© 2020. Published by The Company of Biologists Ltd | Journal of Cell Science (2020) 133, jcs240341. doi:10.1242/jcs.240341

REVIEW

The importance of water and hydraulic pressure in cell dynamics

Yizeng Li[1,2], Konstantinos Konstantopoulos[3,4], Runchen Zhao[3,4], Yoichiro Mori[5] and Sean X. Sun[1,4,6,*]

ABSTRACT

All mammalian cells live in the aqueous medium, yet for many cell biologists, water is a passive arena in which proteins are the leading players that carry out essential biological functions. Recent studies, as well as decades of previous work, have accumulated evidence to show that this is not the complete picture. Active fluxes of water and solutes of water can play essential roles during cell shape changes, cell motility and tissue function, and can generate significant mechanical forces. Moreover, the extracellular resistance to water flow, known as the hydraulic resistance, and external hydraulic pressures are important mechanical modulators of cell polarization and motility. For the cell to maintain a consistent chemical environment in the cytoplasm, there must exist an intricate molecular system that actively controls the cell water content as well as the cytoplasmic ionic content. This system is difficult to study and poorly understood, but ramifications of which may impact all aspects of cell biology from growth to metabolism to development. In this Review, we describe how mammalian cells maintain the cytoplasmic water content and how water flows across the cell surface to drive cell movement. The roles of mechanical forces and hydraulic pressure during water movement are explored.

KEY WORDS: Cell size regulation, Hydraulic pressure, Hydraulic resistance, Ion homeostasis, Osmotic engine model, Osmotic pressure, Two-phase model

Introduction

While the precise mass fraction of water in the cell cytoplasm probably depends on the cell type, water is universally the most abundant molecule in the cell. The next most abundant cytoplasmic components (in terms of overall number and concentrations) are ions: K^+, Na^+, Cl^-, H^+, HCO_3^-, PO_4^- and Ca^{2+} (Fig. 1). Small molecules, such as ATP, taurine, amino acids and glucose are next, and only then do we reach macromolecules, such as proteins and genetic material. A detailed discussion of quantitative estimates of cell components is given in an excellent book (Milo and Phillips, 2015). However, an immediate question following this observation is, why this particular overall composition? Given that nothing in biology makes sense except in the light of evolution, it is reasonable to assume that this composition is similar to the primordial environment where life first evolved. Indeed, there are good reasons to believe that the proto-cell developed in the specialized environment of volcanic pools with high concentrations of K^+ (Mulkidjanian et al., 2012). The operations of essential proteins of life, including the ribosome, DNA polymerase and RNA polymerase, appear to require high concentrations of K^+. Life went to the Na^+-rich ocean environment and became global when the proto-cell developed the Na/K exchanger. The Na/K exchanger is an active ion pump (ATPase) that exports three Na^+ and imports two K^+ ions, and maintains high K^+ and low Na^+ levels in the cytoplasm (Gadsby, 2009). It is one of many active ion exchangers that helps to maintain the ionic composition of the cytoplasm. Therefore, the ionic content of the cytoplasm is actively regulated by the cell, which ultimately impacts critical biochemical processes such as protein translation (Rozov et al., 2019), as well as biophysical features, such as cell size, cell shape and cell motility.

By varying degrees, the plasma membranes of many mammalian cells are permeable to water (Reuss, 2012; Farinas and Verkman, 1996; Farinas et al., 1997). Owing to free-energy driving forces arising from the entropy of mixing, solutes, such as ions, molecules and proteins, generate an osmotic pressure, which drives the flow of water from regions of lower solute concentration to regions of high solute concentration (Atkins, 1990; Reichl, 2016). At physiologically relevant solute concentrations (<1 M), the osmotic pressure is proportional to the solute concentration (as defined by van't Hoff's equation): $\Pi=\gamma RTc$, where c is the solute concentration in molars and R is the gas constant, T is the temperature and γ is an activity coefficient that corrects for non-ideal behavior (but is generally close to 1) (Atkins, 1990). In physiology, osmotic pressure generated by macromolecules (proteins and polymers) is also called the oncotic pressure and is responsible for maintaining water content in circulatory systems such as the vasculature (Mitchison, 2019). It is important to note that the osmotic pressure is conceptually different from the hydraulic (or hydrostatic) pressure, which drives water away from regions of high water density. These two pressures oppose each other, and at chemical equilibrium, the free energy per water molecule is equal across the permeable cell membrane, and the osmotic pressure difference is equal to the hydraulic pressure difference (Fig. 1). The osmotic pressure or entropy of mixing also has nothing to do with any attractive interaction between water and solutes (Jaynes, 1992). Fundamentally, osmotic pressure is an entropic force derived from the ability to distinguish different types of objects in the system (Jaynes, 1992). Given that the cell must maintain high K^+ and low Na^+ concentrations, there is a continuous exchange of Na^+ with K^+ and their associated negative ions even when the overall solute concentration in the cytoplasm is constant (pump and leak) (Hoffmann et al., 2009). Therefore, ion concentrations are never in equilibrium. There is always a concentration gradient of Na^+ and K^+ across the cell boundary.

When the cell size and shape are steady, in addition to the chemical equilibrium of water, there must also be a mechanical equilibrium of forces at the cell surface. Chemical equilibrium of water requires that the water osmotic pressure is equal to hydraulic pressure, and therefore the overall solute concentration must also correspond to the hydraulic pressure at mechanical equilibrium. In addition, at equilibrium, there is a hydraulic pressure difference,

[1]Department of Mechanical Engineering, Johns Hopkins University, Baltimore, MD 21218, USA. [2]Department of Mechanical Engineering, Kennesaw State University. Marietta, GA 30060, USA. [3]Department of Chemical and Biomolecular Engineering, Johns Hopkins University, Baltimore, MD 21218, USA. [4]Institute of NanoBioTechnology, Johns Hopkins University, Baltimore, MD 21218, USA. [5]Department of Mathematics and Department of Biology, University of Pennsylvania, Philadelphia, PA 19104, USA. [6]Center for Cell Dynamics, Johns Hopkins University, Baltimore, MD 21218, USA.

*Author for correspondence (ssun@jhu.edu)

Y.L., 0000-0002-3120-727X; K.K., 0000-0001-8915-2403; Y.M., 0000-0002-4851-5148; S.X.S., 0000-0002-9077-7088

Journal of Cell Science (2020) 133, jcs240341. doi:10.1242/jcs.240341

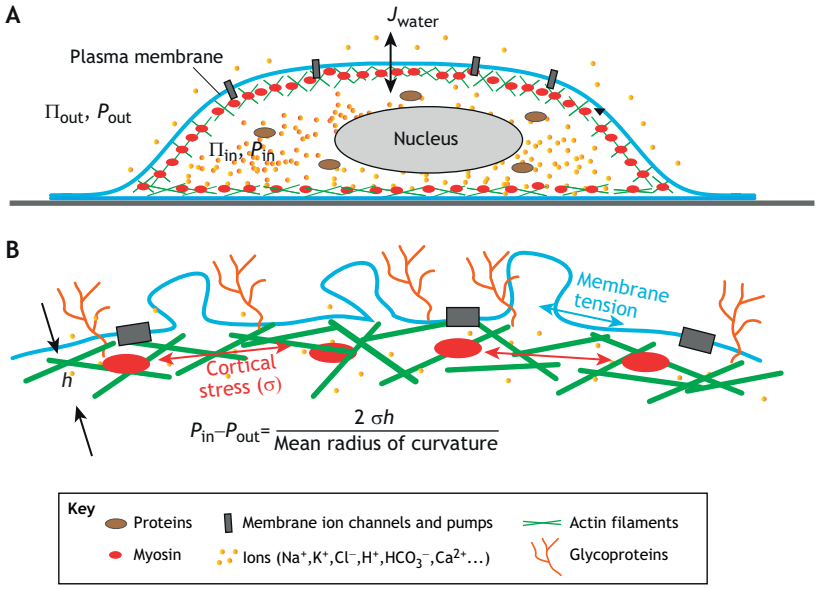

Fig. 1. The cell volume control system. (A) Owing to the difference in the solute concentration, the cytoplasm of a cell has a higher osmotic pressure (Π_{in}) and hydraulic pressure (P_{in}) than the immediate surroundings. The osmotic pressure arises from the mixing entropy of water with solutes, and in the cell, the highest concentrations of solutes are ions and small molecules, followed by proteins. Membrane ion channels, transporters and pumps regulate ion concentrations in the cytoplasm. At equilibrium, the excess osmotic pressure inside the cell (Π_{in}) is balanced by the excess hydraulic pressure (P_{in}), i.e. $[(P_{in}-P_{out})-(\Pi_{in}-\Pi_{out})]=0$. If this balance is perturbed, water will flow across the permeable cell surface, with the water flux $J_{water}\propto-[(P_{in}-P_{out})-(\Pi_{in}-\Pi_{out})]$. (B) Excess hydraulic pressure in the cytoplasm must be balanced by mechanical tension in the cell surface. This force balance means that the pressure difference is proportional to the tension in the surface (σh) and inversely proportional to the surface curvature (Young–Laplace law; Eqn 1 in the main text). The continuous turnover of F-actin and actin-associated proteins in the cortex means that the excess hydraulic pressure is mostly sustained by actin contractile stress in the cortex (Eqn 1), which is generated by myosin and other crosslinking proteins. The membrane tension is typically low at mechanical equilibrium, but can increase dramatically during osmotic shock or mechanical perturbations. Active adaptation by the cell to these type of shocks will restore the membrane tension and cell surface tension to a homeostatic value (Yao et al., 2020; Stewart et al., 2011).

