The Biophysicist

The Biophysics of Cell Migration: Biasing Cell Motion with Feynman Ratchets

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ABSTRACT The concepts and frameworks of soft matter physics and the laws of thermodynamics can be used to describe relevant developmental, physiologic, and pathologic events in which directed cell migration is involved, such as in cancer. Typically, this directionality has been associated with the presence of soluble long-range gradients of a chemoattractant, synergizing with many other guidance cues to direct the motion of cells. In particular, physical inputs have been shown to strongly influence cell locomotion. However, this type of cue has been less explored despite the importance in biology. In this paper, we describe recent in vitro works at the interface between physics and biology, showing how the motion of cells can be directed by using gradient-free environments with repeated local asymmetries. This rectification of cell migration, from random to directed, is a process reminiscent of the Feynman ratchet; therefore, this framework can be used to explain the mechanism behind directed cell motion.

KEY WORDS motility; mechanosensing and motility; adaptive primary literature; learning goals; fundamental concepts and techniques; researchers in biophysics-related education; teachers and students of foundational courses in the biophysics-related sciences

I. INTRODUCTION

Cell migration plays an essential role in many biologic phenomena, either individually or collectively (1). In the absence of any external cue, cells typically move by displaying random trajectories with no preferential direction (2–4). On the contrary, under certain stimuli, cells can bias their motion and migrate directionally toward a particular direction (5–9). This directionality is fundamental in many developmental, physiologic, and pathologic processes. As an example, during embryogenesis (i.e., the formation of an embryo), cells migrate collectively to generate different tissues and organs (10, 11). Similarly, during wound healing, epithelial cells collectively migrate to repair and close the injury (12). Finally, during tumor progression, individual or clusters of cancer cells migrate directionally to invade first the surrounding tumoral tissue and, next, the vasculature and lymphatics, initiating metastasis (Fig 1a) (13). Other physiopathologic processes in

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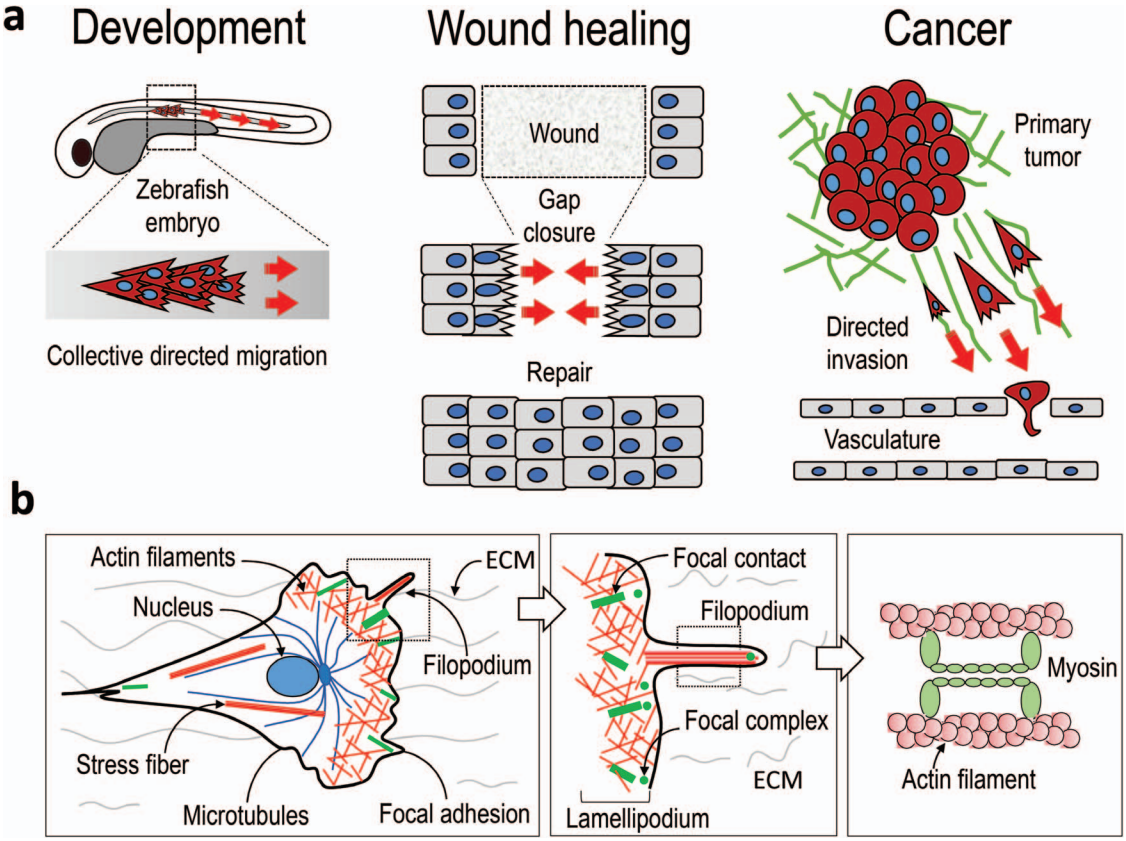

Fig 1. Directed cell migration during developmental, physiologic, and pathologic events. (a) Left: During zebrafish development, mesenchymal cells interact with the ECM and migrate collectively during the formation of the neural crest. Middle: During wound healing, cells polarize in response to the segregated cytokines and migrate directionally to close the injury. Right: During cancer progression, tumor cells detach from the primary tumor in a phenomenon known as an epithelial-to-mesenchymal transition due to the presence of growth factors gradients (e.g., vascular endothelial growth factor). (b) Scheme showing the essential components of the cell cytoskeleton in a polarized migrating cell.

which directed cell migration is involved include an immune response (e.g., the migration of immune cells, such as macrophages, toward the infection region) (14), mental disorders (e.g., defects in neuronal migration during development) (15), or vascular disease (e.g., anomalies in vascular smooth muscle cell migration) (16), among others. Typically, all these developmental and physiopathologic processes in which directed cell migration is involved are explained due to the presence of long-range biochemical gradients (e.g., cytokines or growth factors) in a process denoted as chemotaxis (17, 18), which results from the activation or inhibition of specific genes. The cycle of cell migration due to chemotaxis is typically described as follows: (a) first, the cell polarizes (i.e., there is an asymmetric reorganization of the cell structure, components, and function) due to the presence of an external chemoattractant; (b) next, there is an elongation of cell membrane protrusions, such as filopodia, in the direction of the chemoattractant; (c) these protrusions establish new adhesions (denoted as "focal adhesions") with the extracellular matrix (ECM), which are used by the cell to apply mechanical (traction) forces; and (d) finally, the adhesions at the rear of the cell detach, allowing the cell body to move (18). Throughout this cycle, the cell uses a diverse repertoire of cytoskeleton structures and molecules to adhere and migrate, including actin filaments (F-actin), microtubules, focal adhesions (contacts and complexes), or the molecular motor myosin (Fig 1b). Note that cell migration in vivo is a very complex and integrated mechanism, where biochemical gradients act together with many other guidance

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cues to direct cell locomotion (19). Other types of gradients (e.g., changes in stiffness; durotaxis (20), surface adhesion; haptotaxis (21), and topography; topotaxis (22); see also Appendix A) and gradient-free cues that are related to the structural properties of the local cellular microenvironment (e.g., changes in curvature; curvotaxis (23), electric properties; galvanotaxis (24), and others; see Appendix A) can also influence the migration of cells. Therefore, the contribution of other multidisciplinary approaches and concepts must be considered to expand our knowledge in the mechanism of directed cell migration.

