells completely [77].

### pH-dependent integrin-mediated outside-in signaling

By modulating number and strength of integrin bonds, pericellular pH potentially affects cell migration also by integrin-mediated outside-in signaling. Bound integrins, especially the β-subunits, signal via Src family kinases, the focal adhesion kinase (FAK), and a number of small G proteins in order to regulate PI3K-AKT, MAPK, ITAM-PLC/$Ca^{2+}$, and RhoA-ROCK signaling pathways whose intricate interplay fine-tunes the migratory machinery [105, 145]. Following the (pH-dependent) binding of extracellular ligands to the integrin dimer, an activated subunit of a heterotrimeric G protein, $G\alpha_{13}$, binds to the integrin β subunit and thus (i) enhances integrin outside-in signaling and (ii) feeds back to GPCR (G protein–coupled receptor)–stimulated RhoA activation [46, 145]. Four members of the group of GPCRs, namely GPR4, TDAG8 (GPR65), OGR1 (GPR68), and G2A (GPR132), are proton sensors [149], and at this point, it is worth mentioning that TDAG8 has been shown to act through $G\alpha_{13}$/Rho signaling in blood cancer cells [61]. Hence, it is conceivable that the pericellular pH affects integrin-mediated outside-in signaling not only directly through the number and through the strength of focal adhesions but also indirectly by modulating integrin-mediated signaling via subunits of G proteins activated by proton-sensing GPCRs.

Also in astrocytes, integrin-mediated outside-in signaling leads to the activation and localized recruitment of Cdc42, a member of the Rho GTPase family. Cdc42 then generates a persistent polarity of the migrating astrocyte by promoting (i) Rac-dependent protrusion and (ii) PKCζ/dynein-dependent reorientation of the Golgi, the microtubule organization center, and the microtubule network towards the leading edge [33].

## Cell–cell adhesion

Cell–cell interaction depends on $pH_e$ and is modulated by NHE1 as well, however in opposite direction to their effects on cell–matrix interactions [53]. While acidic $pH_e$ and strong NHE1 activity strengthen cell–matrix adhesion, they weaken cell–cell adhesion (Fig. 3). In view of tumor diseases, this is relevant for the metastatic process because in solid tumor tissue the interstitial pH is considerably lower than in the surrounding healthy tissue and decreases from the tumor edge to the tumor center [57]. Indeed, low cell–cell adhesion forces facilitate detachment of cells from primary melanoma (MV3) spheroids. Thus, a synergistic interplay between pH-dependent cell–matrix and cell–cell adhesions harmonizes different steps of the metastatic cascade. Protons secreted by NHE1 promote metastasis by first facilitating cell detachment from the primary tumor and subsequently modulating cell–matrix interactions to drive cell migration and invasion [53].

## Cell invasion and metastasis—activity of matrix proteases

Cell invasion is based on migration and defines the ability of cells to navigate through the ECM within a tissue or to infiltrate neighboring tissues by crossing the basement membrane that usually separates them. For an invading cell to have sufficient space, the ECM must be remodeled, if not locally digested. The task of ECM remodeling including cleavage

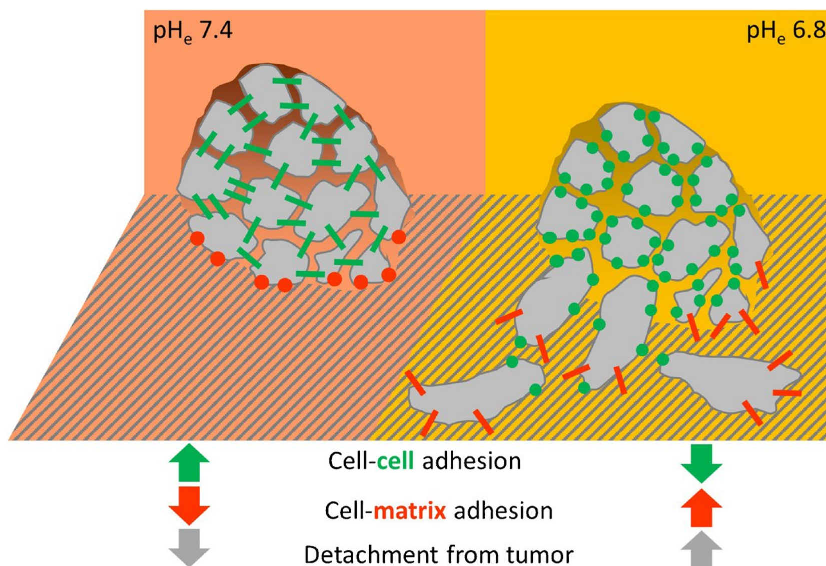


**Fig. 3** Extracellular acidification weakens cell–cell adhesion and stimulates cell–matrix adhesion at the same time. These simultaneous effects are thought to synergistically promote metastasis by facilitating both the detachment of single cells from a primary tumor and the invasion of the surrounding tissue [53]

of most ECM components is performed by matrix-degrading proteases [89]. The pH optimum for the activity of a number of ECM-degrading proteases such as cathepsins B [45], D [12], L [29], and S [13] is clearly more acidic than the physiological $pH_e$ of 7.3–7.4 measured in normal and healthy tissues [44]. In addition to pure enzymatic activity, pH sensitivity of both half-life/stability and activation from inactive precursors (pro-proteases) also contributes to pH-dependent proteolytic activity of matrix proteases. Thus, a low $pH_e$ favors the activity of interacting proteolytic cascades, which in turn convert pro-matrix metalloproteinases (proMMPs) into active MMPs [73]. Human stromelysin-1, also known as matrix metalloproteinase-3 (MMP3), is involved in the activation of pro-MMP1, -7, -8, and -9 [27] and exhibits its maximum activity in a narrow range of pH values ranging from pH 5.75 to 6.25 [54]. Interestingly, the activity of MMP3 relies on its own protonation, i.e., protonation of its $His^{224}$ [54, 60], whereas the activity of MMP2 depends on the extent to which its substrate fibrinogen is protonated [106]. Not only the stability and activity, but also the expression and secretion of ECM-degrading enzymes may depend on $pH_e$. Thus, an acidic environment stimulates the expression of MMP9 through calcium influx–triggered phospholipase D-mitogen-activated protein kinase signaling and acidic sphingomyelinase activation [65, 66] and induces the release of cathepsin B [130].

Because NHE1 contributes greatly to the pericellular pH nanoenvironment, it is hardly surprising that it also affects the secretion and activity of ECM-degrading proteases [16, 47, 171]. Noteworthily, stable transfection with an ion translocation-defective NHE1 reduces both gene expression and activity of MMP9 [119], and in breast cancer cells, NHE1 inhibition blocks the CD44-dependent increase in cathepsin B maturation and activity [10].

In conclusion, the pericellular pH nanoenvironment of migrating/invading cells with a more acidic $pH_e$ either at the very edge of the lamellipodium or at the tips of invadopodia protruding into the ECM leads to localized pH-sensitive proteolytic activity very close to the membrane (Fig. 2). Interestingly, an integrin $\beta_1$-mediated adhesion to ECM proteins and a pH-dependent collagen I-digesting activity of cysteine peptidases at the surface of the brain-eating amoeba *Naegleria fowleri* are assumed to play a critical role in their invasion of the central nervous system [58, 176]. At first glance, it may seem contradictory that acidification at the cell surface causes the cell to bind more strongly to the matrix, while at the same time digesting the matrix to which the cell is supposed to adhere. However, it is thought that digestion of and adhesion to the matrix do not occur at the very same spot. While the ECM at the front of the lamellipodial/invadopodial structures needs to be softened and dissolved, focal adhesion sites acting as anchors and counterforce are created and stabilized a little further back, for example, at the base of the emerging invadopodia [141, 154].

## Intracellular pH nanodomains locally modulate essential components of the migratory machinery

### Cytosolic constituents of focal adhesion complexes

As NHE1 activity acidifies adhesion foci at the cell surface, it simultaneously—and just as locally—alkalizes those submembranous regions of the cytosol that house the cytoplasmic domains of the adhesion molecules, their numerous structural and signaling interaction partners, and other proteins involved in the formation, maturation, and disassembly of focal adhesions [21, 90]. A number of these molecules react in a pH-dependent manner.

#### Talin

The adaptor protein talin, also called the master of integrin adhesions [71], links integrins directly to actin [109]. One of its several actin bindings sites is pH-dependent, with a more than twofold greater affinity of F-actin binding at pH 6.5 compared to pH 7.5 [152]. This is concomitant with a shorter lifetime of focal adhesions combined with a higher migratory rate at higher intracellular pH ($pH_i$) and a decreased focal adhesion turnover accompanied by a reduced migratory activity at lower $pH_i$ values [152]. The pH dependence of their mechanical stability was confirmed on isolated focal adhesions by means of bead-pulling experiments employing a magnetic microneedle apparatus [5]. Correspondingly, the cytosolic pH gradient in migrating cells with more alkaline $pH_i$ values in the front region allows for higher focal adhesion turnover and actin dynamics at the cell front, supporting sampling of the surrounding substrate, actin treadmilling, and lamellipodial growth. The more acidic $pH_i$ towards the trailing edge stabilizes the integrin- and talin-mediated connection between the plasma membrane and F-actin ensuring the efficient and complete retraction of the rear end. Talin's $histidine^{2418}$ was identified as an essential element of the pH-dependent molecular switch regulating F-actin binding [152].

#### Vinculin, paxillin, kindlin, and zyxin

Vinculin being part of the talin-vinculin axis is another major constituent of focal adhesions. It regulates and transmits mechanical forces between the cytoskeleton and adhesion receptors [101], for instance, by maintaining talin in its extended conformation [19]. However, observations on its pH dependence are contradictory [102, 112].

In addition to vinculin, paxillin, kindlin, and zyxin are typical constituents of focal adhesion complexes [62, 63].

Although a pH-dependent role of these proteins in focal adhesion assembly and cell motility has not been shown explicitly, at least paxillin and zyxin may well be pH-dependent. They are furnished with several c-terminal LIM domains that bind to mechanically stressed (tension, compression, twist-bend coupling) actin filaments [186]. The recruitment of paxillin to $\alpha_v\beta_3$ integrin–positive focal adhesions also depends on LIM domains [126]. The activity of these LIM domains is likely to depend on pH. In the small flowering plant *Arabidopsis* (*Brassicaceae* family), a group of these LIM domains can affect actin-bundling in a pH-dependent manner, probably due to an accumulation of acidic amino acids at their C-termini [104].

#### Focal adhesion kinase (FAK)

FAK is a central regulator of focal adhesion remodeling. It binds to the cytoplasmic domain of integrin β subunits and transduces growth factor and integrin signals for survival [1], adhesion dynamics [180], and migration [147]. FAK is directly sensitive to physiological changes in pH. An alkaline pH causes deprotonation of FAK-$His^{58}$ and thus drives conformational changes that modulate the accessibility of $Tyr^{397}$ to enable its autophosphorylation [21], which is required for the subsequent Src-mediated phosphorylation of the catalytic $Tyr^{576}$ and $Tyr^{577}$ [17, 83]. Conversely, a substitution of $His^{58}$ by alanine with its hydrophobic, non-reactive methyl side chain allows autophosphorylation and cell spreading at low $pH_i$, too [21].

### Cytoskeletal dynamics and contractility

Cell migration relies on coordinated periodic lamellipodial protrusion and rear end retraction, accomplished by a highly dynamic regulation of actin structures at the leading edge and an appropriate actomyosin-mediated contractility at the rear end. $pH_i$ controls both actin dynamics in the lamellipodium and actomyosin-mediated contractility towards the cell rear.

#### Gelsolin and actin-depolymerizing factors/cofilin

The actin-regulatory, cytoplasmic gelsolin plays a crucial role in remodeling the actin cytoskeleton during cell migration by severing, capping, and uncapping actin filaments. Its F-actin-severing activity is modulated by phosphatidylinositol-4,5-bisphosphate (PIP2) binding and requires a high (between $10^{-6}$ and $10^{-5}$ mol $L^{-1}$) intracellular $Ca^{2+}$ concentration $[Ca^{2+}]_i$ and a low $pH_i$ [35, 162, 189]. The low $pH_i$ leads to local structural changes caused by protonation of two histidines ($His^{29,\,151}$) and one aspartate ($Asp^{109}$) within the first of six subunits. While protonation of all three is essential for pH-dependent actin-severing activity, protonation of $His^{151}$ directly affects filament binding because it resides right at the gelsolin/actin interface [35].