$\Delta P=P_{in}-P_{out}$, across the cell boundary, due to the elevated osmotic pressure in the cytoplasm (Fig. 1A). This excess hydraulic pressure must be balanced by mechanical tension in the cell surface. Of note, for cells with walls, such as bacteria, yeast and plant cells, the cell wall can help to sustain this pressure difference. For mammalian cells, the cell surface is structurally complex (Fig. 1B) and comprises: (i) the plasma membrane, which separates the cytoplasm from the extracellular environment; (ii) the cytoskeletal cortex underneath the membrane (Chugh et al., 2017), and (iii) extracellular glycoproteins, which form a substantial network that further influences the physical and ionic properties of the cell surface (Gahmberg and Tolvanen, 1996; Varki, 2017). Nevertheless, force balance demands that the tension in this cell surface layer must be equal to the hydraulic pressure difference (ΔP) times the mean curvature (according to the Young–Laplace law) (Tao and Sun, 2015):

$$\Delta P=\frac{2\tau}{R}\approx\frac{2\sigma h}{R}, \quad (1)$$

where R is the radius of mean surface curvature, and $\tau\approx\sigma h$ is the tension in the stress-bearing cell surface, σ is the stress, and h is the thickness of the surface. σh has units of force per length, or tension. Because the membrane is 5 nm in thickness, much thinner than the cytoskeletal cortex or the glycoprotein layer, which are hundreds of nanometers in thickness (Clark et al., 2013), and stress at equilibrium should be relatively uniform, the membrane tension is proportional to its thickness and should be small (Tao et al., 2017; Tao and Sun, 2015). Most of the pressure difference is balanced by tension in the cortex. The glycoproteins, which are highly charged, may also form a loose network that sustains some tension and alter the local ionic environment at the cell surface. For instance, heparan sulfate is involved in regulating cell galvanotaxis or directional sensing of electric fields (Huang et al., 2017). Moreover, because the actomyosin cortical layer is effectively a fluid on long time scales (>10 s, due to polymerization and turnover) (Fritzsche et al., 2013), most of the tension must be sustained from active stresses that are generated by force-producing molecules in the cortex, e.g., non-muscle myosin II and transient addition of actin cross-linking proteins, which generate contractile forces (Walcott and Sun, 2010; Sun et al., 2010) in the cortex. However, the force balance condition in Eqn 1 would change significantly when there are other normal forces at the cell surface, for example, from adhesion proteins, such as integrins that contact substrates or cadherins that are bound to other cells, or from externally applied forces from the extracellular matrix (ECM) or other cells. The normal component of these forces would modify the left-hand side of Eqn 1 and therefore alter cell surface curvature on the right (Tao et al., 2017; Li et al., 2017).

Here, fundamental unanswered questions are what sets the overall solute concentration, ionic content and water content in the cytoplasm, and does the cytoplasmic composition change depending on cell type, cell cycle and/or environmental variables? Ion fluxes of channels, transporters and pumps and their dependence on ion concentration, transmembrane voltage, available ATP, membrane tension and other factors, such as Ca^{2+}, are sometimes known (Yellin et al., 2018; Li et al., 2015; Russell, 2000). Therefore, it may be possible to model this system of ion homeostasis mathematically. Indeed, models of this cell volume (i.e. the cell water and/or ion content) control system were first developed for red blood cells in the 1960s and have since been extended, modified and analyzed in many studies (Tosteson and Hoffman, 1960; Weinstein, 1992; Jakobsson, 1980; Mori, 2012; Armstrong, 2003; Jiang and Sun, 2013). These models, collectively known as pump-leak models, can explain the general characteristics

of cell volume and ionic concentration homeostasis at short time scales. However, cell volume is also regulated dynamically during cell growth and division, or in response to external environmental changes such as osmotic stress (Hoffmann et al., 2009). The mechanisms by which this happens must involve a means for the cell to sense its volume, together with multiple feedback mechanisms that allow the cell to modulate its solute content and active tension. These mechanisms are beginning to be elucidated, but they are still poorly understood. For example, a change in cell water and/or ion content alters the cytoplasmic hydraulic pressure, which impacts the cortical stress (Stewart et al., 2011). Changes in ion content and Ca^{2+} fluxes may also modulate cytoskeletal processes and active contraction in the cortex (He et al., 2018; Zhao et al., 2019). These elements are somehow combined to establish a steady state where cell hydraulic pressure, water content, volume, pH and ionic composition are maintained. For most mammalian cells, which do not have cell walls, the numerical value of ΔP appears to range from a few hundred to a few thousand pascals (Petrie et al., 2014; Sao et al., 2019; Perez-Gonzalez et al., 2019). For bacteria, fungi and plant cells, ΔP (the turgor pressure) can approach megapascal values (Sun and Jiang, 2011; Beauzamy et al., 2015; Altenburg et al., 2019). There is also evidence that the hydraulic pressure difference, ΔP, depends on the cell type and signaling pathway activity. For example, when YAP1, a downstream effector of the mechanosensitive Hippo signaling pathway that controls organ size and cell growth, is knocked out, the cytoplasmic pressure is significantly lower (Perez-Gonzalez et al., 2019).

The intracellular and extracellular ionic composition, together with the types of ion channels present on the membrane, sets the resting membrane voltage (Yellin et al., 2018; Ermentrout and Terman, 2010). This membrane potential, which is defined as the intracellular voltage minus the extracellular voltage, results from a charge imbalance across the cell membrane capacitor. The membrane capacitance is very small, thus only a very small charge imbalance is needed to create a membrane potential; the cytosol as a whole can safely be considered electroneutral. The resting membrane potential is generated primarily by two factors: (i) a high intracellular K^+ concentration with respect to the outside, generated by the Na/K pump, and (ii) the presence of passive K^+ channels on the membrane (and the relative absence of other channels). High intracellular K^+ concentration and high K^+ permeability of the membrane system leads to the diffusion of K^+ out of the cell, until the intracellular side has a voltage negative enough with respect to the extracellular space to counterbalance this diffusive tendency. Typical values of the cell membrane voltage range from −50 to −90 mV; this depends on the cell type and reflects the differing ionic concentrations and compositions with regard to ion channels and pumps (Yang and Brackenbury, 2013; Yellin et al., 2018). The resting membrane potential seems to correlate with the differentiation state of the cell, and is also known to fluctuate with the cell cycle (Yang and Brackenbury, 2013). The significance of these observations is unknown, but may be linked to water flow and cell volume regulation, which are the focus of this Review.

Water flux is fundamental in driving the movement of the cell surface

It follows from the above discussion that when the hydraulic pressure is not equal to the osmotic pressure, water will flow across the cell membrane and generate cell surface movement and volume change. The water flux is proportional to the water free-energy difference: $\Delta\mu=\Delta P-\Delta\Pi$, where ΔP is the hydraulic pressure difference and $\Delta\Pi$ is the osmotic pressure difference across the cell boundary (Fig. 1A). The proportionality constant is the permeability of the cell surface. Thus, the rate of change of the cell water content, which at short times (of the order of minutes) is essentially the rate of change in the cell volume, is:

$$\frac{dV}{dt} = SJ_{\text{water}} = -Sa(\Delta P - \Delta\Pi), \tag{2}$$

where S the cell surface area and a is the surface water permeability. We see that when $\Delta P=\Delta\Pi$, there is no water flux. In literature, a is often scaled by RT and the molecular volume of water, giving a scaled permeability that is in units of cm/s (Farinas et al., 1997). Accurate measurements of permeability require accurate measurement of cell volume and cell surface area. The water permeability of pure lipid vesicles has been estimated to be ∼0.01 cm/s (Olbrich et al., 2000; Mathai et al., 2008), while for several mammalian cells, the permeability has been estimated using light scattering and osmotic shock, and is ∼0.001–0.01 cm/s (Farinas and Verkman, 1996; Farinas et al., 1997). The measured permeability can be interpreted as the water velocity (flux) through the surface for an osmolarity difference of 1 M. This flow velocity is also the velocity of cell size increase or cell boundary velocity. The expression of passive water channels, called aquaporins (Agre, 2006), enhances the water permeability of the cell membrane by about a factor of 10 (Farinas et al., 1997). Note that since the measured ΔP is ∼1 kPa, this implies that, at steady state, the osmotic pressure difference between the inside and outside of the cell is only ∼0.5 mM, which is small compared to the overall cytoplasmic ion concentration of ∼300 mM (Milo and Phillips, 2015). We see that for a concentration difference of 1 mM, the cell boundary velocity would be ∼0.1–1 µm/s. This also explains why macromolecules and oncotic pressure can be used to regulate water content. Even though macromolecular concentrations are small when compared to the total ion concentration, they are not negligible when compared to osmotic concentration differences. 1 kPa is also significant because it is equivalent to 1 nanoNewton (nN) per μm^2, which after integrating over the cell surface area, gives a total force of 100 s nN, the same order as the force generated by typical mammalian cells as measured by traction force microscopy (Style et al., 2014). Therefore, the 'force' from osmotic pressure is of the order of forces from actomyosin activity, and can develop from slight changes in solute concentration. Finally, Eqn 2 implies that rapid flows of water will follow sudden changes in osmotic or hydraulic pressure gradients across the cell surface, and potentially underlies phenomena such as mitotic swelling (Zlotek-Zlotkiewicz et al., 2015) and rapid cell volume increase/decrease upon exposure to hormones (Schneider et al., 1997), since both the ion channel activity that sets the osmotic pressure difference and active contraction that determines the hydraulic pressure difference can be regulated by molecular signals.

The cytoplasmic osmotic pressure can vary spatially in the cytoplasm because cells are generally polarized, even in the absence of any external gradients; this means that ion channels, transporters and pumps that modulate ionic concentrations are typically not uniformly distributed on the cell surface. A simple 1D diffusion calculation predicts that if there is a solute influx, I, at the cell leading edge and the opposite solute efflux at the trailing edge and the solute is free to diffuse in the cytoplasm, the solute gradient would be I/D in the cell where D is the solute diffusion coefficient. However, with the exception of a few cases, precise measurements of intracellular ion concentration gradients are lacking (Zeuthen, 1978; Tsien, 1989). Therefore, locally, the ion concentration and osmotic pressure may deviate from the overall average of ∼300 mM. Such concentration changes can be small and be less than 1 mM.

Journal of Cell Science (2020) 133, jcs240341. doi:10.1242/jcs.240341

However, a concentration of 0.5 mM is equivalent to ∼300,000 solute molecules per 1 μm^3 or 1 fL. Given that some ion channels and pumps can allow the passage of ∼10^6 ions per second (Gadsby, 2009; Shieh et al., 2000), only a few of these can generate this kind of concentration changes quickly.

The cytoplasm is also a viscous liquid that contains a fluid-like cytoskeletal network. The central role of the cytoskeleton in cell motility has been studied extensively (Pollard and Borisy, 2003; Mogilner and Oster, 1996). Indeed, there are thousands of papers that describe the role of actin in driving cell movement and cell shape changes in general. It is well known that actin filaments, together with actin-binding proteins and myosin assemblies, form a viscous gel-like fluid that extends and contracts, depending on the local rates of actin polymerization and depolymerization, and the amount of myosin contraction (Fletcher and Mullins, 2010; Murrell et al., 2015). Therefore, the cytoplasm is filled with at least two types of fluid (Li and Sun, 2018) – the cytosol, which is the combination of water, ions, organic molecules and proteins, and the cytoskeleton, which consists of actin, microtubules and intermediate filaments. These two fluids can be considered as two fluidic phases (with two different pressures) that can dynamically interact. For the cytoskeletal phase, the network pressure can be estimated by the equation of state for the network (pressure–material density relationship; for example, the ideal gas law $PV=nRT$). For liquids, gels and polymers, the equation of state is more complex (Rowlinson and Widom, 1982). Any active contractile stresses (such as those from myosin and crosslinking proteins) will modify the equation of state. Again, at steady state where there is no flow, force balance would require that any pressure gradients in the cytoskeleton phase must be balanced by hydraulic pressure gradients. This may be the reason why contractions (or 'negative' pressure) within the F-actin network can generate hydraulic pressure and result in the development of cell blebs (Charras et al., 2005; Charras et al., 2008; Charras et al., 2009), although fluid flow from outside into the cell might also contribute to blebs (Taloni et al., 2015). This may also explain why the measured cytoplasmic hydraulic pressure depends on myosin activity (Sao et al., 2019). Recent papers have demonstrated that flows in the two fluidic phases can generate forces to position organelles, such as the nucleus and the spindle, in the cell cytoplasm (Duan et al., 2020; Deneke et al., 2019). There are also other fluidic phases, such as the mitochondrial network, lipid reservoirs and nucleoli, which consist of molecules that are phase-separated from the cytosol and the cytoskeleton, and which behave as another viscous liquid; these combine to define the overall mechanical state of the cell.