The laws of physics can contribute to deciphering the mechanism at work for cell migration during physiopathologic events. Cell motility has indeed attracted the interest of biophysicists during recent decades (25–27). Biophysicists view migrating cells as perfect active matter and out-of-equilibrium systems due to the dynamic nature of the constituent parts at different hierarchic levels. The high dynamism of cellular processes results from the continuous input of chemical energy within the cells, which is provided by the hydrolysis of adenosine triphosphate (ATP; ATP $\leftrightharpoons$ ADP [adenosine diphosphate] + P_i) and guanosine triphosphate (GTP; GTP $\leftrightharpoons$ GDP [guanosine diphosphate] + P_i) highly available inside the cells. The released energy is used to fuel a diverse variety of mechanochemical cellular processes, such as force generation during cell migration. In this case, molecular motors, such as myosin, undergo conformational changes by transforming the chemical energy of ATP hydrolysis to produce mechanical work; this makes molecular motors operate far from equilibrium. At a higher hierarchic level, together with several associated proteins, these active molecules can self-organize into more complex subcellular structures. Examples include the polymerization or depolymerization of microtubules and actin filaments, which are the building blocks necessary for cell polarization and locomotion (28). Overall, these nonequilibrium processes can be described by using the laws and concepts of soft matter and statistical physics. By doing this, critical mechanisms of cell migration have been revealed and modeled, making significant contributions to biology (27). Similarly, new experimental approaches have been developed to accurately investigate directed cell motion in the absence of long-range gradients, and novel cell locomotion modes have been reported (Appendix A) (6, 29–35). These works have benefited from progress in micro- and nanofabrication techniques, optical imaging, and quantitative cell biology tools, as well as from the introduction of descriptive physical modeling.

In this paper, we discuss recent studies in the field of physics of cell migration. The main objective is to illustrate, in a simple scientific and pedagogic manner, how physics concepts and frameworks inspired by the Feynman ratchet can be used to obtain and describe the directed motion of cells in the total absence of gradients. It is not the aim of this work to describe in great detail the discussed articles, as it involves a vast number of complex questions. Instead, we give a general overview of the main physical principles applied to the biologic system under investigation to stimulate the critical thinking of the readers. For a detailed description of the mentioned works or specific concepts, the readers may refer to the original papers and references therein.

II. SCIENTIFIC AND PEDAGOGIC BACKGROUND

A. The Feynman ratchet

To illustrate the impact of such physics-based approaches, we will start by briefly describing a simple in vitro experiment in which long-term cell directionality was observed in the absence of biochemical gradients. For this, we fabricated a microenvironment with repeated local asymmetries (namely, an array of asymmetric micro-sized triangles) made of adhesive motifs where cells adhered (30). In this scenario, the symmetry of the cell was broken (i.e., the cell polarized), perturbing the inherently stochastic distribution of membrane protrusions of cells seeded on isotropic two-dimensional environments. Instead, pro-

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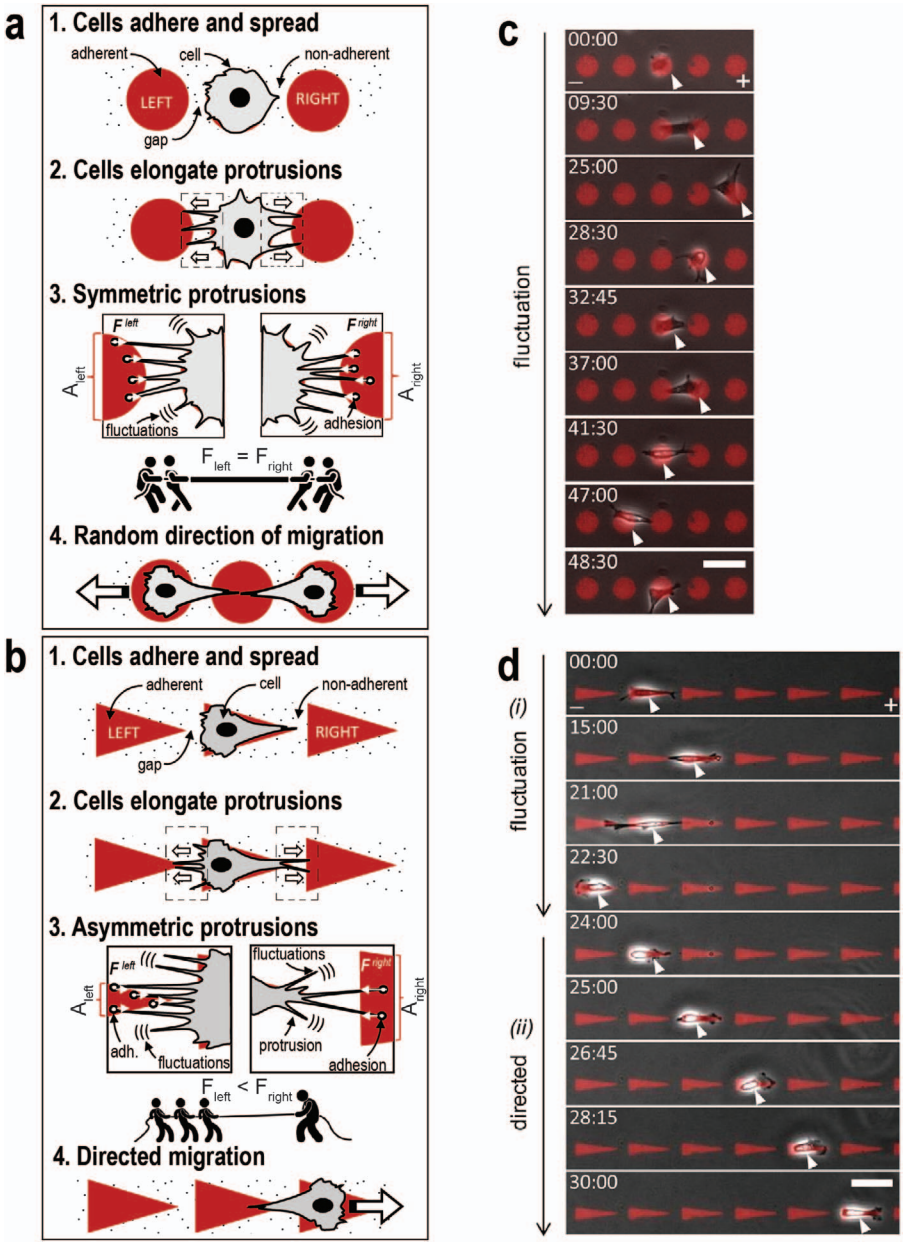

Fig 2. Directed cell migration by ratchetaxis. (a and b) Scheme describing the basic mechanisms of (a) nonbiased (symmetric spots) versus (b) biased (asymmetric triangles) cell migration on the basis of protrusion activities and tug-of-war forces. (c and d) Time-lapse sequence of an NIH/3T3 cell seeded on an array of (c) symmetric spots and (d) asymmetric triangles of fibronectin (in red). The nonpatterned dark regions contain a molecule (PLL-g-PEG), where cells cannot adhere. In (d), cells initially fluctuate on the micropatterned motifs and then migrate directionally. Scale bars: 100 μm. Reproduced with permission from (30, fig 3).

trusion activity was modified, guiding the migration of cells toward a preferential direction and biasing cell motion (Fig 2a,b) (30). Note that in this process, no biochemical gradient was present. Further details are provided in the next section.

This new type of cell migration mode has been denoted as ratchetaxis due to the similarity of the process with the Feynman ratchet. This concept was popularized in physics by Richard Feynman during his famous *Lectures in Physics* at the California Institute of Technology (Caltech; Pasadena; see Table 1). Therein, he defined a new paradigm to rectify the motion of fluctuating objects that are far from the equilibrium, such as cells (36). In particular, Feynman showed that nondirectional motion driven by fluctuations (thermal

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Table 1. Relevant online supporting resources.