Being a member of the actin-depolymerizing factor (ADF)/cofilin family, cofilin is another pH sensor acting on actin dynamics [38, 187]. Increased assembly of a branched actin filament network drives membrane protrusion at the leading edge of migrating cells [8, 117]. In the outgrowing lamellipodium, a zone of fast F-actin polymerization subjacent to the leading edge is followed by an area of virtually complete depolymerization of actin filaments a few micrometers back, generating a 2–4-μm treadmilling actin array adjoining the leading edge [118, 179]. Actin monomers originating from the depolymerization process recycle to the zone of polymerization to keep the treadmill running. Cofilin (i) facilitates the fragmentation of actin filaments in the depolymerization zone resulting in an increase in actin monomers, and (ii) debranches filaments to generate new free barbed ends for nucleation by the Arp2/3 complex [8]. A rather alkaline pH, as found in the front end of migrating cells due to local NHE1 activity [90, 95], leads to deprotonation of cofilin's $His^{133}$, thus weakening the bond between PI[4, 5]P2 in the inner leaflet of the plasma membrane and cofilin [38]. This results in the release of active cofilin from the membrane followed by increases in both actin free barbed ends and available actin monomers [64]. Similarly, in invadopodial structures as well as in lamellipodia, cortactin-bound cofilin is released at more alkaline pH due to a reduced cortactin-cofilin binding affinity, again increasing barbed end generation, while a decrease in local pH pushes cofilin to (re)bind to cortactin [94].

To emphasize the cross-species universality of pH (gradient)–dependent protrusion growth and motility mechanisms, it should be mentioned here that the growing pollen tube of *Arabidopsis thaliana* (*Brassicaceae* family) establishes a pH gradient along the direction of growth and that isovariants of actin-depolymerizing factor (ADF) with different pH sensitivity drive pollen tube growth by acting pH dependently and thus locally in different regions of the growing tube [178]. Similar observations were made earlier in growing lily pollen tubes, where the pH-sensitive ADF together with actin-interacting protein localize to the cortical actin fringe region. Acidification with sodium acetate causes actin filament destabilization in the apical and subapical region, a stabilization of actin-fibers in the distal region, and an inhibition of pollen tube growth by 80% [88].

#### Actin self-assembly and contractility of the actomyosin cytoskeleton

The self-assembly of actin is also pH-dependent. In nematode spermatozoa, faster assembly and lower G-actin

concentrations occur at lower pH values [177], such as those found in the rear end of migrating cells [95]. In addition, the contractility of reconstituted active actin systems is tightly controlled by local pH [74]. Contractility increases as pH decreases. This is due to the fact that the intrinsic cross-bridge strength of myosin-II is pH-dependent and thus accountable for a sharp transition of the actin/myosin-II activity from *non-contractile* to *contractile* by a pH change of not more than 0.1 [74]. Consequently, a cytosolic pH gradient of up to 0.2 pH units [95] allows cell rear retraction in migrating cells while maintaining a flow with strongly reduced overall contractility at the protruding cell front.

Interestingly, $pH_i$ also precisely controls the assembly of the unique major sperm protein (MSP) filament system of migrating nematode sperm cells. The pseudopodium exhibits a $pH_i$ gradient with pH 0.15 units higher at the leading edge, where fiber complexes assemble, than at the base, where disassembly takes place [70].

### Cdc42

The small GTPases Rac, Rho, and Cdc42 are hierarchically linked to each other and play key roles in both establishing cell polarity and directional cell migration [92, 132]. The cell front contains higher active Rac and lower active Rho concentrations, while the rear end has lower active Rac and higher active Rho concentrations. Cdc42 is recruited to the leading edge where a guanine nucleotide-exchanger factor (GEF) catalyzes GTP binding by Cdc42. GEF binding to phosphoinositol 4,5-bisphosphate is pH-dependent and, as shown in fibroblasts, requires $H^+$ efflux by NHE1 activity [39]. For RasGRP1, a Ras-specific GEF, $His^{212}$ has been identified as a pH sensor that activates RasGRP1 when the inside of the cell becomes less acidic. When the charge on $His^{212}$ changes from positive (protonated) to neutral (deprotonated), the RasGRP1 protein opens up to bind to DAG (diacylglycerine) at the membrane [174].

## Molecular pH sensors: basic and acidic amino acids

The previous paragraphs insinuate that over the last two decades (de)protonable basic and acidic amino acids, particularly histidine and aspartate, have emerged as molecular pH sensors that translate pH changes into conformational changes of proteins and thus affect their catalytic activity, substrate binding, stability, interaction, aggregation, and localization [151]. The protonation of proteins can be considered a reversible posttranslational modification, analogous to phosphorylation, methylation, and ubiquitination. This proton-mediated posttranslational modification represents a certain signaling specificity as it applies to only a minority of sites in selective proteins that titrate within the physiological pH range [135]. Relevant examples in addition to the above-mentioned are the epidermal growth factor receptor (EGFR), the transcription factor p53, the signaling and adherens junction protein β-catenin [184, 185], and the proton-sensing G protein–coupled receptors OGR1 and GPR4 [91].

EGFR exhibits pH-sensitive kinase activity and confers increased pathway activation at higher $pH_i$ when arginine at position 776 is mutated to histidine. This results in increased proliferation, accelerated malignant transformation [185], and possibly the acquisition of migratory capabilities as typical of cancer cells with higher $pH_i$ values.

Similarly, p53 shows a pH-sensitive transcriptional activity when displaying the recurrent somatic mutation from $arginine^{273}$ to histidine [59, 185]. The transcriptional activity and cell death in response to DNA damage are decreased at higher $pH_i$.

With regard to cell motility, β-catenin is of interest for various reasons. Activation of β-catenin down-regulates cell–cell junction–related genes, induces epithelial-to-mesenchymal transition [68], regulates genes implicated in migration such as paxillin [2], and stabilizes the front-rear polarity of migrating cells [173]. Degradation of β-catenin needs phosphorylation of N-terminal residues for recognition by the E3 ligase β-TrCP. Not the phosphorylation step itself but the concomitant binding to β-TrCP depends on pH. A higher $pH_i$ induces increased β-TrCP binding and thus decreases β-catenin stability [184]. Interestingly, in contrast to EGFR or p53, it is not a mutation of arginine to histidine, but a somatic mutation of $His^{36}$ to arginine [37] that allows β-catenin to bypass pH sensitivity, i.e., the pH-sensitive β-TrCP recognition (and degradation), which then results in increased Wnt pathway activity in cancer cells [14].

$pH_e$ and its changes can be detected and transduced by the proton-sensing G protein–coupled receptors OGR1 and GPR4 [91]. While OGR1 signals via inositol phosphates (IP), GPR4 elicits cAMP signaling. Both IPs and cAMP are central players in cell migration [55, 56, 168] and reach their maximum production at $pH_e$ 6.8. Five histidines ($His^{17, 20, 84, 169, 269}$), including the interactions of $His^{17}$ with $His^{84}$ and $His^{20}$ with $His^{269}$, allow for the $pH_e$-dependent OGR1-mediated IP- and GPR4-mediated cAMP production [91].

In the end, being one of the major regulators of cellular pH homeostasis, even NHE1 itself displays a cluster of histidine residues in the proximal C-terminal cytoplasmic domain. This histidine cluster regulates pH-dependent $PI(4, 5)P_2$ binding and transporter activity [181].

## pH taxis—directional migration along a pH gradient

When the extracellular bulk $pH_e$ is in the form of a gradient, it can serve as a directional stimulus. Such gradients are found within tumors, in cutaneous wounds, and at inflammatory sites.

At the interface between human prostate tumors grown in mice and the surrounding normal tissue, $pH_e$ increases about 0.4 units (~6.9 to ~7.3) over 1 mm towards the normal tissue [41], and within human colon adenocarcinoma xenografts, $pH_e$ decreases by about 0.7 units (~7.4 to ~6.7) over ~350 nm from a tumor blood vessel [52]. Although hardly measured explicitly in vivo in the human body, the presence of such gradients is highly probable in a number of solid human tumors in situ. The nanoprobe ONM-100, which emits fluorescence at pH values below 6.9 while its fluorescence is quenched at pH values above 6.9, has been successfully applied to identify breast, esophageal, and colorectal tumors, and squamous cell carcinomas in the head and neck region, prior to surgical resection. The images used to localize these tumors actually imply centrifugal fluorescence gradients within the cancer tissue [84, 175].

In chronic cutaneous wounds, $pH_e$ increases considerably from the wound edge (below 6.5) to the wound center (above 7.4) over distances of less than 1 cm [136].

At inflammatory sites, e.g., during the course of inflammatory responses against bacteria in peripheral tissues, $pH_e$ can be as low as 5.5. This drop in $pH_e$ can be caused by (i) a shift to glycolytic metabolism in response to tissue hypoxia resulting from the damage of small blood vessels and the metabolic activity of infiltrating leukocytes, (ii) a massive production of protons by neutrophils during the activation of the respiratory burst, and (iii) the accumulation of short-chain fatty acids produced by bacteria [32].

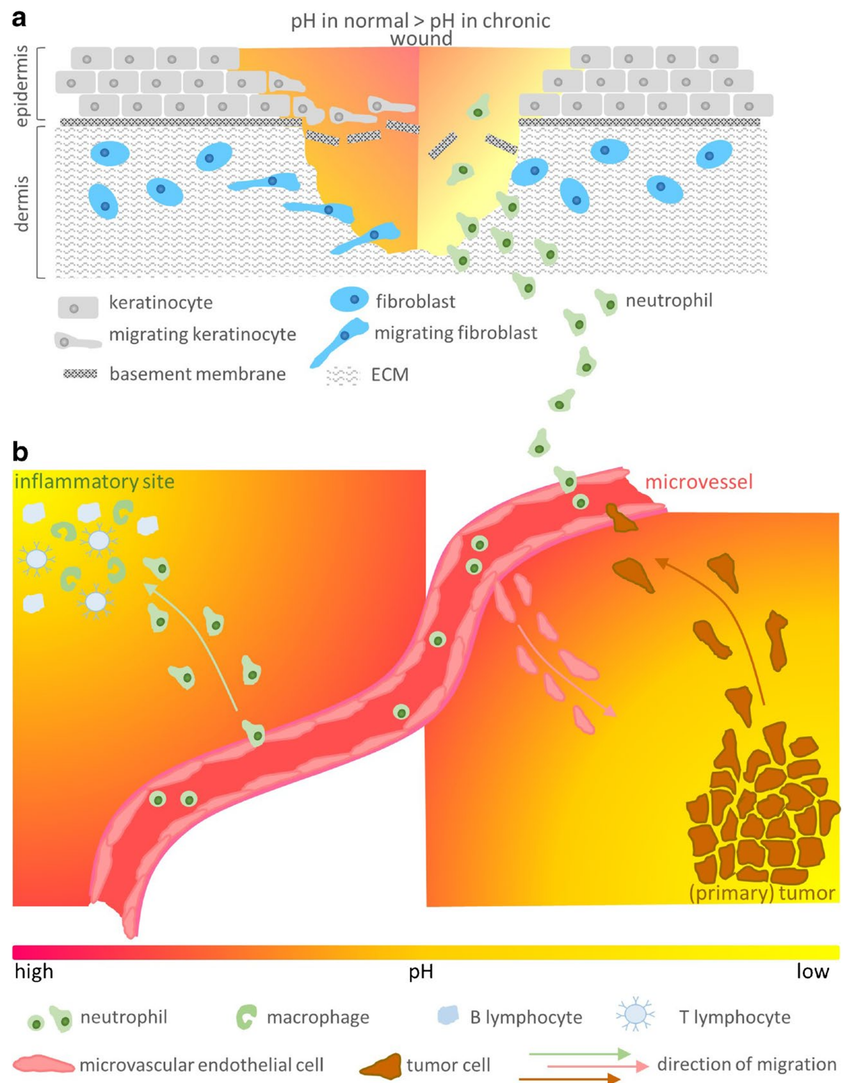


**Fig. 4** Directional cell migration along pH gradients. **a** In a normal wound (left half), epidermal keratinocytes and dermal fibroblasts migrate towards the more alkaline $pH_e$ in the center of the wound in order to close it. In a chronic wound (right half), a quite acidic pH at the wound margin prevents keratinocytes and fibroblasts from migrating into the wound. Instead, neutrophils are attracted by the low pH. Once inside the wound area, their presence including the local secretion of inflammatory mediators fuels the inflammatory process and thus prevents wound healing. **b** In general, neutrophils migrate towards inflammatory sites that typically are acidic (left). Microvascular endothelial cells also migrate to tissue areas with an acidic pH value, while metastatic tumor cells migrate away from the acidic tumor tissue to regions with a physiological pH value near blood and lymph vessels. Please see the section on pH taxis for further details and references