If we accept that the cytoplasm consists of two or more phases, then a kinematic law of cell motion can be derived from mass conservation alone. Consider the cell boundary at time t and $t+\Delta t$ (Fig. 2A). The boundary has moved by the distance $v_{cell}\Delta t$, creating a new volume element. Since there are no empty spaces in a cell, this new volume element must be occupied by material fluxes (Fig. 2A), that is, flux of water from the outside of the cell, J_{water}, flow of cytosol into this element, $v_{cytosol}$, flow of cytoskeletal network into this element, v_n, or the creation of new cytoskeletal network from polymerization, J_n. Therefore the cell boundary velocity is equal to (Li and Sun, 2018; Tao et al., 2017):

$$v_{cell} = J_{water} + J_n + v_{cytosol}\,\theta_{cytosol} + v_n\,\theta_n, \qquad (3)$$

where $\theta_{n,cytosol}$ are the volume fractions of the network phase and cytosol phase, respectively. The local flux of water is determined by the local hydraulic and osmotic pressures, that is $J_{water} = -a(\Delta P - \Delta\Pi)$,

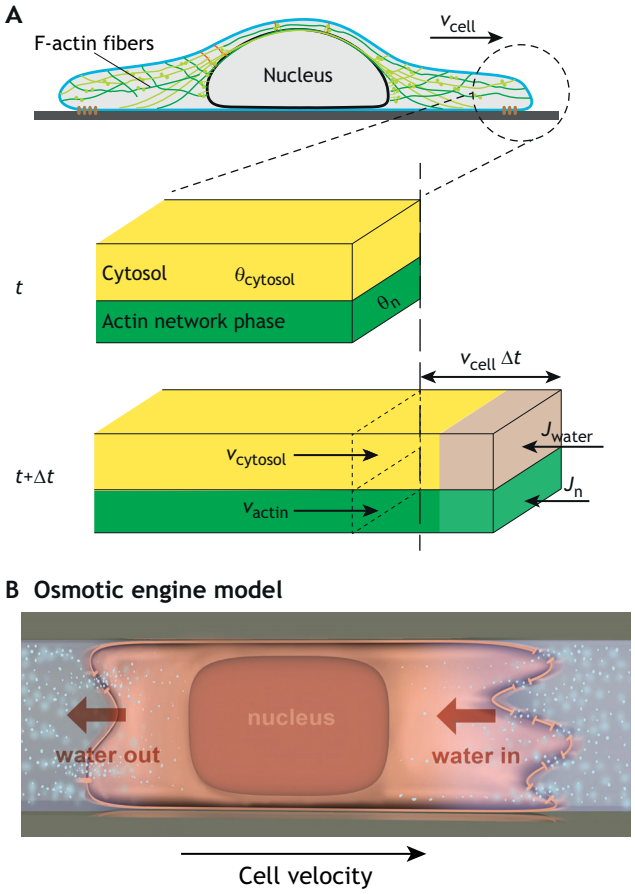

Fig. 2. Water-flux-based cell motility. (A) The cell cytoplasm consists of a cytosol phase (volume fraction $\theta_{cytosol}$; water and dissolved solutes) and cytoskeletal network phase (volume fraction θ_n; actin, microtubules and crosslinkers). The two phases interact with each other mechanically. When the leading edge of the cell advances by the amount $v_{cell}\Delta t$ in time Δt, the newly created space can only be filled by four possible sources of mass flux: cytoplasmic flows into the space ($v_{cytosol}$), flow of cytoskeletal network (v_n) or of newly polymerized cytoskeletal network from polymerization flux (J_n), or water flux from outside (J_{water}). $v_{cytosol}$ and v_n are determined by the mechanical force balance in the cytoplasm, whereas J_n and J_{water} are controlled by the cell. (B) J_{water} is proportional to hydraulic pressure and osmotic pressure gradients (see Fig. 1). If the cell is able to pump solutes (ions) into the cell at the leading edge and pump solutes out at the trailing edge, water flux through the cell will follow. This leads to the forward translocation of the cell body, referred to as the osmotic engine model (OEM). Reprinted from Stroka et al. (2014b) with permission from Elsevier.

and therefore depends on local ion channel activity. Actin polymerization flux J_n is controlled by actin nucleators, signaling pathways and also mechanical forces (Footer et al., 2007; Parekh et al., 2005; Mogilner and Oster, 2003; Hirata et al., 2008). The remaining unknowns are the cytoplasmic flow velocities and their respective volume fractions. These velocities are determined by the mechanics of the fluidic phases and forces that develop in these systems as well as potential active network forces, such as myosin contraction (Li and Sun, 2018; Li et al., 2019; Mogilner et al., 2018; Keren et al., 2009).

When examining the extension of the cell boundary during motility (Fig. 2), it is interesting to also contemplate the motion of the plasma membrane. During cell translocation, the membrane can be stationary with respect to the cell, that is, move with the same speed as the cell, or it can be stationary with respect to the lab-frame and treadmill forward. A third possibility is a tank-treading motion where the flow of the apical membrane velocity is forward and that

of the basal membrane is reverse. Indeed, vesicle trafficking can facilitate treadmilling of membrane forward (O'Neill et al., 2018), whereas forces owing to friction with the substrate can slow down the movement of the basal membrane. These different types of membrane flow behavior have been measured in migrating cells (Lee et al., 1990; Traynor and Kay, 2007; Kucik et al., 1989; Dai and Sheetz, 1995). There does not appear to be a significant flow of membrane with respect to the cell during cell migration. It is also known that mechanical behavior of the membrane is critical for cell polarization and cell motility (Shi et al., 2018; Houk et al., 2012; Keren et al., 2009). Indeed, membrane tension has been shown to serve as a global inhibitory signal for protrusion, which coupled to local activation, leads to cell polarity (Houk et al., 2012). In addition, in the absence of any chemotactic signals, Ca^{2+} currents through membrane channels are also essential for spontaneous cell polarity (Wei et al., 2009).

Actin-independent cell migration and the influence of hydraulic resistance on cell speed

Eqn 3 implies that cells can move by either actin polymerization or water flux. Indeed, there is experimental evidence that actin is dispensable during migration under confinement (Balzer et al., 2012; Stroka et al., 2014b; Panopoulos et al., 2011). For instance, in narrow, confined microfluidic channels, mouse S180 fibrosarcoma cells with a depolymerized actin network are able to migrate with the same speed as cells with an intact cytoskeleton, whereas other cell types (human metastatic MDA-MB-231 breast cancer cells) show a reduction in cell speed, but continue to move even after actin is completely depolymerized (Stroka et al., 2014b). In microchannels, MDA-MB-231 cells show a polarized distribution of aquaporins and NHE1 (a Na and H exchanger; Putney et al., 2002). Moreover, if the osmolarity of the external medium at the leading and trailing ends of the cell are perturbed independently, different cell speeds and cell boundary velocities are observed (Stroka et al., 2014a). Cells retained their asymmetrical distribution of ion transporters (NHE1 and potentially others) after the disruption of the actin network; at the leading end, the cell is passaging ions (perhaps mostly Na^+ and Cl^-) into the cell, whereas at the trailing end, the cell is transporting ions out of the cell. This constant flux of ions establishes a slight gradient of solute concentration in the cell and therefore generates a constant flux of water through the cell. This transport of water leads to a forward translocation of the cell body in this so-called osmotic engine model (OEM) of cell migration (Fig. 2B) (Stroka et al., 2014b; Shoji and Kawano, 2019; Hoffmann et al., 2009). Another parallel mechanism is the uptake of water not through the membrane, but through micropinocytosis (Moreau et al., 2019). In this case, large endocytic vesicles containing extracellular fluid are taken up by the cell, which also leads to cell movement in confined channels.

The remaining question is why the cell uses water-driven mode of motility in confined channels. One possibility is that the geometry of thin longitudinal channels facilitates the polarization of aquaporins and ion channels and/or pumps. Also, in 2D, water is free to move around the cell while, in confined channels, water cannot easily flow around the cell and instead must go through the cell. The different speeds of cells in the absence of actin polymerization suggests that the relative contributions of actin and water to cell speed depends on cell type; therefore, the question arises as to what is the determining factor for a cell to switch between the actin- and water-based mode of cell migration. Accumulating recent experiments suggest that cells are sensitive to the hydraulic resistance they experience (Prentice-Mott et al., 2013; Zhao et al., 2019; Srivastava et al., 2020; Moreau et al., 2019). Hydraulic resistance is the effective fluidic resistive force the cell experiences as it moves (Li and Sun, 2018; Prentice-Mott et al., 2013). If water does not flow through the cell, it must be pushed out of the way around the cell. This is easy to accomplish on flat 2D substrates where the hydraulic resistance, which is proportional to the water viscosity, is very low (Fig. 3). However, in confined channels, in order to push the water forward, the cell must push the entire column of water, which requires overcoming frictional forces between water flow and the channel walls. Therefore, the hydraulic resistance in confined channels is very high and is also proportional to the channel length (L in Fig. 3A) and the channel cross-sectional area (Fig. 3A). Based on theoretical modeling of a two-phase model of the cytoplasm, the cytosol flow velocity $v_{cytosol}$ is directly related to the external hydraulic resistance (Li and Sun, 2018). Therefore, even for fixed J_{water} and J_n, the final cell speed depends on the hydraulic resistance. Indeed, as the hydraulic resistance increases, the contribution of J_{water} to cell speed increases. This provides a possible explanation for why cells can rely on OEM for motility inside confining channels with a high hydraulic resistance, as opposed to using actin in 2D (Li and Sun, 2018). This also indicates that the water-based osmotic engine is a polarized manifestation of the cell volume control system discussed in the Introduction. The same ion channels or pumps involved in maintaining the cell water and/or ion content, when positioned in a spatially polarized manner, also drive directional water influx and efflux, and OEM. Actin- and myosin-mediated contraction play prominent roles in cell polarization, and therefore indirectly influence J_{water} and OEM, pointing to a crosstalk between OEM and the cell cytoskeleton in cell motility. Modeling also indicates that higher metabolic costs are incurred in order for a cell to move by using OEM under high hydraulic resistance conditions than in 2D cell culture conditions (Li et al., 2019). This prediction agrees with the elevated cytoplasmic ATP concentrations measured in cells moving in collagen microchannels (Zanotelli et al., 2018). Moreover, cells in 3D matrices experience higher hydraulic resistance due to the porous nature of the ECM that prevents easy fluid flow (Maity et al., 2019). Here, the matrix fibers generate additional resistance to fluid flow, and higher matrix density can increase hydraulic resistance by several orders of magnitude (Fig. 3C; Maity et al., 2019). Therefore, OEM-based motility may be more prominent in these conditions.