Concept	Description	URL
The Feynman lectures on physics, volume I	Lectures performed by Richard Feynman at Caltech addressed to students. Brownian motion is described in chapter 41 and the ratchet and pawl in chapter 46.	https://www.feynmanlectures.caltech.edu/I_toc.html
The Feynman ratchet	Richard Feynman describes the working principles of the ratchet and pawl device.	https://www.youtube.com/watch?v=M3fYbCatKbA
Actin polymerization in cell protrusions	Description of the main molecular composition of cell protrusions (actin and myosin) and the mechanism of force generation.	https://www.youtube.com/watch?v=-UApgQ2zulU
Chemotaxis	Directed migration of a cell by chemotaxis. This movie shows a neutrophil chasing bacteria, releasing a chemoattractant that is sensed by the immune cell.	https://www.youtube.com/watch?v=xe5q_z9Z9SE

motion) could be "rectified" (i.e., from random to directed) by breaking the spatiotemporal symmetry of the system (29). To illustrate the working mechanism of this complex statement, Feynman described the so-called ratchet and pawl machine (Table 1), a simple device consisting of a saw-type wheel in which a pawl engages, allowing wheel rotation only in one direction (Appendix B). This hypothetical microscopic experiment showed how to extract mechanical work by using the thermal fluctuations of gas molecules as energy. Briefly, the experiment consisted of a box containing flat vanes isolated at T_1. These vanes were connected through an axis with the aforementioned asymmetric saw-type wheel (ratchet) and pawl located inside another reservoir at a temperature T_2. The axis contained another wheel in its middle, holding a load. Due to thermal motion, the air molecules located in the box at T_1 hit the vanes on both sides, making it oscillate. However, due to the asymmetric shape of the ratchet wheel, the pawl is only permitted to turn in one direction. Strikingly, one could imagine that these thermal fluctuations could be used to extract work perpetually violating the second law of thermodynamics. However, Feynman demonstrated that this violation did not occur, showing the thermodynamic requirements for this principle to work. Importantly, he gave a framework for understanding the "rectification" of motion when out-of-equilibrium objects fluctuated in the presence of local asymmetries. Note that in thermodynamic equilibrium (i.e., temperature constant and not changing with time, $T_1 = T_2$), thermal fluctuations prevented the ratchet from generating work. The thermal fluctuations at this length scale caused the pawl to stochastically fluctuate, allowing the ratchet to move also in the opposite (counterclockwise) direction (Appendix B). When lifted, the wheel could rotate clockwise or counterclockwise with the same rate probability, thus preserving the principle of detailed balance. This principle states that at thermal equilibrium, every kinetic process must be balanced by its exact opposite. In other words, this means that the system has time-reversal symmetry. Overall, the take-home message is that at equilibrium, the effect of thermal noise is symmetric, even in an anisotropic environment.

Far from equilibrium ($T_1 \neq T_2$), thermal fluctuations can be rectified obtaining work, because if $T_2 < T_1$, the fluctuations of the pawl are relatively infrequent. In contrast, the higher temperature of T_1 will give to the vanes the needed energy to rotate the wheel in the "correct" direction. Note that this device needs a continuous input of energy to work to maintain the system far from equilibrium, i.e., $T_1 \neq T_2$. One may imagine that due to the asymmetric shape of the pawl, the device may also work in equilibrium. However, if the temperature on both sides is equal ($T_1 = T_2$), as stated previously, the pawl will also fluctuate due to thermal motion allowing the rotation of the wheel in both directions. As a result, no net average motion will be obtained in the long

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run. Appendix B gives further details about the working mechanism of the Feynman ratchet, in particular the case of $T_2 > T_1$, where the ratchet can rotate in the "opposite" direction. It also lists the requirements needed to produce useful work via the rectification of thermal Brownian noise.

III. MATERIALS AND METHODS

A. Micropatterning

A microcontact printing technique was used for patterning an array of fibronectin motifs (37). For this, a polydimethylsiloxane (PDMS) stamp (Sylgard 184; Dow Corning, Midland, MI) was used. The stamp was fabricated by mixing prepolymer and cross-linker solutions in a 10:1 ratio (w/w). Then, the PDMS mixture was degassed and poured in a SU-8 mold (MicroChem, Newton, MA) fabricated by standard ultraviolet photolithography (MJB3 mask aligner; SUSS MicroTec, Munich, Germany), which contained different motifs (i.e., asymmetric triangles and symmetric spots). The sample (SU-8 mold + PDMS) was then cured at 70 °C for 4 h. After curing, the micropatterned PDMS stamp was released from the mold. The stamp was activated by plasma treatment and inked with a 10 μg/mL rhodamine-labeled fibronectin solution (an adhesive ECM protein) for 45 to 60 min (Cytoskeleton, Denver, CO). Next, the inked stamp was dried and placed in contact for 5 min with a glass coverslip to transfer the fibronectin pattern. Prior to patterning, the glass coverslip was functionalized with 3-(mercapto)propyltrimethoxysilane (Fluorochem, Hadfield, Derbyshire, UK) by vapor phase for 1 h and cured at 70 °C for 4 h. Next, the PDMS stamp was released, and the patterned coverslip cleaned with phosphate-buffered saline (PBS), double-deionized water, and 10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) (in this order). Nonpatterned regions were blocked with poly-(L-lysine)-*g*-poly(ethylene glycol) (PLL-g-PEG; 0.1 mg/mL; SurfaceSolutions, Dübendorf, Switzerland) in 10 mM HEPES for 30 min. Finally, the functionalized samples were rinsed with PBS twice prior to cell seeding.

B. Optical microscopy

An inverted optical microscope (Olympus CKX41, Tokyo, Japan) was used for short-term (1 image/30 s) and long-term imaging (1 image/5 min) acquisition. For short- and long-term imaging, 40× (0.65 numeric aperture) and 4× (0.25 numeric aperture) phase-contrast air objectives were used, respectively. The microscope was located inside an environmental cage to keep physiologic conditions (37 °C and 5% CO_2).

C. Cell culture

Mouse NIH/3T3 fibroblasts (ATCC™ CRL-1658) were used for the described experiments. Cells were grown in high-glucose Dulbecco's Modified Eagle Medium (Invitrogen, Reims, France) supplemented with 10% bovine calf serum (BCS; Sigma-Aldrich, Lyon, France) and 1% penicillin–streptomycin antibiotic solution (Invitrogen) at 37 °C and 5% CO_2. Cells were detached from standard culture dishes by using 0.25% trypsin–EDTA solution (Invitrogen) and deposited on top of the micropatterned glass coverslips at a low density (1×10^4 cells/cm^2). After 20 min, the old medium was replaced with a fresh one containing 1% BCS to reduce the deposition of ECM proteins around the micropatterned region. For detailed information, the readers may refer to the methods section of the original references.

IV. RESULTS

A. Ratchetaxis: directing cell migration in gradient-free environments by asymmetric local cues

The ratchet and pawl concept to extract useful work has striking consequences in different research fields, including cell and developmental biology, and in out-of-equilibrium systems, such as cells. Indeed, it is possible to design a ratchet-inspired experiment where the Brownian motion of cells can be rectified (or converted) into unidirectional motion in the total absence of biochemical gradients (soluble, chemotaxis, or surface, haptotaxis). As men-

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tioned previously, recent experimental evidence has validated this hypothesis, showing that the thermodynamic laws and concepts from soft matter physics can be used to build an assay to obtain directed cell migration following a ratchetaxis locomotion mode. To illustrate this, we seeded motile cells (NIH/3T3 fibroblasts) on top of a microfabricated adhesive array (fibronectin) with motifs displaying an asymmetric triangular morphology and separated by a small nonadherent gap (see Fig 2). As a control, we also patterned an array of symmetric adhesive spots. These motifs were patterned on top of a glass surface by microcontact printing, a technique that uses a topographically structured elastomeric "stamp" containing the aforementioned triangular (and circular) motifs to deposit on the surface an ink (in this case, fibronectin) by physical contact (see section III). In this scenario, cells adhered to the fibronectin motifs and extended thin membrane protrusions, mainly filopodia, at both sides (front and rear) of the cell (see Fig 2a,b). These protrusions fluctuated (i.e., elongated and retracted stochastically) toward the adjacent motifs (asymmetric triangles versus symmetric spots), exploring the surrounding microenvironment to find a docking site and initiate locomotion. The biologic origin of these fluctuations is related to the actomyosin cytoskeleton of the cell, a highly dynamic network of protein filaments within the cell (Appendix C). Indeed, the elongation (polymerization) of actin filaments, as well as the motion of molecular motors (myosin), a specific type of molecule that transforms chemical energy into mechanical work, can be also described as a Brownian ratchet (Appendix B).

The ability of the cell to engage focal adhesions was controlled by the gap distance and the available adhesive area on both sides of the cell (left versus right). If the gap was too long, the cell was unable to migrate to the neighboring motifs. For cells seeded on asymmetric triangles, there was more available adhesive area on the right side ($A_{\text{right}} > A_{\text{left}}$; wide side of the adjacent triangle), which resulted in the adhesion of stable protrusions and the consequent application of traction forces (see Fig 2b). In contrast, the number of extended protrusions toward the left side (narrow side of the neighboring triangle) was larger, resulting in a higher probability of finding a docking site. However, these protrusions were less stable (detached faster); therefore, the applied traction force was lower. Overall, a combination of both parameters (available adhesive area and the probability of adhesion) determined the final direction of cell motion in a tug-of-war force mechanism. In this regard, the pointed edge direction (right) was favored due to the formation of cell adhesions that applied stronger traction forces toward this side. Finally, the entire symmetric scenario (array of spots) did not provide any bias in the direction of cell migration.