Depending on the cell type and the physiological context, cells migrate either towards or away from the lower pH (Fig. 4b). Chinese hamster ovary (CHO) cells engineered to express $\alpha_v\beta_3$ integrin and primary bovine retinal microvascular endothelial cells (MVECs, endogenously expressing $\alpha_v\beta_3$ integrin), both seeded on fibronectin, migrate towards the acidic end of a $pH_e$ gradient, ranging from pH 7.5 to 6.0, due to a preferential polarization towards the lower pH [114]. The migration velocity is steadily decreasing as cells move towards the lower pH, confirming that there is an optimum pH for migratory activity as shown for human melanoma (MV3) cells [155]. Integrin activation including outside-in signaling in response to acidic $pH_e$ may represent one of the major potential mechanisms underlying pH taxis. In this way, acidic $pH_e$, as found in hypoxic tissue, could stimulate MVEC migration and angiogenesis in order to ensure sufficient vascularization and oxygen and nutrient supply. However, it has not been clarified yet to what extent mere pH-dependent cell–matrix and cell–cell interactions, including integrin-mediated downstream signaling, can fulfill the task of a pH detector. It can be almost certainly ruled out that this mechanism plays an exclusive role in pH detection. Accordingly, as for endothelial cells, acidic pH was found to downregulate vascular endothelial growth factor receptor-2 (VEGF-2) and thus impede VEGF-mediated migration [34]. In the latter study, however, the experimental set up did not include a $pH_e$ gradient, but instead the cells were exposed to a homogenous bulk pH, and furthermore, both EC sprouting and tubulogenesis remained unaffected by extracellular acidity.

Human metastatic breast cancer cells (MDA-MB-231 cells) seeded on type I collagen migrate in the direction of higher $pH_e$ values in a gradient of 0.2–0.3 pH units/mm [166]. The slope of this gradient would correspond to a gradient of 0.02 units per single cell length and thus would only be a tenth of the NHE1-generated intra- and pericellular pH gradients in MV3 cells [95, 157]. Given the observations that in MV3 cells (i) the pH gradient at the cell surface is stabilized by the glycocalyx [77], and (ii) the protons extruded by polarized distributed NHE1 have a stronger impact on migration than those present in the bulk solution [161], it is difficult to visualize a mechanism by which an externally applied $pH_e$ gradient of 0.02 units could prevail the intrinsic, pericellular pH gradient of 0.2 units. The question of how the shallow ambient $pH_e$ gradient modifies the steeper pH gradient in immediate proximity to the cell surface in order to either enhance or mitigate directed cell migration has remained unanswered to date. An involvement of both the glycocalyx as a cell surface–buffering compartment accepting/releasing protons [77, 157] and proton-sensing G protein–coupled receptors [159, 183] seems possible. pH-dependent MMP activity (please be referred to the above paragraph on cell invasion) and the pH-dependent properties of substrate components including pH-dependent steps of ECM genesis such as collagen fibrillogenesis [51, 76, 82], fiber crosslinking [23], or self-assembly of laminin [40] should be kept in mind as well. Independently of the underlying mechanism, for a cancer cell to intravasate and metastasize, it is essential to move away from the acidic primary tumor towards the most alkaline regions surrounding blood and lymph vessels (Fig. 4b).

In chronic cutaneous wounds, $pH_e$ rises with increasing distance from the wound edges. The strongly acidic margins of the wound ($pH \leq 6.5$) prevent healing by reducing the viability, proliferation, and migration velocity of keratinocytes [78, 136]. Thus, the centripetal keratinocyte recruitment along the $pH_e$ gradient from the acid wound periphery towards the more alkaline wound center ($pH \geq 7.4$) is critically low (Fig. 4a). Of note, this $pH_e$ gradient mirrors the wound's NHE1 expression profile that shows a centrifugally rising NHE1 expression (136). Simply increasing $pH_e$ from below 6.5 to 6.8 (i) enables keratinocytes to migrate directionally towards the higher $pH_e$ in the wound center [136], (ii) increases wound closure in an in vitro fibroblast wound healing assay, and (iii) accelerates re-epithelialization [78]. pH-sensing G protein–coupled receptors are thought to play a role not only in tumor cell migration and metastasis but also in the wound healing process [183]. To what extent proton-sensing G protein–coupled receptors contribute to the detection of an external $pH_e$ gradient and pH tactic behavior is yet to be investigated in detail. In consideration of the chronic wound's characteristic pH profile, both modulating $pH_e$ by applying pH-restoring hydrogel-like dressings [75] and the application of $pH_e$ responsive wound dressings have been recognized as a potential therapeutic strategy [50, 103].

Superimposition of a chemical and a $pH_e$ gradient, as found at sites of inflammation, most efficiently stimulates NHE1-dependent, Cdc42-mediated directional migration of neutrophils [111]. Formylated peptides and complement molecule C5a, both secreted at the site of inflammation, induce neutrophil chemotaxis. Even in the absence of these chemoattractants, neutrophils tend to migrate from $pH_e$ 7.5 towards lower pH values, however lose their directionality at $pH_e$ 7.2 already. In the presence of a C5a gradient, an additionally superimposed $pH_e$ gradient supports neutrophil chemotaxis in the direction of the C5a source and the lower $pH_e$. While directionality is strongly inhibitable with the NHE1 inhibitor cariporide and steadily decreases towards $pH_e$ values as low as 6.5, the migration velocity itself remains nearly unaffected by $pH_e$ and is comparably less affected by cariporide, suggesting that the steering mechanism (chemotaxis) rather than the migration motor (velocity) depends on proper ambient pH and NHE1 activity. In addition to $pH_e$, the intermediate chemoattractant leukotriene $B_4$ ($LTB_4$) is required for efficient chemotaxis towards the end-target chemoattractant C5a. The secretion

of $LTB_4$ from activated neutrophils also depends on NHE1 activity and $pH_i$ and thus indirectly on $pH_e$. It decreases with falling $pH_e$ and $pH_i$ or when NHE1 activity is inhibited [111]. NHE1 activity as a requirement for chemotaxis has been shown also for polymorphonuclear leukocytes in an fMLP gradient [127]. In summary, a shallow proton gradient with $pH_e$ values decreasing from blood vessels towards the inflamed tissue may accelerate extravasation of stimulated neutrophils and then direct them to the inflammatory site (Fig. 4b). Once in the acidic inflammatory microenvironment, neutrophil chemotaxis is impaired while migration velocity is only slightly reduced, allowing cells to ramble on-site through the inflamed tissue.

In quite a number of cell types, NHE1 turns out to be essential for directional migration although other acid/base regulating transporters such as $Na^+$-$HCO_3^-$ cotransporters (NBCs) are also present in the plasma membrane. This holds true even in the absence of a directional stimulus as shown for Madin-Darby canine kidney (MDCK) cells. Whereas $Na^+$-$HCO_3^-$ co-imported by NBC1 can compensate for a lack in NHE1 activity with respect to cellular pH homeostasis and volume regulation, in the absence of a functional NHE1, NBC1 does not provide the cell polarity required for directional cell migration [140]. This mechanistic predominance of NHE1 over NBCs reduces the dependence on the $CO_2$/$HCO_3^-$ carried fraction of the extracellular buffer capacity. This could be particularly important under in vivo conditions, where the buffering capacity of the open $CO_2$/$HCO_3^-$ system can vary considerably depending on $CO_2$ production in the tissue relative to its removal, e.g., by the blood stream. Thus, the dominance of NHE1 over NBCs could ensure continuous and smooth directional migration in the face of a potentially unstable extracellular $HCO_3^-$ concentration. Furthermore, it emphasizes the relevance of a tightly controlled pH profile directly at the cell surface. The pH nanodomains locally generated by NHE1 [90] and its cytoskeletal anchoring, both affecting adhesion, related signaling events, and cytoskeletal dynamics, are virtually certain to regulate asymmetric signals that establish polarity and a differential, coordinated focal adhesion remodeling at the cell front and rear [30]. However, what is the underlying mechanism that would master NHE1 targeting, that is, how is directional trafficking of NHE1 towards the cell front regulated, and how is the cell front as such established in the first place? A redistribution of randomly distributed NHE1, accompanied by the formation of a polarized morphology and the acquisition of the ability to migrate, has been observed in cervical cancer cells upon exposure to EGF [20]. Nevertheless, the mechanism behind the directed trafficking of NHE1 in single cells, especially in the absence of any directional stimulus, remains unexplained to date and needs further examination.

## Does pH contribute to self-guidance mechanisms?

The fact that migrating cells themselves generate directional information via a dynamic interplay of cell-intrinsic and cell-extrinsic regulators and thus have more control over their directionality than previously assumed has been reviewed recently [188]. In terms of single cell migration, there are three different self-guidance mechanisms [188], and pH strongly affects all three of them.

First, "subcellular symmetry breaking" is based on intracellular traveling waves of the cytoskeleton and signaling events via the phosphatidylinositol-4.5-bisphosphate system [7, 43]. The resulting break in the symmetry of the cell cortex then defines sites of cellular protrusions and lamellipodial outgrowth [100]. This symmetry breaking includes the above-mentioned pH-dependent cytoskeletal dynamics and its pH-dependent components gelsolin, ADFs/cofilin, actin self-assembly, actomyosin contractility, and also the small GTPase Cdc42. Hence, the intracellular pH gradient along the direction of movement with higher $pH_i$ values at the cell front or in protrusions, e.g., invadopodia, and lower $pH_i$ values towards the rear end [90, 95] most likely contributes to the perpetuation of "subcellular symmetry breaking," if not even its generation.

Second, during "self-generated chemotaxis," cells create their own local, dynamic gradients by breaking down, sequestering, or scavenging surrounding attractant molecules [110, 133]. In this way, a given, yet non-detectable gradient can be amplified in the immediate vicinity of the cells and thus becomes detectable. Normally, chemotactic cells identify attractant gradients by comparing attractant receptor occupancy between their front and rears. Differences as low as 1% can be distinguished. However, beyond distances of 0.5 up to 1 mm, the given gradients contain zones that would be either too saturating or too shallow to cause the detectable 1% occupancy difference [169]. At this point, the amplification mechanism comes into play to promote long-range chemotaxis and even allow cells to navigate mazes [170]. An almost perfect example for the role of acid extruders and $pH_e$ in "self-generated chemotaxis" is the above-mentioned NHE1, $pH_e$, $pH_i$, and Cdc42 dependence of directional neutrophil migration [111].

Third, cells create "self-organized extracellular scaffolds" by exerting forces towards the ECM. The resulting changes in the ECM's physical properties and its structure, such as either parallelizing or disarranging ECM fibers, impact stiffness, order, topology, and porosity, and can then act as guidance cues to drive directional cell migration [22, 24, 172]. The strength of $\alpha_2\beta_1$ integrin–mediated adhesions to collagen type I depends on cell surface

pH, and at $pH_e$ 6.8 or when NHE1 activity is stimulated by intracellular acidification with propionic acid, human melanoma (MV3) cells pull on ECM fibers potentially rearranging them [155]. In addition to adhesion-affected re-arrangement of the matrix, local digestion of the ECM by pH- and thus NHE1-dependent MMP activity contributes to "self-organized extracellular scaffolds" as well [16, 47, 154, 171].

In conclusion, once the polarized distribution of (net) acid extruders such as NHEs or NBCs is established, their activity may perpetuate $pH_i$ and $pH_e$ gradients along the direction of movement. A concerted action of pH-regulating and pH-dependent proteins as well as locally controlled osmotic swelling and shrinkage [139] may generate an intrinsically self-sustaining process making one think of a perpetual motion machine. Hence, pH does have an essential share in self-guidance. To what extent direct pH sensing by lipids in membranes [3] and subsequent signaling contributes to keeping the migration machinery going is quite an appealing question that certainly demands further investigation.