Cell surface mechanosensing as an organizer of cell motility and cell volume regulation

Mechanically, the presence of high hydraulic resistance during migration also implies that the hydraulic pressure at the cell leading edge is different from the far-field ambient value (P_∞ in Fig. 3A). This is a consequence of the physics of viscous flows, where flow velocities are directly proportional to pressure gradients (Pozrikidis, 2011). If the cell in the channel is not transporting water through the cell, then the external fluid must also move forward at the same speed as the cell; this implies that there is a slightly higher pressure directly in front of the cell (P_f) than P_∞. This pressure gradient is proportional to the channel length. Indeed, recent experiments showed that when cells are exposed to different channels and hydraulic resistances, they exhibit a preference in the direction of their migration, usually preferring the channel with the lowest hydraulic resistance (Zhao et al., 2019; Pretice-Mott et al., 2013) (Fig. 3B). This means that the direction of cell movement is influenced by the hydraulic resistance, and possibly the hydraulic pressure immediately in front of the cell. This form of pressure

Journal of Cell Science (2020) 133, jcs240341. doi:10.1242/jcs.240341

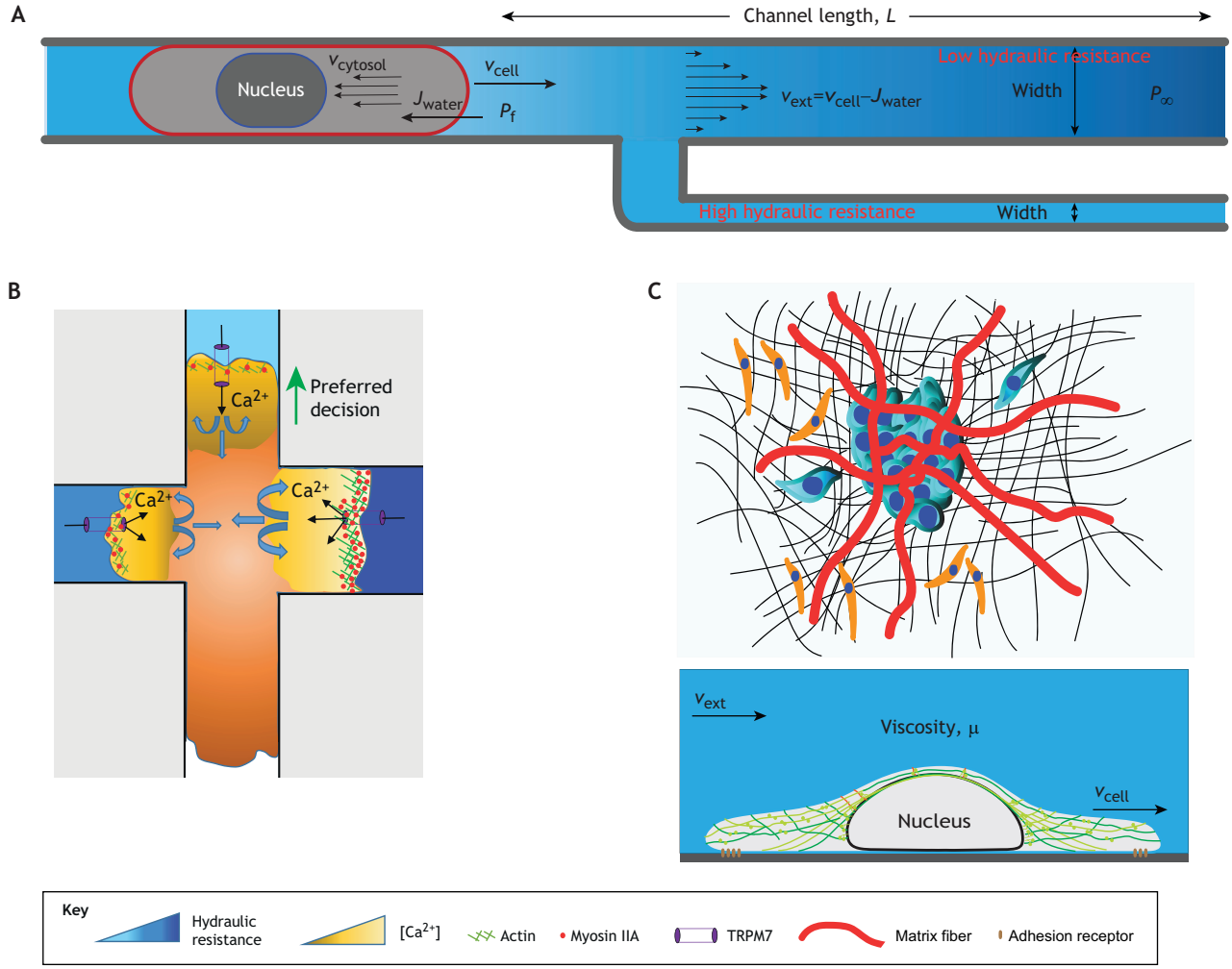

Fig. 3. Cell movement under confinement and hydraulic pressure sensing. (A) The hydraulic resistance experienced by the cell depends on the microenvironment. In a confined microchannel, the hydraulic resistance is proportional to the external fluid velocity, $v_{ext}=(v_{cell}-J_{water})$, and the dimension of the channel. Narrower channels have higher hydraulic resistance. The hydraulic resistance is also related to the hydraulic pressure immediately in front of the cell leading edge (P_f). If the hydraulic resistance is high, then $P_f>P_\infty$, the ambient pressure value. (B) Cells in confining channels sense hydraulic resistance by sensing the hydraulic pressure in front of the cell P_f. Pressure sensing appears to depend on the mechanosensitive TRPM7 Ca^{2+} channel. Reprinted from Zhao et al. (2019), where it was published under a CC-BY-NC 4.0 license, with permission from The American Association for the Advancement of Science. (C) For cells migrating in 3D matrices, the hydraulic resistance depends on the porosity of the surrounding matrix, as well as the stiffness of the matrix fibers (top). Blue, tumor cells; orange, fibroblasts. Cells migrating on flat 2D surface experience low hydraulic resistance, which is proportional to the viscosity (μ) of the surrounding fluid (bottom).

sensing during migration is called barotaxis (Moreau et al., 2019). Indeed, when the mechanosensitive cation channel TRPM7 is either blocked or knocked out, cells lose their directional preference for channels with low hydraulic resistance and instead enter the channels with the largest cross-sectional area irrespective of their prevailing hydraulic resistance (Zhao et al., 2019). Chelating Ca^{2+} or blocking myosin II function has a similar effect on the migration direction. Along these lines, when cells on 2D surfaces are exposed to changes in hydraulic pressure of only a few pascals, their cytoplasmic Ca^{2+} levels show a strong response that is dependent on TRPM7 (Zhao et al., 2019; Liu et al., 2015). Therefore, cells are sensitive to the hydraulic resistance around them and make decisions to polarize in a Ca^{2+}-dependent manner. This sensitivity is connected to hydraulic pressure external to the cell and is actively sensed.

Based on Eqn 1, hydraulic pressure is also linked to the cortical tension. When cells are subjected to external mechanical force, there is also an active response that depends on cellular Ca^{2+} dynamics (He et al., 2018). For example, when cells are mechanically compressed, Rho activity and myosin contraction have been found to instantly decrease in a Ca^{2+}-dependent manner (He et al., 2018). In contrast, when cells are mechanically stretched, myosin contraction increases (Tao and Sun, 2015; Koride et al., 2014; Zhao et al., 2007; Luo et al., 2013). Furthermore, entry of cells into and their motility in short confining channels, which compresses and stretches cells in the apicobasal and longitudinal direction, respectively, results in elevated RhoA activity and myosin II contractility (Mistriotis et al., 2019). Moreover, when cells are subjected to sudden changes in hydraulic pressure, active responses by the cell are also observed (Ju et al., 2009; Stover and Nagatomi, 2007; Kao et al., 2017; Hui et al., 2014; Liu et al., 2019). These experiments, together with hydraulic resistance experiments in channels, indicate that cellular mechanosensation has a universal basis and involves membrane-tension-sensitive ion channels and Ca^{2+} currents, as well as myosin and its associated pathways. Ca^{2+}, as a secondary messenger, also affects many passive and active ion

Journal of Cell Science (2020) 133, jcs240341. doi:10.1242/jcs.240341

channels (Ranade et al., 2015). Therefore, the same cell volume regulation system that controls cellular ion and water homeostasis is likely to regulate OEM, as well as cell polarization and the hydraulic response.

Water dynamics in tissues

Active transport of water that underlies OEM-based cell migration and cell volume regulation is also prominent during tissue development and morphogenesis (Leonavicius et al., 2018; Latorre et al., 2018). For instance, the kidney is a specialized organ that is responsible for retaining 99% of the water in the body. Kidney epithelial cells in the proximal tubule are responsible for this water reabsorption activity and act by pumping Na^+ and Cl^- ions across the epithelium (Weinstein, 2000). The molecular details of this machinery are partially understood. This water-absorption machinery involves ion channels and pumps, and is broadly the same as those driving OEM, but the control mechanisms are likely to be different. Since OEM is predicted to drive cell movement and movement is related to forces, theoretical calculations suggest that is there is also mechanical force generation during the passaging of water across the kidney epithelium (Choudhury et al., 2019 preprint) (Fig. 4). Indeed, this force, in the form of an apical–basal hydraulic pressure difference, was quantified for kidney epithelium using a permeable microfluidic method (Choudhury et al., 2019 preprint). Similarly, MDCK II cells grown on impermeable substrates can generate dynamic fluid-fill domes (Yang et al., 2019). These domes are generated from active pumping of water across the epithelium into the domes, and according to OEM predictions, the hydraulic pressure is higher inside the dome. This elevated hydraulic pressure can drive epithelial shape changes, that is, morphogenesis.