The abovementioned explains the selection of cell directionality for the first motif (short-term period), but not the long-term migration of cells, i.e., along several motifs. The latter can be imagined in two different ways: (a) the mechanism of exploring the neighboring motifs through protrusion fluctuations and the available adhesive area is repeated for each adhesive motif; or (b) after initiating the first-step motion, the cell keeps a "memory" (or polarization) of its direction and migrates directionally for a long time interval, which is denoted as persistence time τ_p. This parameter adapted from polymer physics (time duration over which a mobile region persists from $t = 0$ to $t = \tau_p$, where the state of the mobility changes) describes the extension during which the cell migrates directionally without stopping or switching direction. We found that indeed the periodic arrangement of the triangular motifs supported the directional migration of cells by maintaining its polarization, in contrast to spots configuration, where cells fluctuated right and left stochastically (Fig 2c,d) (31). In a follow-up work, we questioned ourselves about what would happen if during the directed motion by ratchetaxis, cells encountered a reversed motif (i.e., pointing toward the opposite direction). Would the cells ignore the reversed geometry and migrate over it by "inertia," or would the cells stop, fluctuate,

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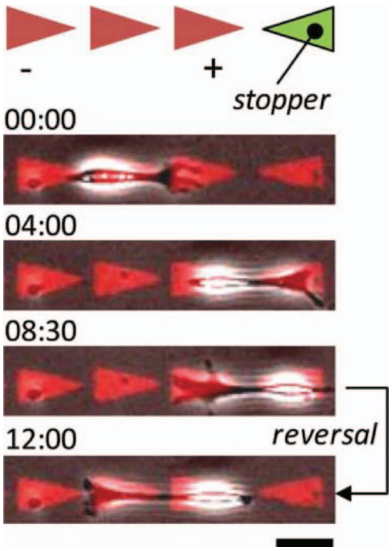

Fig 3. Ratchetaxis and reversal of cell motion. Time-lapse sequence of an NIH/3T3 cell moving directionally by ratchetaxis in a series of asymmetric triangles. The cell reverses its motion after reaching the reversed stopper adhesive motif. Scale bar: 50 μm. Reprinted with permission from (31, fig 5) © 2015 Taylor & Francis Group, LLC.

and reverse their motion? First, it must be stressed that at these length scales, we are at low Reynolds (Re) numbers (Re $<<$ 1), meaning that viscous forces predominate over inertial ones (Appendix D). Therefore, the migration of cells is not related to inertia but the interplay between the local adhesive microenvironment and protrusions activity, suggesting a reversal in cell motion in this scenario. We tested this hypothesis and observed that cells migrating toward the pointed edge in an array of triangular fibronectin motifs paused and reversed their motion (Fig 3). However, cells migrated for short periods along this new direction due to the new cell polarization. The mechanism of asymmetric protrusion activity described above restored the preferential orientation of cell motion in the right direction. The cells were capable of temporally keeping a memory (polarization) of its previous motion but also were capable of exploring the surrounding environment on each motif by integrating all spatial cues to decide into which direction to move.

The analogy with the Feynman ratchet and pawl is straightforward. The array of asymmetric motifs work as the sawlike (ratchet) wheel. The fluctuations in cell protrusions are analogous to the Brownian motion of the air molecules hitting the vane located in a box at a temperature $T_1 \neq T_2$ (this is equivalent to a cell, which is an out-of-equilibrium system due to the continuous input of energy through the hydrolysis of ATP–GTP, the "fuel" molecules). The asymmetric engagement of protrusion adhesions in either direction (left or right) is equivalent to pawl, allowing the rotation of the wheel in only one direction (clockwise or counterclockwise). Finally, the net directed migration toward one direction is analogous to the rotation of the wheel raising the load. To challenge this vision, the modification of the morphology of the adjacent motifs would result in a perturbation of cell directionality. Indeed, as briefly mentioned previously, it was shown that when symmetric motifs were used, such as an array of spots, no preferential direction of cell motion was observed (see Fig 2c); cells displayed a random movement, fluctuating right and left. The reason behind this stochastic behavior is that the available adhesive area and protrusion activity are exactly the same on both sides of the cell. Thus, no spatiotemporal asymmetry is present. Overall, the take-home message from these experiments is that the symmetry of the system needs to be broken to extract useful work.

Note that in vivo, the existence of ratchetaxis and its synergistic interaction with other cues to guide cell motion, is plausible due to the complexity of the native scenario, which is full of mechanical obstacles. To test this interplay in vitro, a recent assay was used to investigate how cells behaved when seeded in a microenvironment containing a topographic ratchetlike microarray (inducing the migration of cells in one direction) and a long-range haptotactic surface gradient of an adhesive protein (fibronectin) (32). Briefly, when cells were seeded in an environment in which both the fibronectin gradient and the ratchet topography were oriented toward the same direction (i.e., cooperating), an amplification in cell directionality was observed, resulting from an enhancement of cell polarization (see Fig 4). In this case, the mechanism of rectification was different compared with the previous case in which the

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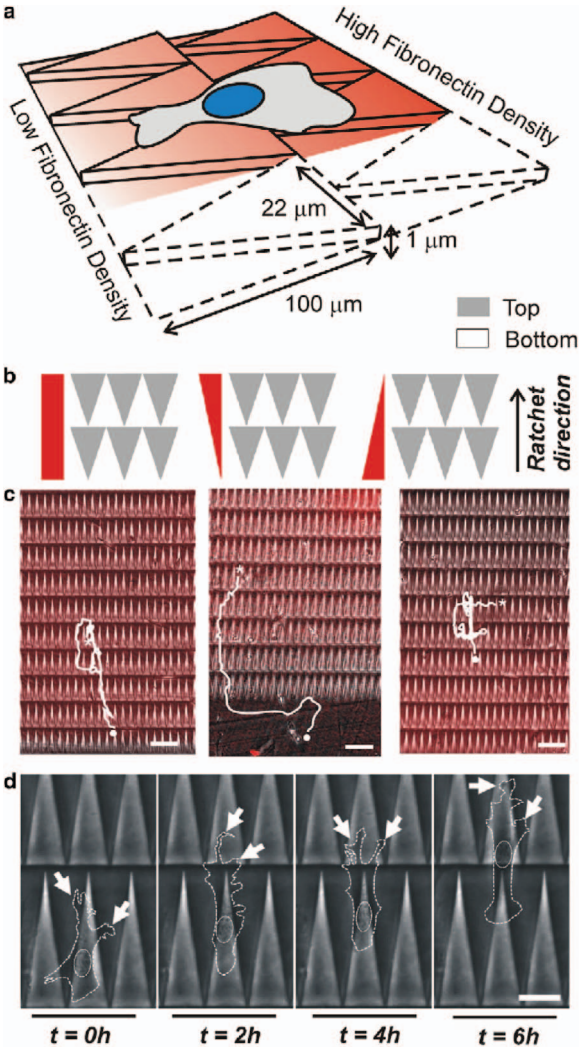

Fig 4. The interplay between ratchetaxis and an external biochemical gradient in guiding cell motion. (a) Experimental setup consisting of a polymeric surface with ratchetlike topographic features coated with a fibronectin surface gradient. (b) Experimental conditions: topographic ratchet coated with (left) homogeneous fibronectin layer; (middle) fibronectin gradient in the same direction to the ratchet; and (right) fibronectin gradient in the opposite direction to the ratchet. (c) Cells migrating on the combined topographic and biochemical surfaces. In white, the cell trajectory is highlighted. When the ratchet and the gradient are pointing toward the same direction, both cues cooperate, and the directed migration of cells is enhanced. When the gradient is pointing in opposite directions, both cues are competing, and cells fluctuate. Scale bar: 100 μm. (d) Detailed image of a cell migrating along with a single ratchet unit, highlighting the critical role of protrusions and the cell nucleus. Scale bar: 20 μm. Reproduced with permission from (32, fig 1).

asymmetry in both the adhesive area and protrusion activity determined the direction of cell migration. Here, the mechanical interaction between the cell nuclei with the walls of the ratchet structures worked as a boat rudder to orient cell polarization and protrusion activity guiding cell migration. Next, when both cues were oriented in opposite directions (i.e., competing), the cells started to fluctuate without any preferential direction; the directionality was lost. Overall, this work shows that the complexity of the in vivo cellular microenvironment not only provides adhesion sites for cells to migrate but also topographic features to guide cell motion mechanically. For this reason, it is easy to imagine that ratchetaxis may synergistically interact with hapto- and chemotaxis to guide cell motion in vivo.