## Acid priming

Several cell types, even metabolically highly active tumor cells with generally upregulated net-acid extruding transporters compared with normal cells [42], e.g., human melanoma [161], lung carcinoma, and normal bronchial epithelial cells [146], rat prostate carcinoma cells [124], or neutrophils [67, 111], respond to acidic $pH_e$ exposure by lowering their $pH_i$ values. Assuming that the intracellular acidification is a direct consequence of the extracellular acidification because (net) acid extruders such as NHEs and NBCs cannot work efficiently enough against the increasing extracellular proton concentration, the intracellular protons may act as messengers via cytosolic proteins with integrated molecular proton sensors, predominantly histidine and aspartate residues (see above). This procedural sequence would not require the presence of proton-sensing G protein–coupled receptors in the plasma membrane, but may be prone to interfering effects caused by pH-sensitive cation channels. As reviewed by Pethö et al. [115], acidic $pH_e$ stimulates $Ca^{2+}$ (TRPV1; TRPC4, 5; $P_2X_{1-4}$) and $Na^+$ (ASICs) conducting channels. While the resulting $Na^+$ influx and the concomitant changes in the $Na^+$ gradient across the plasma membrane may affect the activity of $Na^+$-dependent acid/base regulators and consequently $pH_i$, a rise in $[Ca^{2+}]_i$ can elicit $Ca^{2+}$ signaling, e.g., via $Ca^{2+}$/calmodulin-dependent protein kinases, and thus stimulate migration. However, in pancreatic stellate cells (PSCs) exposed to $pH_e$ 6.6 overnight, $[Ca^{2+}]_i$ is significantly lower, $[Na^+]_i$ significantly higher, and the membrane potential strongly hyperpolarized compared to cells kept at $pH_e$ 7.4 which impels the $Na^+/Ca^{2+}$ exchanger NCX1 that usually removes $Ca^{2+}$ from the cytosol to function in reverse mode. According to this, NCX1 inhibition stimulates PSC migration at $pH_e$ 7.4 because $Ca^{2+}$ ions are retained in the cytosol, whereas it abates migration at $pH_e$ 6.6 due to a blocked $Ca^{2+}$ entry [85].

On the other hand, $Ca^{2+}$ signaling is modulated by $pH_i$. Due to slow diffusion and buffer sharing, alterations in $pH_i$ and $[Ca^{2+}]_i$ can be compartmentalized. Free $Ca^{2+}$ ions and free protons compete for binding to the same cytoplasmic $Ca^{2+}/H^+$ buffer molecules with sufficient mobility, i.e., low molecular weight, such as histidyl peptides, e.g., carnosine, and ATP [165]. In this way, $Ca^{2+}$ bound to such a buffer molecule can be recruited uphill from anywhere in the cytosol to localized acidic nanodomains where it is then released. This spatial $Ca^{2+}/H^+$ coupling is likely to be of general importance in local cell signaling [165]. Such a $Ca^{2+}/H^+$ coupling could also explain the, probably $Ca^{2+}$/calmodulin-mediated, activation of PI3K in cells whose migration is stimulated by an acidic environment without the involvement of acid-sensing ion channels (ASICs), e.g., glioblastoma cells or neutrophils [25, 97].

Long-term exposure to an acidic environment can initiate long-lasting effects including an aggressive migratory behavior observable even hours after the cells' return to physiological pH values. This acidic priming with "memory effect" is a matter of particular interest because it enhances the metastatic potential of cancer cells by allowing for malignant behavior in a physiological environment far away from the primary tumor [123]. In this context, Thews and Riemann [167] have recently reviewed the various outside-in signaling pathways induced by acid exposure and the signaling effects mediated by intracellular acidification. A low $pH_i$ resulting from extracellular acidification causes the release of reactive oxygen species (ROS) from mitochondria. ROS induces MAPK activation and stimulates gene expression via p38 and the transcription factor CREB. The increased transcriptional activity of CREB can persist for 24 h after returning the cells back to normal pH [124]. However, while the acidosis-induced increase in ROS seems imperative for an increased motility of rat prostate cancer cells, the augmented phosphorylation of ERK1/2 and p38 is not required [123]. Nevertheless, 48 h exposure of human triple-negative breast cancer (MDA-MB-231) and mouse mammary carcinoma (4T1) cells to $pH_e$ 6.4 caused changes in the expression and splicing of 2752 genes, including a number of those affecting cell motility, potentially controlled through a specific set of RNA binding proteins and downstream of pH-induced chromatin modifications [128]. Similarly, in human pancreatic ductal adenocarcinoma cell lines, long-term (1-month) acidic pressure ($pH_e$ 6.6) selects cells with enhanced migration and invasion abilities induced by epithelial-mesenchymal transition (EMT), intensifying their metastatic potential when re-exposed to $pH_e$ 7.4, and

in acid-selected human pancreatic ductal adenocarcinoma (PANC-1) cells, genes relevant to proliferation, migration, EMT, and invasion are upregulated [4].

In summary, acid exposure can bring about a "memory effect" that is induced by pH-affected signaling including ROS production and accompanied by transcriptome rewiring. This enables particularly tumor cells to acquire a more malignant and metastatic phenotype in an acidic environment such as primary tumor tissue and to then maintain a pronounced migratory and invasive behavior for several hours up to even more than a day after leaving the acidic tissue.

## Conclusion

Both $pH_e$ and $pH_i$ have a hand in single cell migration. $pH_e$ strongly affects cell adhesion and cell morphology, and under optimum conditions supports the establishment of a polarized morphology, a prerequisite for migration. $pH_i$ has an impact on a number of focal adhesion components, actin polymerization, and actomyosin contractility. There are considerable differences between species, cell types, and cell lines in terms of pH-dependent morphology, optimum adhesion, migration and invasion, and the direction of pH taxis. The set of expressed adhesion molecules alone can make a difference, because different integrin-matrix interactions exhibit different pH optima. Human melanoma (MV3) cells express $\alpha_2\beta_1$ integrins that mediate optimum cell migration on collagen type I at a bulk $pH_e$ of 7.0–7.2 and basal NHE1 activity producing a cell surface pH of 7.2–7.4 at the leading edge [90, 157]. In comparison, in human breast cancer (MCF-7) cells expressing both $\alpha_2\beta_1$ integrins and a constitutively active receptor tyrosine kinase (ErbB2), cell surface pH is 7.05, and only an increase to 7.2 by means of NHE1 inhibition leads to optimum migration on collagen I [80]. This confirms a cell surface pH of ~ 7.2 to be optimal for $\alpha_2\beta_1$ integrin–mediated cell migration, independently of the cell type. Thus, not in spite of, but because of the species- and cell type-dependent differences in the pH characteristics of motility parameters, it is tempting to see the function of pH/protons in the regulation of cell motility to be a widespread if not even cross-species universal mechanism. Given (i) the multitude of pH-sensitive molecules functioning as adjustable setscrews of the migration machinery, (ii) their mutual, pH-dependent interactions, and (iii) the pH-sensitive activity of MMPs, protons are modulators of migration and drivers of invasion, and, therefore, can be considered intra- and intercellular messengers. Mechanistically, (de)protonation of acidic and basic amino acids, particularly histidine and aspartate, translates pH into catalytic activity, substrate binding, stability, interaction, aggregation, and localization of proteins.

An interesting aspect in relation to therapeutic strategies is the extracellular pH as an adjustable screw that could be taken advantage of in order to accelerate wound healing, control inflammatory processes, and reduce invasion and metastasis. This implies that the molecular mechanisms underlying pH-regulated cell migration, which harbor great potential as therapeutic targets, need to be further investigated. In this context, the proton-sensing GPCRs, ASICs, and the proton-sensing members TRPV1 and TRPV4 from the transient receptor potential channel vanilloid subfamily certainly deserve further in-depth consideration.

**Author contributions** CS wrote the manuscript and prepared the figures.

**Funding** Open Access funding enabled and organized by Projekt DEAL.

**Data availability** This declaration is not applicable

## Declarations

**Ethical approval** This declaration is not applicable

**Competing interests** The author has no relevant financial or non-financial interests to disclose.

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## References

1. Almeida EAC, Ilić D, Han Q, Hauck CR, Jin F, Kawakatsu H, Schlaepfer DD, Damsky CH (2000) Matrix survival signaling: from fibronectin via focal adhesion kinase to C-Jun $Nh_2$-terminal kinase. J Cell Biol 149:741–754. https://doi.org/10.1083/jcb.149.3.741
2. Amini-Nik S, Cambridge E, Yu W, Guo A, Whetstone H, Nadesan P, Poon R, Hinz B, Alman BA (2014) β-Catenin-regulated myeloid cell adhesion and migration determine wound healing. J Clin Invest 124:2599–2610. https://doi.org/10.1172/JCI62059
3. Angelova MI, Bitbol A-F, Seigneuret M, Staneva G, Kodama A, Sakuma Y, Kawakatsu T, Imai M, Puff N (2018) pH sensing by lipids in membranes: the fundamentals of pH-driven migration, polarization and deformations of lipid bilayer assemblies. Biochim Biophys Acta Biomembr 1860:2042–2063. https://doi.org/10.1016/j.bbamem.2018.02.026
4. Audero MM, Carvalho TMA, Ruffinatti FA, Loeck T, Yassine M, Chinigò G, Folcher A, Farfariello V, Amadori S, Vaghi C,