The active pumping of water, therefore, is another way for cells to generate mechanical force that is transverse to the epithelium. The elevated pressure at the back of the cell is effectively a pushing force and can promote tissue expansion. For instance, during early mammalian development, a cluster of cells called the blastocyst must expand and develop a lumen to burst out of the zona pellucida, a shell surrounding the early embryo (Leonavicius et al., 2018). This lumen expansion has been shown to rely on elevated pressure inside the lumen. Another example is the mammary gland organoid; here, expansion of the organoid in the matrix and generation of a fluid-filled lumen also requires an elevated pressure (Yang et al., 2019). The elevated pressure is not particularly high – of the order 100–300 Pa, which is about 0.1–0.3% of the atmospheric pressure. However, after multiplying by the typical cell surface area, this pressure difference translates to a force of 50–100 nN per cell. This force is similar to traction force generated by actomyosin contraction (Style et al., 2014; Wang and Li, 2009). Therefore, the OEM model of pressure and/or force generation might be as significant as actomyosin contraction with regard to force generation.

Furthermore, the same kinematic conditions that drive cell boundary movement (see Fig. 2A) are also relevant in tissues during the collective movement of a group of cells. If the epithelium is mature and cells have established junctional complexes such as gap junctions (Kumar and Gilula, 1996; Nielsen et al., 2012), the cytoplasm of neighboring cells are directly connected. Fluid, ions and other molecules from one cell can flow into neighboring cells connected by gap junctions (Fig. 5). This flow of material will drive the movement of the cell–cell boundary and thus the relative movement of cells with respect to each other. Here, pressure differences in neighboring cells, both with regard to osmotic and hydraulic pressure, can drive this flow and contribute to cell collective motion. Therefore, water flows and hydraulic pressures generated by ion fluxes are also important in tissue morphodynamics, and can be used by cells collectively to generate forces and movement.

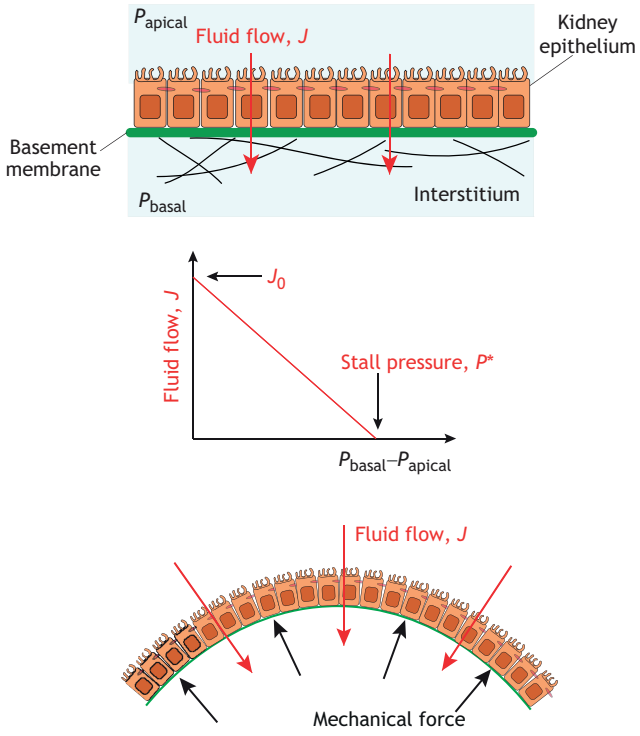

Fig. 4. Mechanics of water transport across the kidney epithelium. Kidney epithelial cells can pump fluid from the lumen (apical region) to the interstitium (basal region). The fluid flux (red arrows) depends on the pressure difference across the epithelium (P_{basal}-P_{apical}). The relationship between flux and pressure difference (middle panel) is reminiscent of mechanical fluid pumps, which generate the highest pump flux when the pressure difference between outlet and inlet is zero and that declines when the pressure difference increases. This pump performance curve has a stall pressure (or head pressure, P^*), at which the fluid flux is zero. This behavior can be contrasted with that of a filter, which requires a higher pressure on the apical side to generate an apical–basal flux. The stall pressure is regulated by biochemical pathways and pressure-sensing mechanisms of kidney epithelial cells, and is ∼100–300 Pa. The pressure gradient during fluid passage will generate a mechanical force in the opposite direction as fluid flow (bottom panel, black arrows). The magnitude of this force is ∼10–100 nN per cell.

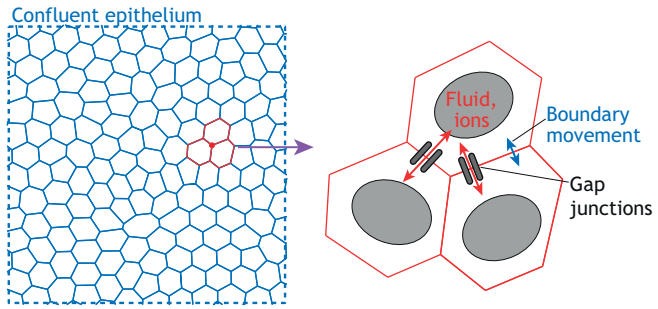

Fig. 5. Role of water flux in collective cell movement. The collective movement of groups of cells is driven by fluid and ion transport through gap junctions between cells. Fluid movement (driven by pressure gradients) and ion or molecule movement (driven by concentration gradients) across the cell–cell boundary result in boundary movement, and, ultimately, the collective movement of cells. The cytoplasms of confluent cells are effectively connected.

Conclusions and perspectives

Water is a central molecule of life and an essential component of the cytoplasm. When water is free to flow in and out of the cell, its overall cytoplasmic content must be carefully controlled. Since water follows solute transport, the cell controls its water content by controlling its solute concentration. Moreover, it is reasonable to conjecture that the cell controls the concentrations of all of the essential ions of life, including K^+, H^+ and Ca^+, so that there is a consistent chemical environment for proteins. The combined homeostatic system can achieve constant ion concentrations and a constant cell volume for a given protein and amino acid content. Indeed, simple modeling shows that within such a system, protein and amino acid concentrations are also constant, therefore as the cell grows by increasing protein content, the cell volume increases proportionally with protein content (Tao and Sun, 2015; Yellin et al., 2018).

This system of cell volume and ion content control is also likely responsible for the observed behaviors when cells are subjected to external force, changes in hydraulic pressure or changes in external osmolarity (Tao and Sun, 2015). External pressure and osmolarity changes will directly cause water flux across the cell membrane. Pressure changes and external forces will also influence the force balance at the cell cortex, and likely result in activation of mechanosensitive ion channels, giving rise to a possible feedback system. Any changes in the force balance at the cell surface, for instance from an externally applied force, can activate a multitude of molecules and cell signaling responses. In particular, with regard to ion homeostasis, many of the ion channels are also Ca^{2+} dependent. Furthermore, most mechanosensitive channels, such as transient receptor potential channels (TRPs) are also Ca^{2+} channels (Bouron et al., 2015; Clapham et al., 2001; Venkatachalam and Montell, 2007), implying that Ca^{2+} dynamics could 'encode' the 'control language' of a cell. Ca^{2+} is also required for spontaneous polarization of the cell (Wei et al., 2009) and controls the activity of many ion channels and pumps, as well as regulating cytoskeletal dynamics. What the roles of Ca^{2+} are is an important open question for cell mechanics, and fully elucidating this in cells may be a key to better understanding of cell volume regulation, ion homeostasis and cell mechanosensation.

Another open area that requires more investigation is the interplay between the F-actin phase and the water phase. We have discussed that the F-actin network can mechanically interact with the cytoplasmic fluid, and their relative flow velocities can set the cell boundary speed. But there are additional couplings as well. F-actin is known to be involved in establishing cell polarity, and spatially localization of ion channels or pumps that drive water movement is likely to depend on the cytoskeleton via the vesicular trafficking system. Moreover, processes such as macropinocytosis that also take up water involve the actomyosin machinery (Moreau et al., 2019). Therefore, water and cytoskeleton systems have many areas of potential interface, and elucidation of their crosstalk deserves further exploration.

Many important signaling pathways are linked to activities of ion channels, cytoskeleton and myosin in the cell cortex. For example, the Rho–Rac signaling pathway is integral for cell polarization and is connected to myosin contraction and cytoskeletal regulation (Maddox and Burridge, 2003; Jilkine et al., 2007; Amano et al., 1997; Hung et al., 2013). Cytoskeletal tension and activity also influence the Hippo signaling pathway and the nuclear localization of its downstream effectors, the transcription factors YAP and TAZ (Perez-Gonzalez et al., 2019; Meng et al., 2018; Perez-Gonzalez et al., 2018; Dupont et al., 2011), which modulate cell size and growth dynamics through their target genes (Tumaneng et al., 2012; Yu et al., 2015). We also know that there must be feedback from the Hippo pathway to the homeostasis system because the cytoplasmic pressure is lowered when YAP is knocked out (Perez-Gonzalez et al., 2019). The steady-state pressure in a cell should correspond to the net osmotic pressure and the total solute concentration. Therefore, the transcriptional activities of the Hippo pathway could also influence cell osmotic control. Beyond the Hippo signaling pathway, there are likely multiple Ca^{2+}- and mechano-sensitive pathways, which lead to biochemical modifications and expression of transcriptional programs. Therefore, it is reasonable to postulate that signals arising from the volume and ion homeostasis system could also lead to global changes in cell behavior.

Finally, if the cytoplasmic osmotic and hydraulic pressure is mostly constant during the cell cycle, and the cell volume scales linearly with protein content, the volume regulation system provides a way for the cell to sense its size. From Eqn 1, we see that if the volume regulation system maintains a constant cell osmolarity and ΔP is constant, cell tension must scale with the radius of curvature. Therefore, a bigger cell with a larger radius of curvature will also have higher active tension. The active tension of the cell is mostly generated by myosin contraction. Based on quantitative single-cell measurements of the levels of phosphorylated myosin light chain (pMLC), a marker of cell active tension, the cell tension indeed appears to increase with cell size (Perez-Gonzalez et al., 2018). Moreover, varying cell substrate stiffness, which changes cell size, also results in corresponding changes in the total pMLC content of the cell. Therefore, the cell active tension in Eqn 1, together with the volume homeostasis system, could be a way for the cell to measure its size. Another cell-size-sensing mechanism that is based on the dilution of a fixed number of proteins has also been proposed (Schmoller et al., 2015). These mechanisms may work together to control cell size during cell cycle progression and generate a cell size checkpoint (Ginzberg et al., 2015; Kafri et al., 2013) that regulates cell growth rates and the G1-S transition. This cell size checkpoint may involve a combination of cell tension sensing, possibly through the Hippo pathway and other Ca^{2+}-sensitive pathways and the dilution of key cell cycle proteins as suggested previously (Schmoller et al., 2015). Further investigations are needed in order to fully reveal the cellular pressure and volume control system and to understand its implications for cell growth and cell cycle control.

Competing interests

The authors declare no competing or financial interests.

Funding

This work has been funded in part by National Institutes of Health (grants U54CA210172 and R01GM134542). Y.M. was supported by National Science Foundation (grant DMS-1907583) and the Simons Foundation. Deposited in PMC for release after 12 months.