B. A theoretic model of ratchetaxis

The Brownian motion of particles is a stochastic process described by statistical physics at equilibrium. This type of motion observed in tiny particles was interpreted by Einstein as the result of thermal fluctuations caused by the presence of colliding molecules (e.g., air), as described by the kinetic theory. This theory describes the motion of inanimate particles driven by environmental fluctuations. However, in nature, there are many examples of out-of-equilibrium self-propelling entities, such as cells. In particular, for the case of cells moving by ratchetaxis, several theoretic models deriving from the Brownian motion paradigm have been reported to qualitatively and quantitatively understand this type of locomotion mode. It is not the aim of this section to go in detail through all the proposed models but to give a general overview of which theoretic framework can be used to describe the motion of cells. We will focus in the specific case of cells moving in one-dimensional (1D) ratchetlike environments. The readers interested in this topic may consult the extensive literature available elsewhere (29, 38, 39).

On homogeneous flat surfaces, cell motility is typically modeled using the so-called persistent random walk model, which quantifies the ability of a cell to maintain its directional motion without changing its direction (40). Other types of living and nonliving "random walkers" displaying this type of persistent motion include bacteria, flocking birds, swarm-

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ing fish, or self-propelled microrobots (41). In this type of model, cell trajectories show a random component, resulting from the stochasticity of the cytoskeleton fluctuations. Despite this randomness, cells have a clear directionality during migration, which can be defined by a local averaging of the cell instantaneous speed. Then, cell persistence time is introduced to quantify cell directionality (29). In 1D environments, cells can move directionally at a constant speed but stochastically switch its direction of motion at a certain rate. Then, the cell can be characterized by its position and its direction of motion (moving to the right: +; left: −). In our case, for cells moving in 1D ratchet microenvironments, the cell trajectory can also be discretized in a series of elementary steps, where the cell is capable of moving from one motif of the ratchet to the neighboring one with a certain transition probability p_{ij} (i = +, −). This probability depends on some experimental parameters, namely, the frequency of probing (i.e., how frequent a protrusion adheres on the neighboring motif in the + and − direction) and stabilization lifetime (i.e., how long the protrusion remains adhered) of protrusions. We observed that the direction taken by the cell on each motif depended on the previous direction taken. In 1D ratchets, it is typically found that $p_{++} > p_{+-}$. The probability for a cell to move to the + direction, knowing that the previous step was also in this direction, is larger than its probability to move to the – direction, knowing that the previous one was in the + direction (i.e., there is a bias in motion). From a physical and biologic perspective, this difference can be attributed to the interplay between the asymmetry of the adhesive motifs and the stability of cell polarity. This makes the cell keep a "memory" of its previous step and maintain its directionality. This also means that in a cell seeded on top of entirely symmetric motifs, such as in a sequence of spots, cell polarity is destabilized and protrusion activity dominates, which results in a $p_{ij} = ½$ for all *i,j*. It has the same probability to move toward one direction or the opposite. In specific cases, a cell may migrate directionally for few motifs before switching direction resulting in $p_{ij} \neq ½$. However, after averaging out many cells, a $<p_{ij}> = ½$ is obtained. In contrast, in asymmetric environments $p_{ij} \neq ½$ due to the bias in protrusion activity. Interestingly, these transition probabilities can be encoded in a predictive direction index. This index is based solely on the activity of protrusions, namely, the aforementioned frequency of probing and stability lifetime, and it can be used to predict the direction and distance of cell migration.

Altogether, the characteristics of cell trajectories, such as bias (i.e., the % of cells moving in + and − direction) and persistence time (and length), can be expressed in terms of these transition probabilities, thus demonstrating the strength of physical characterization for the prediction of the cell behavior (29, 30).

V. DISCUSSION

A. Implications of the Feynman ratchet in biophysics

The objective of this work was to highlight how the concepts and frameworks derived from soft matter physics and thermodynamic laws can be used to design an experiment on the basis of the Feynman ratchet paradigm, to direct the motion of cells in the total absence of biochemical (or other types of) gradients. This type of physical cue can act alone or synergy with many other guidance cues, such as chemotactic gradients, to direct the motion of cells (Table 1). Note that in our system, the Feynman ratchet for rectifying cell motion appears on several length scales. At the nanometer scale, this paradigm is used to describe the motion of molecular motors on cytoskeletal filaments (Fig 5a) (42–44). At the micrometric scale, it describes the mechanism of protrusion elongation (Fig 5b) (45), and at the mesoscopic scale (the aim of this paper), it is used to describe the directional motion of cells by using a ratchetlike microarray (Fig 5c) (29–31). Interestingly, this framework has also been applied in more complex in vivo systems to describe the mechanism of embryonic development in *Drosophila* (46, 47), among other applications in biology (29, 48, 49).

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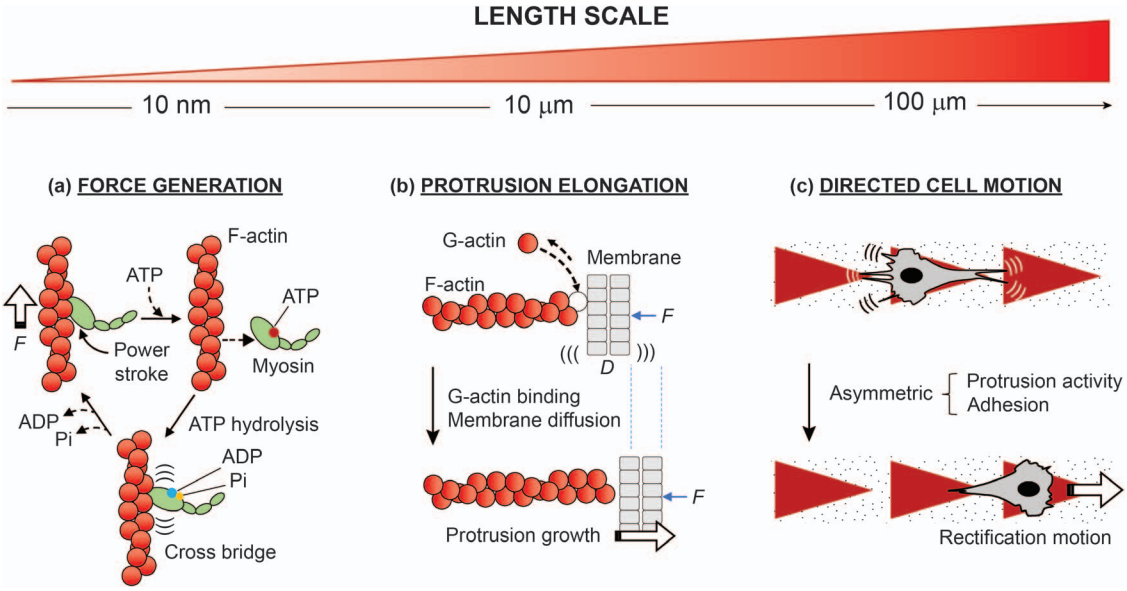

Fig 5. The Feynman ratchet at different length scales. Scheme showing the (a) conformational changes of myosin II during its power stroke due to the ATP hydrolysis cycle, which results in a directional movement along the actin filament. The fluctuations of the myosin head due to thermal motion contribute to the observed biased motion. (b) Polymerization ratchet mechanism of an elongating membrane protrusion (e.g., filopodia). The actin filament polymerizes against a fluctuating cell membrane with a diffusion constant *D* upon which a force *F* acts. Eventually, the generated space is used by a G-actin monomer to bind, resulting in the directed growth of the protrusion, rectifying the Brownian motion. (c) The cell ratchet: A cell seeded in a periodic array of asymmetric adhesive motifs elongates protrusions, which stochastically fluctuate, exploring the surrounding microenvironment. The interplay between asymmetric protrusion activity and available adhesion sites bias the direction of cell motion.