Schwab A, Reshkin SJ, Cardone RA, Prevarskaya N, Fiorio Pla A (2023) Acidic growth conditions promote epithelial-to-mesenchymal transition to select more aggressive PDAC cell phenotypes in vitro. Cancers 15:2572. https://doi.org/10.3390/cancers15092572
5. Beaumont KG, Mrksich M (2012) The mechanostability of isolated focal adhesions is strongly dependent on pH. Chem Biol 19:711–720. https://doi.org/10.1016/j.chembiol.2012.04.016
6. Becker HM, Deitmer JW (2020) Transport metabolons and acid/base balance in tumor cells. Cancers 12:899. https://doi.org/10.3390/cancers12040899
7. Beta C, Kruse K (2017) Intracellular oscillations and waves. Annu Rev Condens Matter Phys 8:239–264. https://doi.org/10.1146/annurev-conmatphys-031016-025210
8. Blanchoin L, Boujemaa-Paterski R, Sykes C, Plastino J (2014) Actin dynamics, architecture, and mechanics in cell motility. Physiol Rev 94:235–263. https://doi.org/10.1152/physrev.00018.2013
9. Boedtkjer E, Bentzon JF, Dam VS, Aalkjaer C (2016) Na+, HCO3- -cotransporter NBCn1 increases pHi gradients, filopodia, and migration of smooth muscle cells and promotes arterial remodelling. Cardiovasc Res 111:227–239. https://doi.org/10.1093/cvr/cvw079
10. Bourguignon LY, Singleton PA, Diedrich F, Stern R, Gilad E (2004) CD44 interaction with Na+-H+ exchanger (NHE1) creates acidic microenvironments leading to hyaluronidase-2 and cathepsin B activation and breast tumor cell invasion. J Biol Chem 279:26991–27007. https://doi.org/10.1074/jbc.M311838200
11. Braatz-Schade K, Haberey M, Stockem W (1973) Correlation between membrane potential, cell shape, and motile activity in *Amoeba proteus*. Exp Cell Res 80:456–458. https://doi.org/10.1016/0014-4827(73)90322-4
12. Briozzo P, Morisset M, Capony F, Rougeot C, Rochefort H (1988) In vitro degradation of extracellular matrix with Mr 52,000 cathepsin D secreted by breast cancer cells. Cancer Res 48:3688–3692
13. Brömme D, Bonneau PR, Lachance P, Wiederanders B, Kirschke H, Peters C, Thomas DY, Storer AC, Vernet T (1993) Functional expression of human cathepsin S in Saccharomyces cerevisiae. Purification and characterization of the recombinant enzyme. J Biol Chem 268:4832–4838. https://doi.org/10.1016/S0021-9258(18)53472-4
14. Bugter JM, Fenderico N, Maurice MM (2021) Mutations and mechanisms of Wnt pathway tumour suppressors in cancer. Nat Rev Cancer 21:5–21. https://doi.org/10.1038/s41568-020-00307-z
15. Burridge K (2018) Focal adhesions: a personal perspective on a half century of progress. FEBS J 284:3355–3361. https://doi.org/10.1111/febs.14195
16. Busco G, Cardone RA, Greco MR, Bellizzi A, Colella M, Antelmi E, Mancini MT, Dell'Aquila ME, Casavola V, Paradiso A, Reshkin SJ (2010) NHE1 promotes invadopodial ECM proteolysis through acidification of the peri-invadopodial space. FASEB J 24:3903–3915. https://doi.org/10.1096/fj.09-149518
17. Calalb MB, Polte TR, Hanks SK (1995) Tyrosine phosphorylation of focal adhesion kinase at sites in the catalytic domain regulates kinase activity: a role for Src family kinases. Mol Cell Biol 15:954–963
18. Canales Coutiño B, Mayor R (2021) Mechanosensitive ion channels in cell migration. Cells Dev 166:203683. https://doi.org/10.1016/j.cdev.2021.203683
19. Case LB, Baird MA, Shtengel G, Campbell SL, Hess HF, Davidson MW, Waterman CM (2015) Molecular mechanism of vinculin activation and nanoscale spatial organization in focal adhesions. Nat Cell Biol 17:880–892. https://doi.org/10.1038/ncb3180
20. Chiang Y, Chou C-Y, Hsu K-F, Huang Y-F, Shen M-R (2008) EGF upregulates $Na^+/H^+$ exchanger NHE1 by post-translational regulation that is important for cervical cancer cell invasiveness. J Cell Physiol 214:810–819. https://doi.org/10.1002/jcp.21277
21. Choi C-H, Webb BA, Chimenti MS, Jacobson MP, Barber DL (2013) pH-sensing by FAK-His58 regulates focal adhesion remodeling. J Cell Biol 202:849–859. https://doi.org/10.1083/jcb.201302131
22. Clark AG, Maitra A, Jacques C, Bergert M, Pérez-Gonzáles C, Simon A, Lederer L, Diz-Muñoz A, Trepat X, Voituriez R, Vignjevic DM (2022) Self-generated gradients steer collective migration on viscoelastic collagen networks. Nat Mater 21:1200–1210. https://doi.org/10.1038/s41563-022-01259-5
23. Claudio-Rizo JA, Delgado J, Quintero-Ortega IA, Mata-Mata JL, Mendoza-Novelo B (2018) Decellularized ECM-derived hydrogels: modification and properties. In: Haider S and Haider A (eds) Hydrogels. IntechOpen Ltd, London. https://doi.org/10.5772/intechopen.78331
24. Conklin MW, Eickhoff JC, Riching KM, Pehlke CA, Eliceiri KW, Provenzano PP, Friedl A, Keely PK (2011) Aligned collagen is a prognostic signature for survival in human breast carcinoma. Am J Pathol 178:1221–1232. https://doi.org/10.1016/j.ajpath.2010.11.076
25. Cortés Franco KD, Brakmann IC, Feoktistova M, Panayotova-Dimitrova D, Gründer S, Tian Y (2023) Aggressive migration in acidic pH of a glioblastoma cancer stem cell line in vitro is independent of ASIC and $K_{Ca}3.1$ ion channels, but involves phosphoinositide 3-kinase. Pflügers Arch 475:405–416. https://doi.org/10.1007/s00424-022-02781-w
26. Csaderova L, Debreova M, Radvak P, Stano M, Vrestiakova M, Kopacek J, Pastorekova S, Svastova E (2013) The effect of carbonic anhydrase IX on focal contracts during cell spreading and migration. Front Physiol 4:271. https://doi.org/10.3389/fphys.2013.00271
27. Cui N, Hu M, Khalil RA (2017) Biochemical and biological attributes of matrix metalloproteinases. Prog Mol Biol Transl Sci 147:1–73. https://doi.org/10.1016/bs.pmbts.2017.02.005
28. Dalous J, Burghardt E, Müller-Taubenberger A, Bruckert F, Gerisch G, Bretschneider T (2008) Reversal of cell polarity and actin-myosin cytoskeleton reorganization under mechanical and chemical stimulation. Biophys J 94:1063–1074. https://doi.org/10.1529/biophysj.107.114702
29. Dehrmann FM, Coetzer TH, Pike RN, Dennison C (1995) Mature cathepsin L is substantially active in the ionic milieu of the extracellular medium. Arch Biochem Biophys 324:93–98. https://doi.org/10.1006/abbi.1995.9924
30. Denker SP, Barber DL (2002) Cell migration requires both ion translocation and cytoskeletal anchoring by the Na-H exchanger NHE1. J Cell Biol 159:1087–1096. https://doi.org/10.1083/jcb.200208050
31. Eble JA, McDougall M, Orriss GL, Niland S, Johanningmeier B, Pohlentz G, Meier M, Karrasch S, Estevão-Costa MI, Lima AM, Stetefeld J (2017) Dramatic and concerted conformational changes enable rhodocetin to block α2β1 integrin selectively. PLoS Biol 15:e2001492. https://doi.org/10.1371/journal.pbio.2001492
32. Erra Díaz F, Dantas E, Geffner J (2018) Unravelling the interplay between extracellular acidosis and immune cells. Mediators Inflamm 2018:1218297. https://doi.org/10.1155/2018/1218297
33. Etienne-Manneville D, Hall A (2001) Integrin-mediated activation of Cdc42 controls cell polarity in migrating astrocytes through PKCzeta. Cell 106:489–498. https://doi.org/10.1016/s0092-8674(01)00471-8

34 Faes S, Uldry E, Planche A, Santoro T, Pythoud C, Demartines N, Dormond O (2016) Acidic pH reduces VEGF-mediated endothelial cell responses by downregulation of VEGFR-2; relevance for anti-angiogenic therapies. Oncotarget 7:86026–86038. https://doi.org/10.18632/oncotarget.13323
35. Fan J-S, Goh H, Ding K, Xue B, Robinson RC, Yang D (2017) Structural basis for pH-mediated regulation of F-actin severing by gelsolin domain 1. Sci Rep 7:45230. https://doi.org/10.1038/srep45230
36. Fish KN (2022) Total internal reflection fluorescence (TIRF) microscopy. Curr Protoc 2:e517. https://doi.org/10.1002/cpz1.517
37. Forbes SA, Beare D, Boutselakis H, Bamford S, Bindal N, Tate J, Cole CG, Ward S, Dawson E, Ponting L, Stefancsik R, Harsha B, Kok CY, Jia M, Jubb H, Sondka Z, Thompson S, De T, Campbell PJ (2017) COSMIC: somatic cancer genetics at high-resolution. Nucleic Acids Res 43(D1):D805–D811. https://doi.org/10.1093/nar/gkw1121
38. Frantz C, Barreiro G, Dominguez L, Chen X, Eddy R, Condeelis J, Kelly MJ, Jacobson MP, Barber DL (2008) Cofilin is a pH sensor for actin free barbed end formation: role of phosphoinositide binding. J Cell Biol 183:865–879. https://doi.org/10.1083/jcb.200804161
39. Frantz C, Karydis A, Nalbant P, Hahn KM, Barber DL (2007) Positive feedback between Cdc42 activity and H+ efflux by the Na-H exchanger NHE1 for polarity of migrating cells. J Cell Biol 179:403–410. https://doi.org/10.1083/jcb.200704169
40. Freire E, Coelho-Sampaio T (2000) Self-assembly of laminin induced by acidic pH. J Biol Chem 275:817–822. https://doi.org/10.1074/jbc.275.2.817
41. Gatenby RA, Gawlinski ET, Gmitro AF, Kaylor B, Gillies RJ (2006) Acid-mediated tumor invasion: a multidisciplinary study. Cancer Res 66:5216–5223. https://doi.org/10.1158/0008-5472.CAN-05-4193
42. Gatenby RA, Gillies RJ (2004) Why do cancers have high aerobic glycolysis? Nat Rev Cancer 4:891–899. https://doi.org/10.1038/nrc1478
43. Gerhardt M, Ecke M, Walz M, Stengl A, Beta C, Gerisch G (2014) Actin and PIP3 waves in giant cells reveal the inherent length scale of an excited state. J Cell Sci 127:4507–4517. https://doi.org/10.1242/jcs.156000
44. Gillies RJ, Raghunand N, Karczmar GS, Bhujwalla ZM (2002) MRI of the tumor microenvironment. J Magn Reson Imaging 16:430–450. https://doi.org/10.1002/jmri.10181
45. Giusti I, D'Ascenzo S, Millimaggi D, Taraboletti G, Carta G, Franceschini N, Pavan A, Dolo V (2008) Cathepsin B mediates the pH-dependent proinvasive activity of tumor-shed microvesicles. Neoplasia 10:481–488. https://doi.org/10.1593/neo.08178
46. Gong H, Shen B, Flevaris P, Chow C, Lam SC, Voyno-Yasenetskaya TA, Kozasa T, Du X (2010) G protein subunit $G\alpha_{13}$ binds to integrin $\alpha_{IIb}\beta_3$ and mediates integrin "outside-in" signaling. Science 327:340–343. https://doi.org/10.1126/science.1174779
47. Greco MR, Antelmi E, Busco G, Guerra L, Rubino R, Casavola V, Reshkin SJ, Cardone RA (2014) Protease activity at invadopodial focal digestive areas is dependent on NHE1-driven pHe. Oncol Rep 31:940–946. https://doi.org/10.3892/or.2013.2923
48. Grinstein S, Woodside M, Waddell TK, Downey GP, Orlowski J, Pouyssegur J, Wong DC, Foskett JK (1993) Focal localization of the NHE-1 isoform of the Na+/H+ antiport: assessment of effects on intracellular pH. EMBO J 12:5209–5218. https://doi.org/10.1002/j.1460-2075.1993.tb06216.x
49. Haase K, Pelling AE (2015) Investigating cell mechanics with atomic force microscopy. J R Soc Interface 12:20140970. https://doi.org/10.1098/rsif.2014.0970
50. Han Z, Yuan M, Liu L, Zhang K, Zhao B, He B, Liang Y, Li F (2023) pH-responsive wound dressings: advances and prospects. Nanoscale Horiz 8:422–440. https://doi.org/10.1039/d2nh00574c
51. Harris JR, Soliakov A, Lewis RJ (2013) In vitro fibrillogenesis of collagen type I in varying ionic and pH conditions. Micron 49:60–68. https://doi.org/10.1016/j.micron.2013.03.004
52. Helmlinger G, Yuan F, Dellian M, Jain RK (1997) Interstitial pH and pO2 gradients in solid tumors in vivo: high resolution measurements reveal a lack of correlation. Nat Med 3:177–182. https://doi.org/10.1038/nm0297-177
53. Hofschröer V, Koch A, Ludwig FT, Friedl P, Oberleithner H, Stock C, Schwab A (2017) Extracellular protonation modulates cell-cell interaction mechanics and tissue invasion in human melanoma cells. Sci Rep 7:42369. https://doi.org/10.1038/srep42369
54. Holman CM, Kan CC, Gehring MR, Van Wart HE (1999) Role of His-224 in the anomalous pH dependence of human stromelysin-1. Biochemistry 38:677–681. https://doi.org/10.1021/bi9822170
55. Howe AK (2004) Regulation of actin-based cell migration by cAMP/PKA. Biochim Biophys Acta 1692:159–174. https://doi.org/10.1016/j.bbamcr.2004.03.005
56. Howe AK (2011) Cross-talk between calcium and protein kinase A in the regulation of cell migration. Curr Opin Cell Biol 5:554–561. https://doi.org/10.1016/j.ceb.2011.05.006
57. Huber V, Camisaschi C, Berzi A, Ferro S, Lugini L, Triulzi T, Tuccitto A, Tagliabue E, Castelli C, Rivoltini L (2017) Cancer acidity: an ultimate frontier of tumor immune escape and a novel target of immunomodulation. Semin Cancer Biol 43:74–89. https://doi.org/10.1016/j.semcancer.2017.03.001
58. Jamerson M, da Rocha-Azevedo B, Cabral GA, Marciano-Cabral F (2012) Pathogenic *Naegleria fowleri* and non-pathogenic *Naegleria lovaniensis* exhibit differential adhesion to, and invasion of, extracellular matrix proteins. Microbiology (Reading) 158:791–803. https://doi.org/10.1099/mic.0.055020-0
59. Joerger AC, Fersht AR (2010) The tumor suppressor p53: from structures to drug discovery. Cold Spring Harb Perspect Biol 2:a000919. https://doi.org/10.1101/cshperspect.a000919
60. Johnson LL, Pavlovsky AG, Johnson AR, Janowicz JA, Man CF, Ortwine DF, Purchase CF 2nd, White AD, Hupe DJ (2000) A rationalization of the acidic pH dependence for stromelysin-1 (matrix metalloproteinase-3) catalysis and inhibition. J Biol Chem 275:11026–11033. https://doi.org/10.1074/jbc.275.15.11026
61. Justus CR, Sanderlin EJ, Dong L, Sun T, Chi J-T, Lertpiriyapong K, Yang LV (2017) Contextual tumor suppressor function of T cell death-associated gene 8 (TDAG8) in hematological malignancies. J Trans Med 15:204. https://doi.org/10.1186/s12967-017-1305-6
62. Kanchanawong P, Calderwood DA (2023) Organization, dynamics and mechanoregulation of integrin-mediated cell-ECM adhesions. Nat Rev Mol Cell Biol 24:142–161. https://doi.org/10.1038/s41580-022-00531-5
63. Kanchanawong P, Shtengel G, Pasapera AM, Ranko EB, Davidson MW, Hess HF, Waterman CM (2010) Nanoscale architecture of integrin-based cell adhesions. Nature 468:580–584. https://doi.org/10.1038/nature09621
64. Katan M, Cockcroft S (2020) Phosphatidylinositol(4,5)bisphosphate: diverse functions at the plasma membrane. Essays Biochem 64:513–531. https://doi.org/10.1042/EBC20200041
65. Kato Y, Lambert CA, Colige AC, Mineur P, Noël A, Frankenne F, Foidart JM, Baba M, Hata R, Miyazaki K, Tsukuda M (2005) Acidic extracellular pH induces matrix metalloproteinase-9 expression in mouse metastatic melanoma cells through the phospholipase D-mitogen-activated protein kinase signaling. J Biol Chem 280:10938–10944. https://doi.org/10.1074/jbc.M411313200
66. Kato Y, Ozawa S, Tsukuda M, Kubota E, Miyazaki K, St-Pierre Y, Hata R-I (2007) Acidic extracellular pH increases