References

Agre, P. (2006). The aquaporin water channels. *Proc. Am. Thorac. Soc.* **3**, 5-13. doi:10.1513/pats.200510-109JH

Altenburg, T., Goldenbogen, B., Uhlendorf, J. and Klipp, E. (2019). Osmolyte homeostasis controls single-cell growth rate and maximum cell size of. *NPJ Syst. Biol. Appl.* **5**, 34. doi:10.1038/s41540-019-0111-6

Amano, M., Chihara, K., Kimura, K., Fukata, Y., Nakamura, N., Matsuura, Y. and Kaibuchi, K. (1997). Formation of actin stress fibers and focal adhesions enhanced by Rho-kinase. *Science* **275**, 1308-1311. doi:10.1126/science.275.5304.1308

Armstrong, C. M. (2003). The Na/K pump, Cl ion, and osmotic stabilization of cells. *Proc. Natl. Acad. Sci. USA* **100**, 6257-6262. doi:10.1073/pnas.0931278100

Atkins, P. W. (1990). *Physical Chemistry*. New York: W. H. Freeman and Company.

Balzer, E. M., Tong, Z., Paul, C. D., Hung, W. C., Stroka, K. M., Boggs, A. E., Martin, S. S. and Konstantopoulos, K. (2012). Physical confinement alters tumor cell adhesion and migration phenotypes. *FASEB J.* **26**, 4045-4056. doi:10.1096/fj.12-211441

Beauzamy, L., Derr, J. and Boudaoud, A. (2015). Quantifying hydrostatic pressure in plant cells by using indentation with an atomic force microscope. *Biophys. J.* **108**, 2448-2456. doi:10.1016/j.bpj.2015.03.035

Bouron, A., Kiselyov, K. and Oberwinkler, J. (2015). Permeation, regulation and control of expression of TRP channels by trace metal ions. *Pflugers Arch.* **467**, 1143-1164. doi:10.1007/s00424-014-1590-3

Charras, G. T., Yarrow, J. C., Horton, M. A., Mahadevan, L. and Mitchison, T. J. (2005). Non-equilibration of hydrostatic pressure in blebbing cells. *Nature* **435**, 365-369. doi:10.1038/nature03550

Charras, G. T., Coughlin, M., Mitchison, T. J. and Mahadevan, L. (2008). Life and times of a cellular bleb. *Biophys. J.* **94**, 1836-1853. doi:10.1529/biophysj.107.113605

Charras, G. T., Mitchison, T. J. and Mahadevan, L. (2009). Animal cell hydraulics. *J. Cell Sci.* **122**, 3233-3241. doi:10.1242/jcs.049262

Choudhury, M. I., Li, Y., Mistriotis, P., Dixon, E. E., Yang, J., Maity, D., Walker, R., Benson, M., Martin, L., Koroma, F. et al. (2019). Trans-epithelial fluid pumping performance of renal epithelial cells and mechanics of cystic expansion. *bioRxiv*, 727313. doi:10.1101/727313

Chugh, P., Clark, A. G., Smith, M. B., Cassani, D. A. D., Dierkes, K., Ragab, A., Roux, P. P., Charras, G., Salbreux, G. and Paluch, E. K. (2017). Actin cortex architecture regulates cell surface tension. *Nat. Cell Biol.* **19**, 689-697. doi:10.1038/ncb3525

Clapham, D. E., Runnels, L. W. and Strübing, C. (2001). The TRP ion channel family. *Nat. Rev. Neurosci.* **2**, 387-396. doi:10.1038/35077544

Clark, A. G., Dierkes, K. and Paluch, E. K. (2013). Monitoring actin cortex thickness in live cells. *Biophys. J.* **105**, 570-580. doi:10.1016/j.bpj.2013.05.057

Dai, J. and Sheetz, M. P. (1995). Axon membrane flows from the growth cone to the cell body. *Cell* **83**, 693-701. doi:10.1016/0092-8674(95)90182-5

Deneke, V. E., Puliafito, A., Krueger, D., Narla, A. V., De Simone, A., Primo, L., Vergassola, M., De Renzis, S. and Di Talia, S. (2019). Self-Organized Nuclear Positioning Synchronizes the Cell Cycle in Drosophila Embryos. *Cell* **177**, 925-941.e17. doi:10.1016/j.cell.2019.03.007

Duan, X., Li, Y., Yi, K., Guo, F., Wang, H., Wu, P. H., Yang, J., Mair, D. B., Morales, E. A., Kalab, P. et al. (2020). Dynamic organelle distribution initiates actin-based spindle migration in mouse oocytes. *Nat. Commun.* **11**, 277. doi:10.1038/s41467-019-14068-3

Dupont, S., Morsut, L., Aragona, M., Enzo, E., Giulitti, S., Cordenonsi, M., Zanconato, F., Le Digabel, J., Forcato, M., Bicciato, S. et al. (2011). Role of YAP/TAZ in mechanotransduction. *Nature* **474**, 179-183. doi:10.1038/nature10137

Ermentrout, B. G. and Terman, D. H. (2010). *Mathematical Foundations of Neuroscience*. Springer.

Farinas, J. and Verkman, A. S. (1996). Cell volume and plasma membrane osmotic water permeability in epithelial cell layers measured by interferometry. *Biophys. J.* **71**, 3511-3522. doi:10.1016/S0006-3495(96)79546-2

Farinas, J., Kneen, M., Moore, M. and Verkman, A. S. (1997). Plasma membrane water permeability of cultured cells and epithelia measured by light microscopy with spatial filtering. *J. Gen. Physiol.* **110**, 283-296. doi:10.1085/jgp.110.3.283

Fletcher, D. A. and Mullins, R. D. (2010). Cell mechanics and the cytoskeleton. *Nature* **463**, 485-492. doi:10.1038/nature08908

Footer, M. J., Kerssemakers, J. W., Theriot, J. A. and Dogterom, M. (2007). Direct measurement of force generation by actin filament polymerization using an optical trap. *Proc. Natl. Acad. Sci. USA* **104**, 2181-2186. doi:10.1073/pnas.0607052104

Fritzsche, M., Lewalle, A., Duke, T., Kruse, K. and Charras, G. (2013). Analysis of turnover dynamics of the submembranous actin cortex. *Mol. Biol. Cell* **24**, 757-767. doi:10.1091/mbc.e12-06-0485

Gadsby, D. C. (2009). Ion channels versus ion pumps: the principal difference, in principle. *Nat. Rev. Mol. Cell Biol.* **10**, 344-352. doi:10.1038/nrm2668

Gahmberg, C. G. and Tolvanen, M. (1996). Why mammalian cell surface proteins are glycoproteins. *Trends Biochem. Sci.* **21**, 308-311. doi:10.1016/S0968-0004(96)10034-7

Ginzberg, M. B., Kafri, R. and Kirschner, M. (2015). Cell biology on being the right (cell) size. *Science* **348**, 1245075. doi:10.1126/science.1245075

He, L., Tao, J., Maity, D., Si, F., Wu, Y., Wu, T., Prasath, V., Wirtz, D. and Sun, S. X. (2018). Role of membrane-tension gated Ca^{2+} flux in cell mechanosensation. *J. Cell Sci.* **131**, jcs208470. doi:10.1242/jcs.208470

Hirata, H., Tatsumi, H. and Sokabe, M. (2008). Mechanical forces facilitate actin polymerization at focal adhesions in a zyxin-dependent manner. *J. Cell Sci.* **121**, 2795-2804. doi:10.1242/jcs.030320

Hoffmann, E. K., Lambert, I. H. and Pedersen, S. F. (2009). Physiology of cell volume regulation in vertebrates. *Physiol. Rev.* **89**, 193-277. doi:10.1152/physrev.00037.2007

Houk, A. R., Jilkine, A., Mejean, C. O., Boltyanskiy, R., Dufresne, E. R., Angenent, S. B., Altschuler, S. J., Wu, L. F. and Weiner, O. D. (2012). Membrane tension maintains cell polarity by confining signals to the leading edge during neutrophil migration. *Cell* **148**, 175-188. doi:10.1016/j.cell.2011.10.050

Huang, Y. J., Schiapparelli, P., Kozielski, K., Green, J., Lavell, E., Guerrero-Cazares, H., Quinones-Hinojosa, A. and Searson, P. (2017). Electrophoresis of cell membrane heparan sulfate regulates galvanotaxis in glial cells. *J. Cell Sci.* **130**, 2459-2467. doi:10.1242/jcs.203752

Hui, T. H., Zhou, Z. L., Qian, J., Lin, Y., Ngan, A. H. and Gao, H. (2014). Volumetric deformation of live cells induced by pressure-activated cross-membrane ion transport. *Phys. Rev. Lett.* **113**, 118101. doi:10.1103/PhysRevLett.113.118101

Hung, W. C., Chen, S. H., Paul, C. D., Stroka, K. M., Lo, Y. C., Yang, J. T. and Konstantopoulos, K. (2013). Distinct signaling mechanisms regulate migration in unconfined versus confined spaces. *J. Cell Biol.* **202**, 807-824. doi:10.1083/jcb.201302132

Jakobsson, E. (1980). Interactions of cell volume, membrane potential, and membrane transport parameters. *Am. J. Physiol.* **238**, C196-C206. doi:10.1152/ajpcell.1980.238.5.C196

Jaynes, E. T. (1992). Gibbs paradox. In *Maximum entropy and Baysian Methods*, (Eds Smith, C. R., Erickson and G. J., Neudorfer, P. O.), pp. 1-22. Kluwer Academic Publishers.

Jiang, H. and Sun, S. X. (2013). Cellular pressure and volume regulation and implications for cell mechanics. *Biophys. J.* **105**, 609-619. doi:10.1016/j.bpj.2013.06.021

Jilkine, A., Marée, A. F. and Edelstein-Keshet, L. (2007). Mathematical model for spatial segregation of the Rho-family GTPases based on inhibitory crosstalk. *Bull. Math. Biol.* **69**, 1943-1978. doi:10.1007/s11538-007-9200-6

Ju, W. K., Kim, K. Y., Lindsey, J. D., Angert, M., Patel, A., Scott, R. T., Liu, Q., Crowston, J. G., Ellisman, M. H., Perkins, G. A. et al. (2009). Elevated hydrostatic pressure triggers release of OPA1 and cytochrome C, and induces apoptotic cell death in differentiated RGC-5 cells. *Mol. Vis.* **15**, 120-134.