Furthermore, it could also be involved in critical pathologic events, such as in cancer progression. In this regard, it is plausible that due to the complexity of the tumor scenario, cells also use ratchetlike paths that are physically favorable to invade the surrounding tumor microenvironment besides using typical migration modes on the basis of chemotaxis. If proven true, this may reveal key insights in the physicochemical mechanism of cancer cell invasion and result in the development of new therapies to combat cancer (29).

Finally, the Feynman ratchet paradigm has influenced other research fields besides biology. In particular, the inherently random motion of synthetic self-propelled microrobots has recently been rectified by using ratchetlike topographic structures (41). In this case, the hydrodynamic interactions of the microrobots with the asymmetric walls broke the time-reversal symmetry of particle trajectories and directed their macroscopic flow. Overall, this work also demonstrates that the ability to control the behavior of other types of active matter systems, such as self-propelled particles, paves the way toward novel applications, opportunities, and developments of advanced microfluidic or lab-on-a-chip systems.

B. The pedagogic context in biophysics

The Feynman ratchet paradigm may have a robust educational component within the biophysics curriculum because it is involved in fundamental biologic phenomena at different length scales. This includes (a) the motion of molecular motors that leads to force generation (e.g., muscle contraction, cell division); (b) the elongation of cellular protrusions (e.g., filopodia); and (c) the long-range cell motility powered by protrusions elongation, which can be rectified by using microfabricated adhesive patterns. All these multiscale ratchet-based biologic events can be explained by using the laws of physics applied to living matter and, therefore, may be taught in different core courses in biophysics. Examples include courses on (a) biophysical approaches to cell biology,

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showing how molecular motors and the actomyosin cytoskeleton govern cell polarity, force generation, and migration resulting in large-scale morphogenesis; (b) complex biologic systems, such as self-organization principles and actin gels and regulation by signaling pathways (e.g., Rho pathway); (c) membrane biophysics showing the molecular and physical laws regulating membrane function and dynamics; or (d) theoretic biophysics and dynamic systems modeling to illustrate the principles related to multiscale modeling, active gel theory, and out-of-equilibrium systems. This manuscript may raise the interest of students who want to explore the physical properties, structures, and behaviors of living matter using the laws of physics to quantify biologic processes (e.g., cell migration) at the molecular, cellular, and tissue scale. This may include fundamental courses providing training in cell physics, statistical physics, or thermodynamics, highlighting the inviolability of the second law of thermodynamics and detailed balance. In more applied educational fields, this work may also be included in the curricula of applied biophysics and bioengineering, where microfabrication and nanotechnology play a fundamental role in understanding cellular morphodynamics and solving biologic questions of interest.

This manuscript has been written in a brief and comprehensive manner, adapting its content, sometimes very complex, to the level of the targeted audience (MSc and young PhD students) with basic multidisciplinary knowledge and training in physics and biology. We are convinced that this format and style is more advantageous because it will attract the interest and enthusiasm of a new generation of interdisciplinary researchers. Importantly, it will motivate the students to look for specific details (in physics, engineering, or biology) in the references and online resources provided (Table 1), thus stimulating critical thinking.

Finally, the multi- and interdisciplinarity of this work spans different communities besides physics, such as cell and developmental biology, which requires basic training to be gathered at the undergraduate level. The instructor may use this paper to point out where the concepts and ideas fit into the body of physics and biology (i.e., to illustrate how biologic phenomena can be described by using the laws and principles of physics). The instructor may also provide a set of problems elucidating some of the ideas of the paper and the references therein to promote discussion. This will univocally offer a very fertile and multidisciplinary environment in biophysics graduate training.

VI. CONCLUSIONS

Herein, we have summarized several recent research articles that show how directed cell migration can be obtained by using concepts and frameworks of physics. Ratchetaxis describes a new mode of cell locomotion to guide the migration of cells by means of the periodic asymmetry of the cellular microenvironment. Indeed, by combining microfabricated surfaces, cell biology tools, and physical principles (the Feynman ratchet), we have shown in a comprehensive manner that it is possible to guide the motion of cells (or other types of out-of-equilibrium particles) toward a particular direction in the total absence of biochemical gradients. The asymmetry of the adhesive motifs modulated the adhesion and dynamics of cell protrusions biasing cell migration. Importantly, the presence and relevance of ratchetaxis still needs to be proven in vivo, but the complex native environment of cells leaves ample space for this to occur. For this, future collaborations between physicists and biologists will be fundamental.

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AUTHOR CONTRIBUTIONS

DC contributed to conceptualization, writing, and editing. DC, SCK, and RLR were involved in overall supervision and funding acquisition.

APPENDIX A. DIRECTED CELL MIGRATION

Directed cell migration is defined as the large-scale displacement of a cell, or group of cells, toward a particular direction in response to an external stimulus (Fig A1A). During directed migration, cell motion is typically maintained over large spatial (from tens of microns to a few millimeters) and temporal (from several minutes to hours) scales. This is in contrast to the small fluctuations observed at short temporal scales, where cells fluctuate by stochastically elongating and shrinking membrane protrusions without migrating. The stimulus can be very heterogeneous, ranging from surface gradients of an adhesive protein from the ECM or physical features of the surface to mechanically guide the cell. Depending on the type of stimulus, the directed motion of the cell receives different names. In Table A1, we describe the main types of directed cell migration modes.

Note that cells can also migrate directionally in the absence of any external stimuli. In this case, the directed migration results from the spontaneous polarization of the cell, which drives its persistent motion toward a particular random direction. This directionality is maintained over length scales of several cell lengths and times. Then, the cell loses its polarization and stochastically switches its direction of motion (with or without stopping), and the process is repeated. At the end, the cell typically displays a (persistent) Brownian-like trajectory with no preferential direction (Fig A1B).

Similar to ratchetaxis, other migration modes can be explained by using physical concepts, such as thermodynamic motion. As an example, haptotaxis can also be considered as a Brownian ratchet (50). Consider an object (such as a membrane vesicle) deposited on a haptotactic gradient. The object will engage adhesion with the surface, whose strength will be determined by the free energy of the system. Due to thermal motion, this object will experience mechanical forces toward the increasing and decreasing gradient concentrations. The slightly stronger adhesion in the up-gradient direction, resulting from a larger difference in free energy (comparing the free energy of the object adhesive surface in contact versus separated), can counteract the thermal energy in the system and resist better the rupture of the adhesive bonds. In contrast, the lower adhesion in the down-gradient direction (thus, lower free energy) will favor the rupture of adhesion bonds. Overall, the particle will have a net motion toward the up-gradient direction, resulting from random agitation and bias in surface adhesion. Finally, a similar procedure is used by adhesive cells to migrate directionally when seeded in surfaces with increasing concentrations of adhesive proteins.

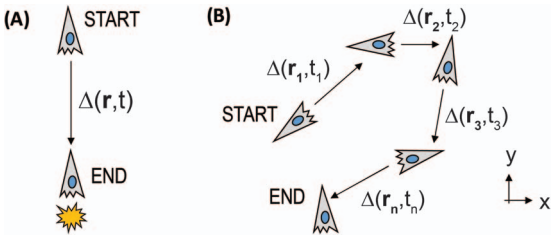

Fig A1. Directed cell migration. (A) Scheme showing the directional migration of a cell for a specific length and time $\Delta(r,t)$ due to the presence of an external (gradient-dependent or gradient-free) stimulus. (B) Persistent directed migration of a cell in an environment without any external stimuli. The persistent migration is maintained for a specific persistence time and length $\Delta(r_i,t_i)$ ($i = 1 \ldots n$) before switching direction toward a random direction, displaying at the end a (persistent) Brownian-like trajectory without any preferential directionality.

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Table A1. Main types of directed cell migration modes.

