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⁺, HCO₃⁻, PO₄⁻ and Ca²⁺ (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⁺ (Mulkidjian 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 (Gadby, 2009). It is one of many active ion exchangers that helps to maintain the ionic composition of the cytoplasm.

The importance of water and hydraulic pressure in cell dynamics
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ΔP = 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):

\[
\Delta P = \frac{2\tau}{R} \approx \frac{2\pi h}{R},
\]

where \( R \) is the radius of mean surface curvature, and \( \pi \approx h \) is the thickness of the surface. \( ah \) 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 uniformly distributed, 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 Ca²⁺ 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; Beazumy 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 compositions 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 \( \Delta P \) 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:

\[
\frac{dV}{dt} = SJ_{\text{water}} = -Sa(\Delta P - \Delta \Pi),
\]

where \( S \) is 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 \( \text{cm/s} \) (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 \( \Delta P \) 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 \( \mu \)m/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 \( \mu \)m², 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.
However, a concentration of 0.5 mM is equivalent to ~300,000 solute molecules per 1 μm³ or 1 fL. Given that some ion channels and pumps can allow the passage of ~10⁶ 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 from 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; Denëke 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, $J_{cytosol}$, flow of cytoskeletal network into this element, $J_{n}$, 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):

$$v_{cell} = J_{water} + J_{n} + v_{cytosol} \theta_{cytosol} + v_{n} \theta_{n},$$

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 $J_{water} = -a(\Delta P - \Delta \Pi)$, 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 $J_{os}$, 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 cross-talk 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 ($P_\infty$ 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 $P_\infty$. 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; Prentice-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
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, 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

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**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, \(v_{\text{ext}} = (v_{\text{cell}} - J_{\text{water}})\), 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 \(\mu\) of the surrounding fluid (bottom).
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 also mechanical force generation during the passage 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 (Pbasal−Papical). 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.](image1)

![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 cytoplasm of confluent cells are effectively connected.](image2)
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 mechanosensitive 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 $\Delta p$ 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
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