Kafri, R., Levy, J., Ginzberg, M. B., Oh, S., Lahav, G. and Kirschner, M. W. (2013). Dynamics extracted from fixed cells reveal feedback linking cell growth to cell cycle. *Nature* **494**, 480-483. doi:10.1038/nature11897

Kao, Y.-C., Jheng, J.-R., Pan, H.-J., Liao, W.-Y., Lee, C.-H. and Kuo, P.-L. (2017). Elevated hydrostatic pressure enhances the motility and enlarges the size of the lung cancer cells through aquaporin upregulation mediated by caveolin-1 and ERK1/2 signaling. *Oncogene* **36**, 863-874. doi:10.1038/onc.2016.255

Keren, K., Yam, P., Kinkhabwala, A., Mogilner, A. and Theriot, J. (2009). Intracellular fluid flow in rapidly moving cells. *Nat. Cell Biol.* **11**, 1219-1224. doi:10.1038/ncb1965

Koride, S., He, L., Xiong, L. P., Lan, G., Montell, D. J. and Sun, S. X. (2014). Mechanochemical regulation of oscillatory follicle cell dynamics in the developing Drosophila egg chamber. *Mol. Biol. Cell* **25**, 3709-3716. doi:10.1091/mbc.e14-04-0875

Kucik, D. F., Elson, E. L. and Sheetz, M. P. (1989). Forward transport of glycoproteins on leading lamellipodia in locomoting cells. *Nature* **340**, 315-317. doi:10.1038/340315a0

Kumar, N. M. and Gilula, N. B. (1996). The gap junction communication channel. *Cell* **84**, 381-388. doi:10.1016/S0092-8674(00)81282-9

Latorre, E., Kale, S., Casares, L., Gómez-González, M., Uroz, M., Valon, L., Nair, R. V., Garreta, E., Montserrat, N., Del Campo, A. et al. (2018). Active superelasticity in three-dimensional epithelia of controlled shape. *Nature* **563**, 203-208. doi:10.1038/s41586-018-0671-4

Lee, J., Gustafsson, M., Magnusson, K. E. and Jacobson, K. (1990). The direction of membrane lipid flow in locomoting polymorphonuclear leukocytes. *Science* **247**, 1229-1233. doi:10.1126/science.2315695

Leonavicius, K., Royer, C., Preece, C., Davies, B., Biggins, J. S. and Srinivas, S. (2018). Mechanics of mouse blastocyst hatching revealed by a hydrogel-based microdeformation assay. *Proc. Natl. Acad. Sci. USA* **115**, 10375-10380. doi:10.1073/pnas.1719930115

Li, Y. and Sun, S. X. (2018). Transition from Actin-Driven to Water-Driven Cell Migration Depends on External Hydraulic Resistance. *Biophys. J.* **114**, 2965-2973. doi:10.1016/j.bpj.2018.04.045

Li, Y., Mori, Y. and Sun, S. X. (2015). Flow-Driven Cell Migration under External Electric Fields. *Phys. Rev. Lett.* **115**, 268101. doi:10.1103/PhysRevLett.115.268101

Li, Y., He, L., Gonzalez, N. A. P., Graham, J., Wolgemuth, C., Wirtz, D. and Sun, S. X. (2017). Going with the Flow: Water Flux and Cell Shape during Cytokinesis. *Biophys. J.* **113**, 2487-2495. doi:10.1016/j.bpj.2017.09.026

Li, Y., Yao, L., Mori, Y. and Sun, S. X. (2019). On the energy efficiency of cell migration in diverse physical environments. *Proc. Natl. Acad. Sci. USA*. **116**, 23894-23900. doi:10.1073/pnas.1907625116

Liu, Y. S., Liu, Y. A., Huang, C. J., Yen, M. H., Tseng, C. T., Chien, S. and Lee, O. K. (2015). Mechanosensitive TRPM7 mediates shear stress and modulates osteogenic differentiation of mesenchymal stromal cells through Osterix pathway. *Sci. Rep.* **5**, 16522. doi:10.1038/srep16522

Liu, S., Tao, R., Wang, M., Tian, J., Genin, G. M., Lu, T. J. and Xu, F. (2019). Regulation of Cell Behavior by Hydrostatic Pressure. *Appl. Mech. Rev.* **71**, 0408031-4080313.

Luo, T., Mohan, K., Iglesias, P. A. and Robinson, D. N. (2013). Molecular mechanisms of cellular mechanosensing. *Nat. Mater.* **12**, 1064-1071. doi:10.1038/nmat3772
Maddox, A. S. and Burridge, K. (2003). RhoA is required for cortical retraction and rigidity during mitotic cell rounding. *J. Cell Biol.* **160**, 255-265. doi:10.1083/jcb.200207130
Maity, D., Li, Y., Chen, Y. and Sun, S. X. (2019). Response of collagen matrices under pressure and hydraulic resistance in hydrogels. *Soft Mat.* **15**, 2617-2626. doi:10.1039/C8SM02143K
Mathai, J. C., Tristram-Nagle, S., Nagle, J. F. and Zeidel, M. L. (2008). Structural determinants of water permeability through the lipid membrane. *J. Gen. Physiol.* **131**, 69-76. doi:10.1085/jgp.200709848
Meng, Z., Qiu, Y., Lin, K. C., Kumar, A., Placone, J. K., Fang, C., Wang, K. C., Lu, S., Pan, M., Hong, A. W. et al. (2018). RAP2 mediates mechanoresponses of the Hippo pathway. *Nature* **560**, 655-660. doi:10.1038/s41586-018-0444-0
Milo, R. and Phillips, R. (2015). *Cell biology by the numbers*. Garland Science.
Mistriotis, P., Wisniewski, E. O., Bera, K., Keys, J., Li, Y., Tuntithavornwat, S., Law, R. A., Perez-Gonzalez, N., Erdogmus, E., Zhang, Y. et al. (2019). Confinement hinders motility by inducing RhoA-mediated nuclear influx, volume expansion, and blebbing. *J. Cell Biol.* **218**, 4093-4111. doi:10.1083/jcb.201902057
Mitchison, T. J. (2019). Colloid osmotic parameterization and measurement of subcellular crowding. *Mol. Biol. Cell* **30**, 173-180. doi:10.1091/mbc.E18-09-0549
Mogilner, A. and Oster, G. (1996). Cell motility driven by actin polymerization. *Biophys. J.* **71**, 3030-3045. doi:10.1016/S0006-3495(96)79496-1
Mogilner, A. and Oster, G. (2003). Force generation by actin polymerization II: the elastic ratchet and tethered filaments. *Biophys. J.* **84**, 1591-1605. doi:10.1016/S0006-3495(03)74969-8
Mogilner, A., Manhart, A., Davis, S. and Moin, P. (2018). Intracellular Fluid Mechanics: Coupling Cytoplasmic Flow with Active Cytoskeletal Gel. *Annu. Rev. Fluid Mech.*, **50**, 347-370. doi:10.1146/annurev-fluid-010816-060238
Moreau, H. D., Blanch-Mercader, C., Attia, R., Maurin, M., Alraies, Z., Sanséau, D., Malbec, O., Delgado, M. G., Bousso, P., Joanny, J. F. et al. (2019). Macropinocytosis Overcomes Directional Bias in Dendritic Cells Due to Hydraulic Resistance and Facilitates Space Exploration. *Dev. Cell* **49**, 171-188.e5. doi:10.1016/j.devcel.2019.03.024
Mori, Y. (2012). Mathematical properties of pump-leak models of cell volume control and electrolyte balance. *J. Math. Biol.* **65**, 875-918. doi:10.1007/s00285-011-0483-8
Mulkidjanian, A. Y., Bychkov, A. Y., Dibrova, D. V., Galperin, M. Y. and Koonin, E. V. (2012). Origin of first cells at terrestrial, anoxic geothermal fields. *Proc. Natl. Acad. Sci. USA* **109**, E821-E830. doi:10.1073/pnas.1117774109
Murrell, M., Oakes, P. W., Lenz, M. and Gardel, M. L. (2015). Forcing cells into shape: the mechanics of actomyosin contractility. *Nat. Rev. Mol. Cell Biol.* **16**, 486-498. doi:10.1038/nrm4012
Nielsen, M. S., Axelsen, L. N., Sorgen, P. L., Verma, V., Delmar, M. and Holstein-Rathlou, N. H. (2012). Gap junctions. *Compr. Physiol.* **2**, 1981-2035.
Olbrich, K., Rawicz, W., Needham, D. and Evans, E. (2000). Water permeability and mechanical strength of polyunsaturated lipid bilayers. *Biophys. J.* **79**, 321-327. doi:10.1016/S0006-3495(00)76294-1
O'neill, P. R., Castillo-Badillo, J. A., Meshik, X., Kalyanaraman, V., Melgarejo, K. and Gautam, N. (2018). Membrane Flow Drives an Adhesion-Independent Amoeboid Cell Migration Mode. *Dev. Cell* **46**, 9-22.e4. doi:10.1016/j.devcel.2018.05.029
Panopoulos, A., Howell, M., Fotedar, R. and Margolis, R. (2011). Glioblastoma motility occurs in the absence of actin polymer. *Mol. Biol. Cell* **22**, 2212-2220. doi:10.1091/mbc.e10-10-0849
Parekh, S. H., Chaudhuri, O., Theriot, J. A. and Fletcher, D. A. (2005). Loading history determines the velocity of actin-network growth. *Nat. Cell Biol.* **7**, 1219-1223. doi:10.1038/ncb1336
Perez-Gonzalez, N., Tao, J., Rochman, N. D., Vig, D., Chiu, E., Wirtz, D. and Sun, S. X. (2018). Cell tension and mechanical regulation of cell volume. *Mol. Biol. Cell* **29**, 21. doi:10.1091/mbc.E18-04-0213
Perez-Gonzalez, N. A., Rochman, N. D., Yao, K., Tao, J., Le, M. T., Flanary, S., Sablich, L., Toler, B., Crentsil, E., Takaesu, F. et al. (2019). YAP and TAZ regulate cell volume. *J. Cell Biol.* **218**, 3472-3488. doi:10.1083/jcb.201902067
Petrie, R. J., Koo, H. and Yamada, K. M. (2014). Generation of compartmentalized pressure by a nuclear piston governs cell motility in a 3D matrix. *Science* **345**, 1062-1065. doi:10.1126/science.1256965
Pollard, T. D. and Borisy, G. G. (2003). Cellular motility driven by assembly and disassembly of actin filaments. *Cell* **112**, 453-465. doi:10.1016/S0092-8674(03)00120-X
Pozrikidis, C. (2011). *Theoretical and computational fluid dynamics*. Oxford University Press.
Prentice-Mott, H. V., Chang, C.-H., Mahadevan, L., Mitchison, T. J., Irimia, D. and Shah, J. V. (2013). Biased migration of confined neutrophil-like cells in asymmetric hydraulic environments. *Proc. Natl. Acad. Sci. USA* **110**, 21006-21011. doi:10.1073/pnas.1317441110
Putney, L. K., Denker, S. P. and Barber, D. L. (2002). The changing face of the Na^+/H^+ exchanger, NHE1: structure, regulation, and cellular actions. *Annu. Rev. Pharmacol. Toxicol.* **42**, 527-552. doi:10.1146/annurev.pharmtox.42.092001.143801
Ranade, S. S., Syeda, R. and Patapoutian, A. (2015). Mechanically Activated Ion Channels. *Neuron* **87**, 1162-1179. doi:10.1016/j.neuron.2015.08.032
Reichl, L. E. (2016). *A modern course in statistical mechanics*. Wiley-VCH.
Reuss, L. (2012). *Water transport across cell membranes*. Chichester: John Wiley and Sons Ltd.
Rowlinson, J. S. and Widom, B. (1982). *Molecular theory of capillarity*. Dover Publications.
Rozov, A., Khusainov, I., El Omari, K., Duman, R., Mykhaylyk, V., Yusupov, M., Westhof, E., Wagner, A. and Yusupova, G. (2019). Importance of potassium ions for ribosome structure and function revealed by long-wavelength X-ray diffraction. *Nat. Commun.* **10**, 2519. doi:10.1038/s41467-019-10409-4
Russell, J. M. (2000). Sodium-potassium-chloride cotransport. *Physiol. Rev.* **80**, 211-276. doi:10.1152/physrev.2000.80.1.211
Sao, K., Jones, T. M., Doyle, A. D., Maity, D., Schevzov, G., Chen, Y., Gunning, P. W. and Petrie, R. J. (2019). Myosin II governs intracellular pressure and traction by distinct tropomyosin-dependent mechanisms. *Mol. Biol. Cell* **30**, 1170-1181. doi:10.1091/mbc.E18-06-0355
Schmoller, K. M., Turner, J. J., Kõivomägi, M. and Skotheim, J. M. (2015). Dilution of the cell cycle inhibitor Whi5 controls budding-yeast cell size. *Nature* **526**, 268-272. doi:10.1038/nature14908
Schneider, S. W., Yano, Y., Sumpio, B. E., Jena, B. P., Geibel, J. P., Gekle, M. and Oberleithner, H. (1997). Rapid aldosterone-induced cell volume increase of endothelial cells measured by the atomic force microscope. *Cell Biol. Int.* **21**, 759-768. doi:10.1006/cbir.1997.0220
Shi, Z., Graber, Z. T., Baumgart, T., Stone, H. A. and Cohen, A. E. (2018). Cell Membranes Resist Flow. *Cell* **175**, 1769-1779.e13. doi:10.1016/j.cell.2018.09.054
Shieh, C. C., Coghlan, M., Sullivan, J. P. and Gopalakrishnan, M. (2000). Potassium channels: molecular defects, diseases, and therapeutic opportunities. *Pharmacol. Rev.* **52**, 557-594.
Shoji, K. and Kawano, R. (2019). Osmotic-engine-driven liposomes in microfluidic channels. *Lab. Chip* **19**, 3472-3480. doi:10.1039/C9LC00788A
Srivastava, N., Traynor, D., Piel, M., Kabla, A. J. and Kay, R. R. (2020). Pressure sensing through Piezo channels controls whether cells migrate with blebs or pseudopods. *Proc. Natl. Acad. Sci. USA* **117**, 2506-2512. doi:10.1073/pnas.1905730117
Stewart, M. P., Helenius, J., Toyoda, Y., Ramanathan, S. P., Muller, D. J. and Hyman, A. A. (2011). Hydrostatic pressure and the actomyosin cortex drive mitotic cell rounding. *Nature* **469**, 226-230. doi:10.1038/nature09642
Stover, J. and Nagatomi, J. (2007). Cyclic pressure stimulates DNA synthesis through the PI3K/Akt signaling pathway in rat bladder smooth muscle cells. *Ann. Biomed. Eng.* **35**, 1585-1594. doi:10.1007/s10439-007-9331-9
Stroka, K. M., Gu, Z., Sun, S. X. and Konstantopoulos, K. (2014a). Bioengineering paradigms for cell migration in confined microenvironments. *Curr. Opin. Cell Biol.* **30**, 41-50. doi:10.1016/j.ceb.2014.06.001
Stroka, K. M., Jiang, H., Chen, S. H., Tong, Z., Wirtz, D., Sun, S. X. and Konstantopoulos, K. (2014b). Water permeation drives tumor cell migration in confined microenvironments. *Cell* **157**, 611-623. doi:10.1016/j.cell.2014.02.052
Style, R. W., Boltyanskiy, R., German, G. K., Hyland, C., Macminn, C. W., Mertz, A. F., Wilen, L. A., Xu, Y. and Dufresne, E. R. (2014). Traction force microscopy in physics and biology. *Soft Mat.* **10**, 4047-4055. doi:10.1039/c4sm00264d
Sun, S. X. and Jiang, H. (2011). Physics of bacterial morphogenesis. *Microbiol. Mol. Biol. Rev.* **75**, 543-565. doi:10.1128/MMBR.00006-11
Sun, S. X., Walcott, S. and Wolgemuth, C. W. (2010). Cytoskeletal cross-linking and bundling in motor-independent contraction. *Curr. Biol.* **20**, R649-R654. doi:10.1016/j.cub.2010.07.004
Taloni, A., Kardash, E., Salman, O. U., Truskinovsky, L., Zapperi, S. and La Porta, C. A. (2015). Volume Changes During Active Shape Fluctuations in Cells. *Phys. Rev. Lett.* **114**, 208101. doi:10.1103/PhysRevLett.114.208101
Tao, J. and Sun, S. X. (2015). Active Biochemical Regulation of Cell Volume and a Simple Model of Cell Tension Response. *Biophys. J.* **109**, 1541-1550. doi:10.1016/j.bpj.2015.08.025
Tao, J., Li, Y., Vig, D. K. and Sun, S. X. (2017). Cell mechanics: a dialogue. *Rep. Prog. Phys.* **80**, 036601. doi:10.1088/1361-6633/aa5282
Tosteson, D. C. and Hoffman, J. F. (1960). Regulation of cell volume by active cation transport in high and low potassium sheep red cells. *J. Gen. Physiol.* **44**, 169-194. doi:10.1085/jgp.44.1.169
Traynor, D. and Kay, R. R. (2007). Possible roles of the endocytic cycle in cell motility. *J. Cell Sci.* **120**, 2318-2327. doi:10.1242/jcs.007732
Tsien, R. Y. (1989). Fluorescence ratio imaging of dynamic intracellular signals. *Acta Physiol Scand Suppl* **582**, 6.
Tumaneng, K., Russell, R. C. and Guan, K. L. (2012). Organ size control by Hippo and TOR pathways. *Curr. Biol.* **22**, R368-R379. doi:10.1016/j.cub.2012.03.003
Varki, A. (2017). Biological roles of glycans. *Glycobiology* **27**, 3-49. doi:10.1093/glycob/cww086
Venkatachalam, K. and Montell, C. (2007). TRP channels. *Annu. Rev. Biochem.* **76**, 387-417. doi:10.1146/annurev.biochem.75.103004.142819