Gradient dependent		Gradient free	
Chemotaxis	Directional migration of cells in response to a soluble gradient of a chemoattractant (52)	Contact guidance or haptokinesis	Guided migration defined by the anisotropy of the substrate topography, such as aligned grooves or fibers (53)
Haptotaxis	Directional migration of cells by means of a surface gradient of cellular adhesion sites (21)	Galvanotaxis or Electrotaxis	Directed migration of cells in response to an electric field (24)
Topotaxis	Directional migration of cells in response to gradients of topographic features (e.g., the density of ECM fibers) (22)	Curvotaxis	Directed migration of cells in response to surface curvature variations at the scale of the cell (23)
Durotaxis	Cell migration is guided directionally by a gradient of extracellular rigidity (20)	Ratchetaxis	Directed migration of cells in response to local periodic asymmetries of the cell microenvironment (29)

APPENDIX B. THE FEYNMAN AND BROWNIAN RATCHET FOR THE RECTIFICATION OF MOTION

The Feynman ratchet is a paradigm, which is used for rectifying the motion of fluctuating objects that are out of equilibrium. Richard Feynman illustrated the working procedure of this mechanism in his famous ratchet and pawl experiment (see Fig B1A and Table 1) (36). Briefly, the ratchet wheel and pawl are located inside a box at a temperature T_2 and the vanes in another box at T_1. In this scenario, the vanes are constantly (and randomly) bombarded by the gas molecules inside the box due to thermal motion, making the axis to rotate clockwise or counterclockwise. We can consider different scenarios:

- If $T_2 = T_1$, thermal fluctuations prevent the ratchet from generating useful work (i.e., rotation of the wheel in one direction lifting a load) because both the vane and the pawl will have Brownian motion. In thermal motion, the probability to be in a configuration is only determined by its energy, so the probability to have the pawl and ratchet positioned right at the tip of the tooth is independent on the direction of motion. At that position, at the apex of the tooth, the thermal motion can equally rotate the ratchet right or left (the same force is needed for either direction at this position), and the symmetry of the rotation is derived. In other words, when the pawl lifts up by thermal motion, there is the same probability to rotate the wheel clockwise or counterclockwise due to the thermal motion of the vanes. This means that, on average, the number of clockwise rotations are cancelled by the counterclockwise ones, thus preserving detailed balance. Therefore, there is no net average motion of the wheel even in the presence of an asymmetric energy potential (see the following).
- If $T_2 < T_1$, the wheel will rotate with higher probability clockwise, because the pawl will fluctuate less frequently and the vanes will often reach the needed energy to rotate the ratchet wheel.
- If $T_2 > T_1$, the wheel will rotate with higher probability counterclockwise, because of the thermal motion of the pawl, which makes it fluctuate frequently. When the pawl is detached, the wheel can freely rotate. Eventually, the pawl bounces down but always in an inclined plane of a ratchet wheel unit, pushing the wheel backward. This cycle is then repeated.

The requirements to produce useful work via the rectification of thermal Brownian noise are the following:

(a) The system must be microscopic to undergo Brownian motion.
(b) There must be an asymmetry in the system (i.e., an asymmetric potential energy profile when the ratchet and pawl are engaged).
(c) There must be a temperature difference (i.e., a constant input of energy), thus breaking thermodynamic detailed balance.

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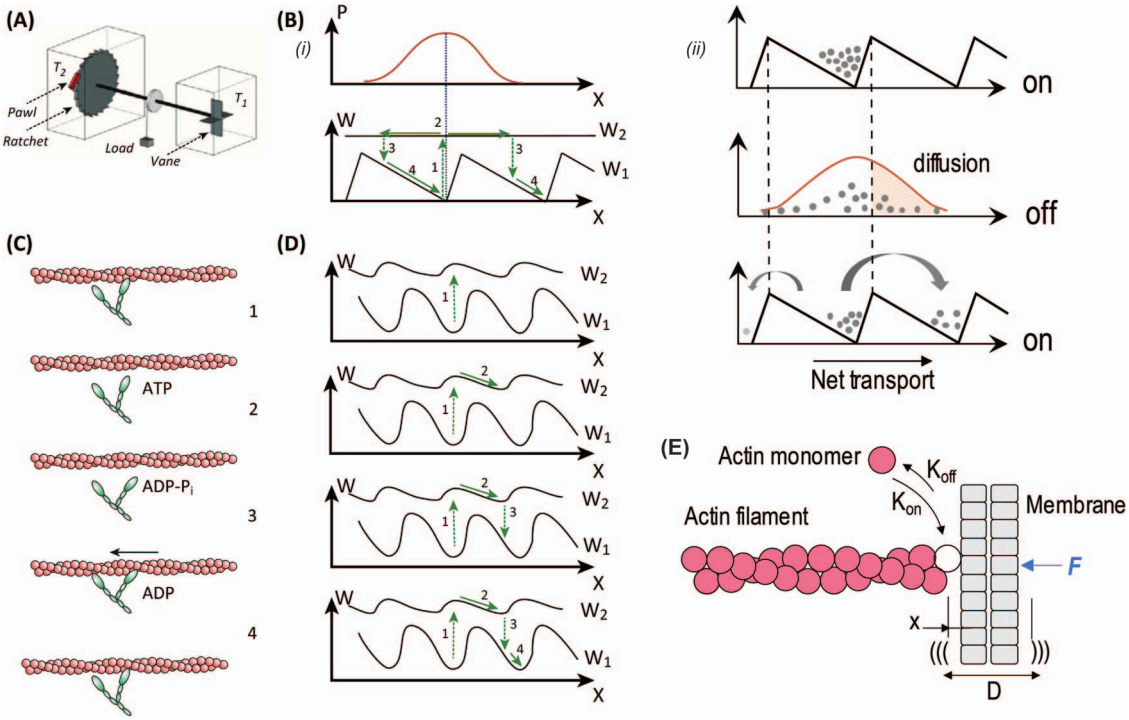

Fig B1. Rectification of motion by means of the Feynman ratchet paradigm. (A) The Feynman ratchet and pawl device. (B) Left: rectification of the motion of diffusing particles by a Brownian ratchet mechanism. The probability distribution P of the presence of Brownian motion is a Gaussian distribution. Right: The flashing sawtooth potential. Initially, the potential is ''on'' with all the particles located around one of the minima. After switching ''off'' the potential, the particles can diffuse freely following a Gaussian probability distribution. After switching the potential ''on'' again, the particles in the highlighted region (right side of the Gaussian curve) slide downhill toward the local minimum on the right side. The other particles slide back to the original minimum. Very few particles can diffuse long enough toward the left side to ''hop'' to the opposite (left) minimum. Overall, the asymmetry of the potential makes the original particle distribution to move, on average, to the right direction, resulting in a net transport. (C) Myosin II mechanical ratchet driven by ATP hydrolysis. The myosin binds to the actin filaments and exerts mechanical force during its power stroke. (D) Energy states (W_1 and W_2) of the molecular motor in (C) interacting with the actin filament. Reproduced with permission from (29). (E) The Brownian ratchet model of actin protrusion elongation. According to this model, an actin filament formed by an array of monomers polymerizes against the cell membrane with a diffusion constant D upon which a force F applies (45). Due to thermal motion, the distance x between the cell membrane and the end of the actin filament fluctuates randomly. Eventually, this distance is large enough for a new G-actin monomer to add on onto the end of the filament, resulting in the filament growth and the outward pushing of the cell membrane. The polymerization rate depends on several parameters, such as the monomer concentration and the association and dissociation rates (K_{on}/K_{off}).

Overall, the Feynman ratchet mechanism provides a framework for understanding the rectification of motion when objects are fluctuating and exposed to noise in environments with repeated local asymmetries. As shown in Figure B1B (left), an object can be initially located in a periodic asymmetric energy state W_1 (engaged pawl) and allowed to switch to a W_2 flat level (disengaged pawl) with a higher energy level ($1 \rightarrow 2$). When the system is switched from W_1 to W_2, the object can diffuse freely on a flat energy landscape, following a Gaussian probability distribution P. When the energy level W_1 is restored, the object can be trapped in the neighboring minimum of energy ($2 \rightarrow 3 \rightarrow 4$). Overall, due to the asymmetric microenvironment, together with the above-mentioned requirements, it can be obtained a bias in the motion of the object. Importantly, note that the motion will not be rectified if the transition from the energy level W_1 to W_2 and from W_2 to W_1 obeyed a thermodynamic detailed balance. If no energy is provided to the system, then the wheel would have the same probability to turn in one or other direction. In this case, the thermal fluctuations can switch the system from W_1 to W_2 (and back) everywhere (the transition rates between both states are the same); therefore, the particles can ''hop'' from one state to the other

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(the arrows 1 and 3 in Fig B1B are bidirectional). Then, the final steady-state distribution is given by the Boltzmann factors, which express the relative probabilities of two states with energies W_2 and W_1 that only depend on the difference of the energy states. This is the same whether energy W_1 refers to the original potential minimum, one to the left or one to the right, so there is no bias in thermal equilibrium. In equilibrium, the Boltzmann rates between these two states are equal (thermodynamic detailed balance is maintained); therefore, no bias is obtained on average. Note finally that this condition would be analogous to an equal temperature (energy) in Figure B1A (left).