calcium influx-triggered phospholipase D activity along with acidic sphingomyelinase activation to induce matrix metalloproteinase-9 expression in mouse metastatic melanoma. FEBS J 274:3171–3183. https://doi.org/10.1111/j.1742-4658.2007.05848.x
67. Khan MA, Philip LM, Cheung G, Vadakepeedika S, Grasemann H, Sweezey N, Palaniyar N (2018) Regulating NETosis: increasing pH promotes NADPH oxidase-dependent NETosis. Front Med 5:19. https://doi.org/10.3389/fmed.2018.00019
68. Kim WK, Kwon Y, Jang M, Park M, Kim J, Cho S, Jang DG, Lee WB, Jung SH, Choi HJ, Min BS, Il Kim T, Hong SP, Paik YK, Kim H (2019) β-Catenin activation down-regulates cell-cell junction-regulated genes and induces epithelial-to-mesenchymal transition in colorectal cancers. Sci Rep 9:18440. https://doi.org/10.1038/s41598-019-54890-9
69. Kim D-H, Wirtz D (2013) Focal adhesion size uniquely predicts cell migration. FASEB J 27:1351–1361. https://doi.org/10.1096/fj.12-220160
70. King KL, Essig J, Roberts TM, Moerland TS (1994) Regulation of the *Ascaris* major sperm protein (MSP) cytoskeleton by intracellular pH. Cell Motil Cytoskel 27:193–205. https://doi.org/10.1002/cm.970270302
71. Klapholz B, Brown NH (2017) Talin – the master of integrin adhesions. J Cell Sci 130:2435–2446. https://doi.org/10.1242/jcs.190991
72. Klein M, Seeger P, Schuricht B, Alper SL, Schwab A (2000) Polarization of Na+/H+ and Cl-/HCO3- exchangers in migrating renal epithelial cells. J Gen Physiol 115:599–608. https://doi.org/10.1085/jgp.115.5.599
73. Koblinski JE, Ahram M, Sloane BF (2000) Unraveling the role of proteases in cancer. Clin Chim Acta 291:113–135. https://doi.org/10.1016/s0009-8981(99)00224-7
74. Köhler S, Schmoller KM, Crevenna AH, Bausch AR (2012) Regulating contractility of the actomyosin cytoskeleton by pH. Cell Rep 2:433–439. https://doi.org/10.1016/j.celrep.2012.08.014
75. Koehler J, Wallmeyer L, Hedtrich S, Goepferich AM, Brandl FP (2017) pH-modulating poly(ethylene glycol)/alginate hydrogel dressings for the treatment of chronic wounds. Macromol Biosci 17:1600369. https://doi.org/10.1002/mabi.201600369
76. Köster S, Leach JB, Struth B, Pfohl T, Wong JY (2007) Visualization of flow-aligned type I collagen self-assembly in tunable pH gradients. Langmuir 23:357–359. https://doi.org/10.1021/la062473a
77. Krähling H, Mally S, Eble JA, Noël J, Schwab A, Stock C (2009) The glycocalyx maintains a cell surface pH nanoenvironment crucial for integrin-mediated migration of human melanoma cells. Pflügers Arch 458:1069–1083. https://doi.org/10.1007/s00424-009-0694-7
78. Kruse CR, Singh M, Targosinski S, Sinha I, Sørensen JA, Eriksson E, Nuutila K (2017) The effect of pH on cell viability cell migration, cell proliferation, wound closure and wound reepithelializaton: in vitro and in vivo study. Wound Repair Regen 25:260–269. https://doi.org/10.1111/wrr.12526
79. Labruyère E, Thibeaux R, Olivo-Marin JC, Guillén N (2019) Crosstalk between *Entamoeba histolytica* and the human intestinal tract during amoebiasis. Parasitology 146:1140–1149. https://doi.org/10.1017/S0031182017002190
80. Lauritzen G, Stock CM, Lemaire J, Lund SF, Jensen JM, Damsgaard B, Petersen KS, Wiwel M, Rønnov-Jessen L, Schwab A, Pedersen SF (2012) The Na+/H+ exchanger NHE1, but not the Na+/HCO3(-) cotransporter NBCn1, regulates motility of MCF7 breast cancer cells expressing constitutively active ErbB2. Cancer Lett 317:172–183. https://doi.org/10.1016/j.canlet.2011.11.023
81. Lehenkari PP, Horton MA (1999) Single integrin molecule adhesion forces in intact cells measured by atomic force microscopy. Biochem Biophys Res Commun 259:645–650. https://doi.org/10.1006/bbrc.1999.0827
82. Li Y, Asadi A, Monroe MR, Douglas EP (2009) pH effects on collagen fibrillogenesis in vitro: electrostatic interactions and phosphate binding. Mater Sci Eng C 29:1643–1649. https://doi.org/10.1016/j.msec.2009.01.001
83. Lietha D, Cai X, Ceccarelli DFJ, Li Y, Schaller MD, Eck MJ (2007) Structural basis for the autoinhibition of focal adhesion kinase. Cell 129:1037–1038. https://doi.org/10.1016/j.cell.2007.05.041
84. Liu R, Xu Y, Xu K, Dai Z (2021) Current trends and key considerations in the clinical translation of targeted fluorescent probes for intraoperative navigation. Aggregate 2:e23. https://doi.org/10.1002/agt2.23
85. Loeck T, Rugi M, Todesca LM, Kalinowska P, Soret B, Neumann I, Schimmelpfennig S, Najder K, Pethő Z, Farfariello V, Prevarskaya N, Schwab A (2023) The context-dependent role of the $Na^+/Ca^{2+}$-exchanger (NCX) in pancreatic stellate cell migration. Pflugers Arch 475:1225–1240. https://doi.org/10.1007/s00424-023-02847-3
86. Loitto V-M, Forslund T, Sundqvist T, Magnusson K-E, Gustafsson M (2002) Neutrophil leucocyte motility requires directed water influx. J Leukoc Biol 71:212–222
87. Loitto V-M, Karlsson T, Magnusson K-E (2009) Water flux in cell motility: expanding the mechanisms of membrane protrusion. Cell Motil Cytoskeleton 66:237–247. https://doi.org/10.1002/cm.20357
88. Lovy-Wheeler A, Kunkel JG, Allwood EG, Hussey PJ, Hepler PK (2006) Oscillatory increases in alkalinity anticipate growth and may regulate actin dynamics in pollen tubes of lily. Plant Cell 18:2182–2193. https://doi.org/10.1105/tpc.106.044867
89. Lu P, Takai K, Weaver VM, Werb Z (2011) Extracellular matrix digestion and remodeling in development and disease. Cold Spring Harb Perspect Biol 3:a005058. https://doi.org/10.1101/cshperspect.a005058
90. Ludwig FT, Schwab A, Stock C (2013) The Na+/H+ exchanger (NHE1) generates pH nanodomains at focal adhesions. J Cell Physiol 228:1351–1358. https://doi.org/10.1002/jcp.24293
91. Ludwig MG, Vanek M, Guerini D, Gasser JA, Jones CE, Junker U, Hofstetter H, Wolf RM, Seuwen K (2003) Proton-sensing G-protein-coupled receptors. Nature 425:93–98. https://doi.org/10.1038/nature01905
92. Lyda JK, Tan ZL, Rajah A, Momi A, Mackay L, Brown CM, Khadra A (2019) Rac activation is key to cell motility and directionality: an experimental and modelling investigation. Comput Struct Biotechnol J 17:1436–1452. https://doi.org/10.1016/j.csbj.2019.10.002
93. Maaser K, Wolf K, Klein CE, Niggemann B, Zänker KS, Bröcker EB, Friedl P (1999) Functional hierarchy of simultaneously expressed adhesion receptors: integrin alpha2beta1 but not CD44 mediates MV3 melanoma cell migration and matrix reorganization within three-dimensional hyaluron-containing collagen matrices. Mol Biol Cell 10:3067–3079. https://doi.org/10.1091/mbc.10.10.3067
94. Magalhaes MA, Larson DR, Mader CC, Bravo-Cordero JJ, Gil-Henn H, Oser M, Chen X, Koleske AJ, Condeelis J (2011) Cortactin phosphorylation regulates cell invasion through a pH-dependent pathway. J Cell Biol 195:903–920. https://doi.org/10.1083/jcb.201103045
95. Martin C, Pedersen SF, Schwab A, Stock C (2011) Intracellular pH gradients in migrating cells. Am J Physiol Cell Physiol 300:C490-495. https://doi.org/10.1152/ajpcell.00280.2010