Walcott, S. and Sun, S. X. (2010). Active force generation in cross-linked filament bundles without motor proteins. *Phys. Rev. E Stat. Nonlin. Soft Matter Phys.* **82**, 050901. doi:10.1103/PhysRevE.82.050901
Wang, J. H. C. and Li, B. (2009). Application of cell traction force microscopy for cell biology research. *Methods Mol. Biol.* **586**, 301-313. doi:10.1007/978-1-60761-376-3_17
Wei, C., Wang, X., Chen, M., Ouyang, K., Song, L. S. and Cheng, H. (2009). Calcium flickers steer cell migration. *Nature* **457**, 901-905. doi:10.1038/nature07577
Weinstein, A. M. (1992). Analysis of volume regulation in an epithelial cell model. *Bull. Math. Biol.* **54**, 537-561. doi:10.1016/S0092-8240(05)80076-X
Weinstein, A. M. (2013). Sodium and chloride transport: Proximal nephron. *In The Kidney: Physiology and pathophysiology*, pp. 1081-1141. 5th edn.: Lippinscott Williams & Wilkins.
Yang, M. and Brackenbury, W. J. (2013). Membrane potential and cancer progression. *Front Physiol* **4**, 185. doi:10.3389/fphys.2013.00185
Yang, J., Duan, X., Fraser, A. K., Choudhury, M. I., Ewald, A. J., Li, R. and Sun, S. X. (2019). Microscale pressure measurements based on an immiscible fluid/fluid interface. *Sci. Rep.* **9**, 20044. doi:10.1038/s41598-019-56573-x
Yao, K., Rochman, N. and Sun, S. X. (2020). CTRL – a label-free artificial intelligence method for dynamic measurement of single-cell volume. *J. Cell Sci.* **133**, jcs245050. doi:10.1242/jcs.245050
Yellin, F., Li, Y., Sreenivasan, V. K. A., Farrell, B., Johny, M. B., Yue, D. and Sun, S. X. (2018). Electromechanics and volume dynamics in nonexcitable tissue cells. *Biophys. J.* **114**, 2231-2242. doi:10.1016/j.bpj.2018.03.033
Yu, F. X., Zhao, B. and Guan, K. L. (2015). Hippo pathway in organ size control, tissue homeostasis, and cancer. *Cell* **163**, 811-828. doi:10.1016/j.cell.2015.10.044
Zanotelli, M. R., Goldblatt, Z. E., Miller, J. P., Bordeleau, F., Li, J., Vanderburgh, J. A., Lampi, M. C., King, M. R. and Reinhart-King, C. A. (2018). Regulation of ATP utilization during metastatic cell migration by collagen architecture. *Mol. Biol. Cell* **29**, 1-9. doi:10.1091/mbc.E17-01-0041
Zeuthen, T. (1978). Intracellular gradients of ion activities in the epithelial cells of the Necturus gallbladder recorded with ion-selective microelectrodes. *J. Membr. Biol.* **39**, 185-218. doi:10.1007/BF01870331
Zhao, X. H., Laschinger, C., Arora, P., Szászi, K., Kapus, A. and Mcculloch, C. A. (2007). Force activates smooth muscle alpha-actin promoter activity through the Rho signaling pathway. *J. Cell Sci.* **120**, 1801-1809. doi:10.1242/jcs.001586
Zhao, R., Afthinos, A., Zhu, T., Mistriotis, P., Li, Y., Serra, S. A., Zhang, Y., Yankaskas, C. L., He, S., Valverde, M. A. et al. (2019). Cell Sensing and decision-making in confinement: the role of TRPM7 in a tug of war between hydraulic pressure and cross-sectional area. *Sci. Adv.* **5**, eaaw7243. doi:10.1126/sciadv.aaw7243
Zlotek-Zlotkiewicz, E., Monnier, S., Cappello, G., Le Berre, M. and Piel, M. (2015). Optical volume and mass measurements show that mammalian cells swell during mitosis. *J. Cell Biol.* **211**, 765-774. doi:10.1083/jcb.201505056