The previously mentioned can also be viewed as a group of diffusing particles trapped in a flashing sawtooth potential (Fig B1B, right), where the energy landscape can switch from a sawtooth shape (on) to a flat form (off). In this case, the detailed balance is broken (i.e., the system is driven away from thermal equilibrium by an external input of energy). After switching to the flat energy profile, the particles start to diffuse by Brownian motion following a Gaussian profile as discussed previously. If this flat profile is "on" long enough before switching back to the sawtooth potential, the particles may diffuse beyond the tip of the energy profile located on the right side of the initial well and "hop" to the neighboring one. Otherwise, the particles will diffuse to the initial state. Note also that the Gaussian profile may also have a nonvanishing overlap with the well located in the left side. This may result in the movement of particles toward this opposite direction. However, due to the asymmetry of the system (sawtooth), the overlap with the well on the right is larger than in the left. As a result, the particles will have, on average, a net motion toward the right. However, and again, if we consider detailed balance condition (thermodynamic equilibrium with no energy provided to the system), there is no net transport of a particle (51).

This framework can also be used to describe the biased motion of myosin II in actin filaments, where the external energy input is given by ATP hydrolysis (Fig B1C,D). Similarly, the same framework can be used for other types of molecular motors (e.g., dynein, kinesin), as well as for the elongation of actin filaments in cellular protrusions, which do not involve molecular motors. The latter follows a Brownian ratchet mechanism because rectification of Brownian motion is indeed needed for the extension of protrusions (actin filament, F-actin). In this case, the actin filament polymerizes against the cell membrane. As nicely described by Peskin et al. (45), the ratchet mechanism is, in this case, the intercalation of actin monomers (G-actin) between the cell membrane and the F-actin end (Fig B1E) (45). When a gap (in the order of a monomer) is opened between both of them due to large thermal fluctuations, a G-actin monomer can bind and polymerize onto the end of the filament. The repetition of this process produces the directed growth of the filament.

APPENDIX C. THE CELL CYTOSKELETON

The cell cytoskeleton is responsible for a diverse variety of fundamental cellular phenomena, including cell motility or division. During these biologic processes, the cytoskeleton organizes in ordered structures. The cytoskeleton is formed by a network of highly dynamic, out-of-equilibrium, and polar protein filaments with actin and microtubules as its main constituents besides the so-called intermediate filaments (see Fig C1). Actin and microtubules are formed by tiny proteins that self-assemble, forming helicoidally (actin) and tubular (microtubules) structures of about 8 to 9 nm (F-actin) and 25 nm in diameter (microtubules) and about tens of micrometers in length. Actin and microtubules monomers (G-actin and tubulin) polymerize (and depolymerize) into large and ordered filaments through multiple linking proteins, with the cell rigidity and elasticity. They are responsible for the transmission of mechanical forces allowing the cell to move. These filaments are dynamic; they are constantly broken down and reformed through precise molecular machinery. They also

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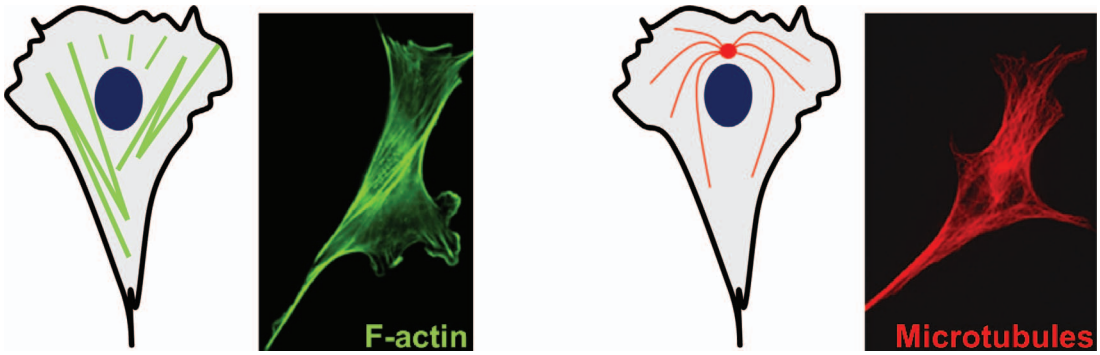

Fig C1. The cell cytoskeleton components (left: actin filaments, F-actin, in green; right: microtubules in red).

have a polarity, which determines the direction of growth. The out-of-equilibrium dynamic properties of the cytoskeleton come from the continuous input of energy provided by the ATP hydrolysis: ATP $\leftrightharpoons$ ADP + P_i. This conversion from ATP to ADP is a crucial aspect of many processes within the cell. This source of energy, together with the hydrolysis of another so-called "fuel" molecule GTP to GDP is used by multiple molecules and proteins within the cell, such as actin and tubulin, to auto-organize in dynamic microfilaments. G-actin binds ATP, which can polymerize to form F-actin making the filament grow. However, F-actin may also hydrolyze its bound ATP to ADP + P_i and start depolymerizing the filament. This cycle is repeated continuously, for instance, during cell migration. Importantly, F-actin has a polarity, which is determinant for its auto-organization. The filament grows in one of its ends and simultaneously shrinks in the other one. This phenomenon is known as treadmilling and plays an important role in cell dynamics. Actin filaments often crosslink with the "motor" protein myosin. Such an active network can apply traction forces necessary for motility by the hydrolysis of ATP. This association is known as actomyosin complex. Microtubules differ from actin filaments regarding its structure; it is not a single filament made of consecutive tubulin monomers, but it is organized in α- and β-tubulin dimers (the subunits of the filaments), which polymerize end-to-end forming protofilaments. These protofilaments (typically 13) auto-assemble into a helixlike structure and organize in a hollow tubelike structure. Microtubules grow all from the same point, the centrosome, an organelle located next to the nucleus. They show a characteristic behavior of switching between phases of growth (with rate $\sim$1 μm/min) and shrinkage (with rate $\sim$10 μm/min) in the (+) end, which is known as dynamic instability. The depolymerization and total shrinkage of microtubules back to the centrosome is known as catastrophe, where afterward, another microtubule grows in another random direction.

APPENDIX D. THE REYNOLDS NUMBER

Cells are microscopic organisms located in a microfluidic environment. This microenvironment is highly viscous and dominates over any other type of inertial forces. Under these circumstances, the physics governing the cell locomotion can be described by a (low) Re number. This is a dimensionless parameter on the basis of the Navier–Stokes equations of fluid physics (describing the motion of an incompressible Newtonian fluid) that expresses the ratio of inertial to viscous forces of an object located inside a fluid (e.g., water, air, or honey). Therefore, the Re number allows for a qualitative description of the flow properties (laminar versus turbulent). Briefly, the inertial forces are characterized by the product of the fluid density ρ times the typical length scale of the object l times the velocity v. The viscous ones are characterized by the viscosity of the fluid η. Overall, the Re number is expressed by Eq. D1.

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$$\mathrm{Re} = \frac{F_{\mathrm{inertial}}}{F_{\mathrm{viscous}}} = \frac{\rho l v}{\eta} \qquad \text{(D1)}$$

High values of Re ($\sim 10^6$) indicate that inertial forces dominate over viscous ones. Lower Re values ($\sim$100) suggest that viscous forces must be considered. Low Re values ($<$1) indicate that viscous forces dominate over inertial ones. In fluid physics, high Re values (typically, Re $>$ 4,000) indicate that the flow of the fluid is turbulent. For Re $<$ 2,000, we have laminar flow, and in between, we have a transition flow. In the particular case of migrating cells seeded into a water-based solution ($\rho_{\mathrm{water}} = 10^3$ kg/m^3 and $\eta_{\mathrm{water}} = 8.9{\cdot}10^{-4}$ Pa·s) with a typical length scale of about 10 μm and migrating speed of 10 μm/h, we obtain a Re $\sim 10^{-8} << 1$. Overall, this very low Re value shows that cells, at this scale, are exposed to a very viscous environment with no inertial effects. This also indicates that for moving, cells always need to apply mechanical forces through focal adhesions and molecular motors to engage locomotion. Similarly, for swimming bacteria, the highly viscous surrounding fluid will cause the bacteria to stop once the flagella stop rotating.

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