96. Martin SG (2015) Spontaneous cell polarization: feedback control of Cdc42 GTPase breaks cellular symmetry. BioEssays 37:1193–1201. https://doi.org/10.1002/bies.201500077
97. Martinez D, Vermeulen M, Trevani A, Ceballos A, Sabatté J, Gamberale R, Álvarez ME, Salamone G, Tanos T, Coso OA, Geffner J (2006) Extracellular acidosis induces neutrophil activation by a mechanism dependent on activation of phosphatidylinositol 3-kinase/Akt and ERK pathways. J Immunol 176:1163–1171. https://doi.org/10.4049/jimmunol.176.2.1163
98. Masaike S, Tsuji Y, Kidoaki S (2023) Local pH mapping in the cell adhesion nano-interfaces on a pH-responsive fluorescence-dye-immobilized substrate. Anal Sci 39:347–355. https://doi.org/10.1007/s44211-022-00239-8
99. Mastrogiovanni M, Di Bartolo V, Alcover A (2022) Cell polarity regulators, multifunctional organizers of lymphocyte activation and function. Biomed J 45:299–309. https://doi.org/10.1016/j.bj.2021.10.002
100. Miao Y, Bhattacharya S, Banerjee T, Abubaker-Sharif B, Long Y, Inoue T, Iglesias PA, Devreotes PN (2019) Wave patterns organize cellular protrusions and control cortical dynamics. Mol Syst Biol 15:e8585. https://doi.org/10.15252/msb.20188585
101. Mierke CT (2009) The role of vinculin in the regulation of the mechanical properties of cells. Cell Biochem Biophys 53:115–126. https://doi.org/10.1007/s12013-009-9047-6
102. Miller GJ, Ball EH (2001) Conformational change in the vinculin C-terminal depends on a critical histidine residue (His-906). J Biol Chem 276:28829–28834. https://doi.org/10.1074/jbc.M102561200
103. Miranda-Calderon L, Yus C, Landa G, Mendoza G, Arruebo M, Irusta S (2022) Pharmacokinetic control on the release of antimicrobial drugs from pH-responsive electrospun wound dressings. Int J Pharm 624:122003. https://doi.org/10.1016/j.ijpharm.2022.122003
104. Moes D, Hoffmann C, Dieterle M, Moreau F, Neumann K, Papuga J, Furtado AT, Steinmetz A, Thomas C (2015) The pH sensibility of actin-bundling LIM proteins is governed by the acidic properties of their C-terminal domain. FEBS Lett 589:2312–2319. https://doi.org/10.1016/j.febslet.2015.07.033
105. Moissoglu K, Schwartz MA (2006) Integrin signalling in directed cell migration. Biol Cell 98:547–555. https://doi.org/10.1042/BC20060025
106. Monaco S, Gioia M, Rodriguez J, Fasciglione GF, Di Pierro D, Lupidi G, Krippahl L, Marini S, Coletta M (2007) Modulation of the proteolytic activity of matrix metalloproteinase-2 (gelatinase A) on fibrinogen. Biochem J 402:503–513. https://doi.org/10.1042/BJ20061064
107. Moore TI, Aaron J, Chew T-L, Springer TA (2018) Measuring integrin conformational change on the cell surface with super-resolution microscopy. Cell Rep 22:1903–1212. https://doi.org/10.1016/j.celrep.2018.01.062
108. Morishita K, Watanabe K, Ichijo H (2019) Cell volume regulation in cancer cell migration driven by osmotic water flow. Cancer Sci 110:2337–2347. https://doi.org/10.1111/cas.14079
109. Muguruma M, Matsumura S, Fukazawa T (1990) Direct interactions between talin and actin. Biochem Biophys Res Commun 171:1217–1223. https://doi.org/10.1016/0006-291X(90)90815-5
110. Muinonen-Martin A, Susanto O, Zhang Q, Smethurst E, Faller WJ, Veltman DM, Kalna G, Lindsay C, Bennett DC, Sansom OJ, Herd R, Jones R, Machesky LM, Wakelam MJO, Knecht DA, Insall RH (2014) Melanoma cells break down LPA to establish local gradients that drive chemotactic dispersal. PLoS Biol 12:e1001966. https://doi.org/10.1371/journal.pbio.1001966
111. Oster L, Schröder J, Rugi M, Schimmelpfennig S, Sargin S, Schwab A, Najder K (2022) Extracellular pH controls chemotaxis of neutrophil granulocytes by regulating leukotriene $B_4$ production and Cdc42 signaling. J Immunol 209:136–144. https://doi.org/10.4049/jimmunol.2100475
112. Palmer SM, Schaller MD, Campbell SL (2008) Vinculin tail conformation and self-association is independent of pH and H906 protonation. Biochemistry 47:12467–12475. https://doi.org/10.1021/bi801764a
113. Paradise RK, Lauffenburger DA, Van Vliet KJ (2011) Acidic extracellular pH promotes activation of integrin α(v)β(3). PLoS One 6:e15746. https://doi.org/10.1371/journal.pone.0015746
114. Paradise RK, Whitfield MJ, Lauffenburger DA, Van Vliet KJ (2013) Directional cell migration in an extracellular pH gradient: a model study with an engineered cell line and primary microvascular endothelial cells. Exp Cell Res 319:487–497. https://doi.org/10.1016/j.yexcr.2012.11.006
115. Pethő Z, Najder K, Carvalho T, McMorrow R, Todesca LM, Rugi M, Bulk E, Chan A, Löwik CWGM, Reshkin SJ, Schwab A (2020) pH-channeling in cancer: how pH-dependence of cation channels shapes cancer pathophysiology. Cancers 12:2484. https://doi.org/10.3390/cancers12092484
116. Piroli ME, Blanchette JO, Jabbarzadeh E (2019) Polarity as a physiological modulator of cell function. Front Biosci (Landmark Ed) 24:451–462. https://doi.org/10.2741/4728
117. Pollard TD, Borisy GG (2003) Cellular motility driven by assembly and disassembly of actin filaments. Cell 112:453–465. https://doi.org/10.1016/s0092-8674(03)00120-x
118. Ponti A, Machacek M, Gupton SL, Waterman-Storer CM, Danuser G (2004) Two distinct actin networks drive the protrusion of migrating cells. Science 305:1782–1786. https://doi.org/10.1126/science.1100533
119. Putney LK, Barber DL (2004) Expression profile of genes regulated by activity of the Na-H exchanger NHE1. BMC Genomics 5:46. https://doi.org/10.1186/1471-2164-5-46
120. Rappel W-J, Edelstein-Keshet L (2017) Mechanisms of cell polarization. Curr Opin Syst Biol 3:43–53. https://doi.org/10.1016/j.coisb.2017.03.005
121. Rauschner M, Lange L, Hüsing T, Reime S, Nolze A, Maschek M, Thews O, Riemann A (2021) Impact of the acidic environment on gene expression and functional parameters of tumors in vitro and in vivo. J Exp Clin Cancer Res 40:10. https://doi.org/10.1186/s13046-020-01815-4
122. Reithmeier RA (2001) A membrane metabolon linking carbonic anhydrase with chloride/bicarbonate anion exchangers. Blood Cells Mol Dis 27:85–89. https://doi.org/10.1006/bcmd.2000.0353
123. Riemann A, Schneider B, Gündel D, Stock C, Thews O, Gekle M (2014) Acidic priming enhances metastatic potential of cancer cells. Pflugers Arch 466:2127–2138. https://doi.org/10.1007/s00424-014-1458-6
124. Riemann A, Schneider B, Ihling A, Nowak M, Sauvant C, Thews O, Gekle M (2011) Acidic environment leads to ROS-induced MAPK signaling in cancer cells. PLoS ONE 6:e22445. https://doi.org/10.1371/journal.pone.0022445
125. Riga A, Castiglioni VG, Boxem M (2020) New insights into apical-basal polarization in epithelia. Curr Opin Cell Biol 62:1–8. https://doi.org/10.1016/j.ceb.2019.07.017
126. Ripamonti M, Liaudet N, Azizi L, Bouvard D, Hytönen VP, Wehrle-Haller B (2021) Structural and functional analysis of LIM domain-dependent recruitment of paxillin to αvβ3 integrin-positive focal adhesions. Commun Biol 4:380. https://doi.org/10.1038/s42003-021-01886-9
127. Ritter M, Schratzberger P, Rossmann H, Wöll E, Seiler K, Seidler U, Reinisch N, Kähler CM, Zwierzina H, Lang HJ, Lang F, Paulmichl M, Wiedermann CJ (1998) Effect of inhibitors of $Na^+/H^+$-exchange and gastric $H^+/K^+$ ATPase on cell volume, intracellular pH and migration of human polymorphonuclear leucocytes. Br J Pharmacol 124:627–638. https://doi.org/10.1038/sj.bjp.0701864

128. Rohani N, Hao L, Alexis MS, Joughin BA, Krismer K, Moufarrej MN, Soltis AR, Lauffenburger DA, Yaffe MB, Burge CB, Bhatia SN, Gertler FB (2019) Acidification of tumor at stromal boundaries drives transcriptome alterations associated with aggressive phenotypes. Cancer Res 79:1952–1966. https://doi.org/10.1158/0008-5472.CAN-18-1604
129. Rose R, Kemper B, Schwab A, Schlatter E, Edemir B (2021) Unexpected localization of AQP3 and AQP4 induced by migration of primary cultured IMCD cells. Sci Rep 11:11930. https://doi.org/10.1038/s41598-021-91369-y
130. Rozhin J, Sameni M, Ziegler G, Sloane BF (1994) Pericellular pH affects distribution and secretion of cathepsin B in malignant cells. Cancer Res 54:6517–6525
131. Saadoun S, Papadopoulos MC, Watanabe H, Yan D, Manley GT, Verkman AS (2005) Involvement of aquaporin-4 in astroglial cell migration and glial scar formation. J Cell Sci 118:5691–5698. https://doi.org/10.1242/jcs.02680
132. Sasaki S, Takahashi R, Luo Y, Chujo K, Sera T, Kudo S (2021) Spatiotemporal distribution of PKCα, Cdc42, and Rac1 before directed cell migration. Biochem Biophys Res Commun 584:26–31. https://doi.org/10.1016/j.bbrc.2021.10.080
133. Scherber C, Aranyosi AJ, Kulemann B, Thayer SP, Toner M, Iliopoulos O, Irimia D (2012) Epithelial cell guidance by self-generated EGF gradients. Integr Biol 4:259–269. https://doi.org/10.1039/c2ib00106c
134. Schneider L, Klausen TK, Stock C, Mally S, Christensen ST, Pedersen SF, Hoffmann EK, Schwab A (2007) H-ras transformation sensitizes volume-activated anion channels and increases migratory activity of NIH3T3 fibroblasts. Pflugers Arch 455:1055–1062. https://doi.org/10.1007/s00424-007-0367-3
135. Schönichen A, Webb BA, Jacobson MP, Barber DL (2013) Considering protonation as a posttranslational modification regulating protein structure and function. Annu Rev Biophys 42:289–314. https://doi.org/10.1146/annurev-biophys-050511-102349
136. Schreml S, Meier RJ, Kirschbaum M, Kong SC, Gehmert S, Felthaus O, Küchler S, Sharpe JR, Wöltje K, Weiß KT, Albert M, Seidl U, Schröder J, Morszeck C, Prantl L, Duschl C, Pedersen SF, Gosau M, Berneburg M, Wolfbeis OS, Landthaler M, Babilas P (2014) Luminescent dual sensors reveal extracellular pH-gradients and hypoxia on chronic wounds that disrupt epidermal repair. Theranostics 4:721–735. https://doi.org/10.7150/thno.9052
137. Schwab A, Gabriel K, Finsterwalder G, Folprecht G, Greger R, Kramer A, Oberleithner H (1995) Polarized ion transport during migration of transformed Madin-Darby canine kidney cells. Pflugers Arch 430:802–807. https://doi.org/10.1007/BF00386179
138. Schwab A, Hanley P, Fabian A, Stock C (2008) Potassium channels keep mobile cells on the go. Physiology 23:212–220. https://doi.org/10.1152/physiol.00003.2008
139. Schwab A, Nechyporuk-Zloy V, Fabian A, Stock C (2007) Cells move when ions and water flow. Pflugers Arch 453:421–432. https://doi.org/10.1007/s00424-006-0138-6
140. Schwab A, Rossmann H, Klein M, Dieterich P, Gassner B, Neff C, Stock C, Seidler U (2005) Functional role of $Na^+$-$HCO_3^-$ cotransport in migration of transformed renal epithelial cells. J Physiol 568:445–458. https://doi.org/10.1113/jphysiol.2005.092957
141. Schwab A, Stock C (2014) Ion channels and transporters in tumour cell migration and invasion. Philos Trans R Soc Lond B Biol Sci 369:20130102. https://doi.org/10.1098/rstb.2013.0102
142. Schwab A, Wulf A, Schulz C, Kessler W, Nechyporuk-Zloy V, Römer M, Reinhardt J, Weinhold D, Dieterich P, Stock C, Hebert SC (2006) Subcellular distribution of calcium-sensitive potassium channels (IK1) in migrating cells. J Cell Physiol 206:86–94. https://doi.org/10.1002/jcp.20434
143. Severin M, Pedersen EL, Borre MT, Axholm I, Christiansen FB, Ponniah M, Czaplinska D, Larsen T, Pardo LA, Pedersen SF (2023) Dynamic localization of the Na+-HCO3- co-transporter NBCn1 to the plasma membrane, centrosomes, spindle and primary cilia. J Cell Sci 136:jcs260687. https://doi.org/10.1242/jcs.260687
144. Shanley LJ, Walczysko P, Bain M, MacEwan DJ, Zhao M (2006) Influx of extracellular $Ca^{2+}$ is necessary for electrotaxis in *Dictyostelium*. J Cell Sci 119:4741–4748. https://doi.org/10.1242/jcs.03248
145. Shen B, Delaney MK, Xiaoping Du (2012) Inside-out, outside-in, and inside-outside-in: G protein signaling in integrin-mediated cell adhesion, spreading, and retraction. Curr Opin Cell Biol 24:600–606. https://doi.org/10.1016/j.ceb.2012.08.011
146. Shi Q, Maas L, Veith C, Van Schooten FJ, Godschalk RW (2017) Acidic cellular microenvironment modifies carcinogen-induced DNA damage and repair. Arch Toxicol 91:2425–2442. https://doi.org/10.1007/s00204-016-1907-4
147. Sieg DJ, Hauck CR, Ilić D, Klingbeil CK, Schaefer E, Damsky CH, Schlaepfer DD (2000) FAK integrates growth-factor and integrin signals to promote cell migration. Nat Cell Biol 2:249–256. https://doi.org/10.1038/35010517
148. Simchowitz L, Cragoe EJ Jr (1986) Regulation of human neutrophil chemotaxis by intracellular pH. J Biol Chem 261:6492–6500. https://doi.org/10.1016/S0021-9258(19)84589-1
149. Sisignano M, Fischer MJM, Geisslinger G (2021) Proton-sensing GPCRs in health and disease. Cells 19:2050. https://doi.org/10.3390/cells10082050
150. Smith IM, Stroka KM (2023) The multifaceted role of aquaporins in physiological cell migration. Am J Physiol Cell Physiol 325:C208–C223. https://doi.org/10.1152/ajpcell.00502.2022
151. Srivastava J, Barber DL, Jacobson MP (2007) Intracellular pH sensors: design principles and functional significance. Physiology 22:30–39. https://doi.org/10.1152/physiol.00035.2006
152. Srivastava J, Barreiro G, Groscurth S, Gingras AR, Goult BT, Critchley DR, Kelly MJS, Jacobson MP, Barber DL (2008) Structural model and functional significance of pH-dependent talin-actin binding for focal adhesion remodeling. Proc Natl Acad Sci USA 105:14436–14441. https://doi.org/10.1073/pnas.0805163105
153. Sterling D, Reithmeier RA, Casey JR (2001) A transport metabolon. Functional interaction of carbonic anhydrase II and chloride/bicarbonate exchangers. J Biol Chem 276:47886–47894. https://doi.org/10.1074/jbc.M105959200
154. Stock C, Cardone RA, Busco G, Krähling H, Schwab A, Reshkin SJ (2008) Protons extruded by NHE1: digestive or glue? Eur J Cell Biol 87:591–599. https://doi.org/10.1016/j.ejcb.2008.01.007
155. Stock C, Gassner B, Hauck CR, Arnold H, Mally S, Eble JA, Dieterich P, Schwab A (2005) Migration of human melanoma cells depends on extracellular pH and $Na^+/H^+$ exchange. J Physiol 567:225–238. https://doi.org/10.1113/jphysiol.2005.088344
156. Stock C, Ludwig FT, Hanley PJ, Schwab A (2013) Roles of ion transport in control of cell motility. Compr Physiol 3:59–119. https://doi.org/10.1002/cphy.c110056
157. Stock C, Mueller M, Kraehling H, Mally S, Noël J, Eder C, Schwab A (2007) pH nanoenvironments at the surface of single melanoma cells. Cell Physiol Biochem 20:679–686. https://doi.org/10.1159/000107550
158. Stock C, Schwab A (2009) Protons make tumor cells move like clockwork. Pflugers Arch 458:981–992. https://doi.org/10.1007/s00424-009-0677-8
159. Stolwijk JA, Wallner S, Heider J, Kurz B, Pütz L, Michaelis S, Goricnik B, Erl J, Frank L, Berneburg M, Haubner F, Wegener J, Schreml S (2023) GPR4 in the pH-dependent migration of melanoma cells in the tumor microenvironment. Exp Dermatol 32:479–490. https://doi.org/10.1111/exd.14735

160. Stroka KM, Jiang H, Chen S-H, Tong Z, Wirtz D, Sun SX, Konstantopoulos K (2014) Water permeation drives tumor cell migration in confined microenvironments. Cell 157:611–623. https://doi.org/10.1016/j.cell.2014.02.052
161. Stüwe L, Müller M, Fabian A, Waning J, Sabine M, Noël J, Schwab A, Stock C (2007) pH dependence of melanoma cell migration: protons extruded by NHE1 dominate protons of the bulk solution. J Physiol 585:351–360. https://doi.org/10.1113/jphysiol.2007.145185
162. Sun HQ, Yamamoto M, Mejillano M, Yin HL (1999) Gelsolin, a multifunctional actin regulatory protein. J Biol Chem 274:33179–33182. https://doi.org/10.1074/jbc.274.47.33179
163. Sutoo S, Maeda T, Suzuki A, Kato Y (2020) Adaptation to chronic acidic extracellular pH elicits a sustained increase in lung cancer cell invasion and metastasis. Clin Exp Metastasis 37:133–144. https://doi.org/10.1007/s10585-019-09990-1
164. Svastova E, Witarski W, Csaderova L, Kosik I, Skvarkova L, Hulikova A, Zatovicova M, Barathova M, Kopacek J, Pastorek J, Pastorekova S (2012) Carbonic anhydrase IX interacts with bicarbonate transporters in lamellipodia and increases cell migration via its catalytic domain. J Biol Chem 287:3392–3402. https://doi.org/10.1074/jbc.M111.286062
165. Swietach P, Youm J-B, Saegusa N, Leem CH, Spitzer KW, Vaughan-Jones RD (2013) Coupled $Ca^{2+}$/$H^+$ transport by cytoplasmic buffers regulates local $Ca^{2+}$ and $H^+$ ion signaling. Proc Natl Acad Sci USA 110:E2064–E2073. https://doi.org/10.1073/pnas.1222433110
166. Takahashi E, Yamaguchi D, Yamaoka Y (2020) A relatively small gradient of extracellular pH directs migration of MDA-MB-231 cells in vitro. Int J Mol Sci 21:2565. https://doi.org/10.3390/ijms21072565
167. Thews O, Riemann A (2019) Tumor pH and metastasis: a malignant process beyond hypoxia. Cancer Metastasis Rev 38:113–129. https://doi.org/10.1007/s10555-018-09777-y
168. Tsujita K, Itoh T (2015) Phosphoinositides in the regulation of actin cortex and cell migration. Biochim Biophys Acta 1851:824–831. https://doi.org/10.1016/j.bbalip.2014.10.011
169. Tweedy L, Knecht DA, Mackay GM, Insall RH (2016) Self-generated chemoattractant gradients: attractant depletion extends the range and robustness of chemotaxis. PLoS Biol 14:e1002404. https://doi.org/10.1371/journal.pbio.1002404
170 Tweedy L, Thomason PA, Paschke PI, Martin K, Machesky LM, Zagnoni M, Insall RH (2020) Seeing around corners: cells solve mazes and respond at a distance using attractant breakdown. Science 369:eaay9792. https://doi.org/10.1126/science.aay9792
171. Vahle A-K, Domikowsky B, Schwöppe C, Krähling H, Mally S, Schäfers M, Hermann S, Shahin V, Haier J, Schwab A, Stock C (2014) Extracellular matrix composition and interstitial pH modulated NHE1-mediated melanoma cell motility. Int J Oncol 44:78–90. https://doi.org/10.3892/ijo.2013.2158
172. Van Helvert S, Storm C, Friedl P (2018) Mechanoreciprocity in cell migration. Nat Cell Biol 20:8–20. https://doi.org/10.1038/s41556-017-0012-0
173. Vassilev V, Platek A, Hiver S, Enomoto H, Takeichi M (2017) Catenins steer cell migration via stabilization of front-rear polarity. Dev Cell 43:463-479.e5. https://doi.org/10.1016/j.devcel.2017.10.014
174. Vercoulen Y, Kondo Y, Iwig JS, Janssen AB, White KA, Amini M, Barber DL, Kuriyan J, Roose JP (2017) A histidine pH sensor regulates activation of the Ras-specific guanine nucleotide exchange factor RasGRP1. Elife 6:e29002. https://doi.org/10.7554/eLife.29002
175. Voskuil FJ, Steinkamp PJ, Zhao T, van der Vegt B, Koller M, Doff JJ, Jayalakshmi Y, Hartung JP, Gao J, Sumer BD, Witjes MJH, van Dam GM, SHINE study group, (2020) Exploiting metabolic acidosis in solid cancers using a tumor-agnostic pH-activatable nanoprobe for fluorescence-guided surgery. Nat Commun 11:3257. https://doi.org/10.1038/s41467-020-16814-4
176. Vyas IK, Jamerson M, Cabral GA, Marciano-Cabral F (2015) Identification of peptidases in highly pathogenic vs. weakly pathogenic *Naegleria fowleri* amebae. J Eukaryot Microbiol 62:51–59. https://doi.org/10.1111/jeu.12152
177. Wang F, Sampogna RV, Ware BR (1989) pH dependence of actin self-assembly. Biophys J 55:293–298. https://doi.org/10.1016/S0006-3495(89)82804-8
178. Wang J, Shen J, Xu Y, Jiang Y, Qu X, Zhao W, Wang Y, Huang S (2023) Differential sensitivity of ADF isovariants to a pH gradient promotes pollen tube growth. J Cell Biol 222:e202206074. https://doi.org/10.1083/jcb.202206074
179. Watanabe N, Mitchison TJ (2002) Single-molecule speckle analysis of actin filament turnover in lamellipodia. Science 295:1083–1089. https://doi.org/10.1126/science.1067470
180. Webb DJ, Donais K, Whitmore LA, Thomas SM, Turner CE, Parsons JT, Horwitz AF (2004) FAK-Src signaling through paxillin, ERK and MLCK regulates adhesion disassembly. Nat Cell Biol 6:154–161. https://doi.org/10.1038/ncb1094
181. Webb BA, White KA, Grillo-Hill BK, Schönichen A, Choi C, Barber DL (2016) A histidine cluster in the cytoplasmic domain of the Na-H exchanger NHE1 confers pH-sensitive phospholipid binding and regulates transporter activity. J Cell Biol Chem 291:24096–24104. https://doi.org/10.1074/jbc.M116.736215
182. Wedlich-Soldner R, Li R (2003) Spontaneous cell polarization: undermining mechanism. Nat Cell Biol 5:267–270. https://doi.org/10.1038/ncb0403-267
183. Weiß KT, Fante M, Köhl G, Schreml J, Haubner F, Kreutz M, Haverkampf S, Berneburg M, Schreml S (2017) Proton-sensing G protein-coupled receptors as regulators of cell proliferation and migration during tumor growth and wound healing. Exp Dermatol 26:127–132. https://doi.org/10.1111/exd.13209
184. White KA, Grillo-Hill BK, Esquivel M, Peralta J, Bui VN, Chire I, Barber DL (2018) β-Catenin is a pH sensor with decreased stability at higher intracellular pH. J Cell Biol 217:3965–3976. https://doi.org/10.1083/jcb.201712041
185. White KA, Ruiz DG, Szpiech ZA, Strauli NB, Hernandez RD, Jacobson MP, Barber DL (2017) Cancer-associated arginine-to-histidine mutations confer a gain in pH sensing to mutant proteins. Sci Signal 10:9931. https://doi.org/10.1126/scisignal.aam9931
186. Winkelman JD, Anderson CA, Suarez C, Kovar DR, Gardel ML (2020) Evolutionarily diverse LIM domain-containing proteins bind stressed actin filaments through a conserved mechanism. Proc Natl Acad Sci USA 117:25532–25542. https://doi.org/10.1073/pnas.2004656117
187. Wioland H, Jegou A, Romet-Lemonne G (2019) Quantitative variations with pH of actin depolymerizing factor/cofilin's multiple actions on actin filaments. Biochemistry 58:40–47. https://doi.org/10.1021/acs.biochem.8b01001
188. Wong M, Gilmour D (2021) Going your own way: self-guidance mechanisms in cell migration. Curr Opin Cell Biol 72:116–123. https://doi.org/10.1016/j.ceb.2021.07.004
189. Yin HL (1987) Gelsolin: calcium- and polyphosphoinositide-regulated actin-modulating protein. BioEssays 7:176–179. https://doi.org/10.1002/bies.950070409

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