Mechanical forces on cellular organelles

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HYPOTHESIS

SUBJECT COLLECTION: MECHANOTRANSDUCTION

Mechanical forces on cellular organelles

Qian Feng* and Benoît Kornmann*

ABSTRACT

The intracellular environment of eukaryotic cells is highly complex and compact. The limited volume of the cell, usually a few hundred femtoliters, is not only occupied by numerous complicated, diverse membranous and proteinaceous structures, these structures are also highly dynamic due to constant remodeling and trafficking events. Consequently, intracellular interactions are more than just opportunities to exchange molecules; they also involve components physically navigating around each other in a highly confined space. While the biochemical interactions between organelles have been intensely studied in the past decades, the mechanical properties of organelles and the physical interactions between them are only beginning to be unraveled. Indeed, recent studies show that intracellular organelles are, at times, under extreme mechanical strain both in widely used experimental systems as well as *in vivo*. In this Hypothesis, we highlight known examples of intracellular mechanical challenges in biological systems and focus on the coping mechanisms of two important organelles, the nucleus and mitochondria, for they are the best studied in this aspect. In the case of mitochondria, we propose that ER–mitochondrial contact sites at thin cell peripheries may induce mitochondrial fission by mechanically constricting mitochondrial tubules. We also briefly discuss the mechano-responsiveness of other organelles and interesting directions for future research.

KEY WORDS: Mechanobiology, Membrane, Mitochondria, Nucleus, Organelle, ER–mitochondria contact sites, Organelle fission, Actin, Dynamin-related protein 1, Mitochondrial fission factor

Introduction

The eukaryotic cell is a compact and dynamic structure. The cytoplasm hosts a large number of components, such as membrane-bound organelles, proteinaceaous organelles and cytoskeletal elements in a limited space – merely 5 picoliters (Valm et al., 2017) – and is thus an extremely crowded place. This is best seen by tomographical methods, such as cryo-electron tomography and block-face or focused-ion beam (FIB) scanning electron microscopy (SEM). Indeed, using these techniques it has been demonstrated how exactly organelles are packed into a dense intermingled meshwork in the cytoplasm of pancreatic β-cells and neurons (Marsh et al., 2001; Wu et al., 2017). Although electron microscopy-based methods are paramount for their resolution in all three dimensions, they do not allow a glimpse into the fourth dimension – time. Live-cell light microscopy can reveal an additional layer of complexity in cytoplasmic organization, where intertwined organelles are continually transported and remodeled, and constantly explore the cytoplasm. For instance, it is estimated that the endoplasmic reticulum (ER) of a typical cell occupies ∼30% of the cytoplasm, but explores 97% of its volume within 15 min (Valm et al., 2017). Such dynamic behaviors make physical encounters between organelles inescapable. These observations raise interesting questions: is there an interest in mixing organelles in the cytoplasm, rather than keeping them in separate territories? How is this dynamic organization achieved? Does it come with additional challenges?

We can speculate about the reasons organelles might mix in the cytoplasm. Proximity between organelles might allow them to exchange metabolites and information. For instance, a large network of organelle contacts exist within the cell. These membrane contact sites allow the exchange of lipids between compartments and the direct transfer of Ca^{2+} ions from one organelle to another, without disturbing the cytosol or other organelles (reviewed in Cohen et al., 2018). Therefore, organelle intermingling appears to be an important feature for the proper function of eukaryotic cells.

However, this highly complex and dynamic organization of organelles in the cytoplasm is not purely the result of random mixing. All organelles reach different spots in the cell by means of cytoskeleton-based transport, using microtubule and actin filaments as the transport railways. Transport is carried out by motor proteins, such as dynein and kinesin, which travel towards microtubule minus- and plus-ends, respectively, whereas myosins transport cargos on actin filaments. Filament subunits, the motors and a large number of motor adaptor proteins further provide regulatory platforms for cargo specificity and the directionality, distance and speed of transport (reviewed in Barlan and Gelfand, 2017; Birsa et al., 2013).

Mitochondria are probably the best-studied organelles when it comes to transport. Mitochondria associate with both microtubule motors for long-distance trafficking, and with myosin 19 for local transport and tethering on actin filaments (Quintero et al., 2009). Interestingly, mitochondrial transport is inhibited by Ca^{2+} (Saotome et al., 2008; Wang and Schwarz, 2009), which might result in the accumulation of mitochondria close to Ca^{2+} sources such as the ER. In addition, both ER and mitochondria are preferentially transported on a subset of microtubules that are acetylated, which may further promote their encounter (Friedman et al., 2010). Thus, one goal of organelle transport might indeed be to facilitate organelle contact.

In this Hypothesis, we will discuss the mechanical challenges faced by intracellular organelles, and the strategies they employ to cope with mechanical stress. The nucleus and mitochondria are discussed as prominent examples, as these organelles have been most extensively studied in mechanobiology. We call attention to the physical/mechanical interactions between organelles at contact sites, and hypothesize that biological phenomena observed at interorganellar contact sites may result, at least in part, from mechanical, instead of biochemical stimulation, such as in the case of ER-induced mitochondrial fission.

Institute of Biochemistry, ETH Zurich, 8093 Zürich, Switzerland.

*Authors for correspondence (qian.feng@bc.biol.ethz.ch; Benoit.Kornmann@bc.biol.ethz.ch)

Q.F., 0000-0002-5504-9843; B.K., 0000-0002-6030-8555

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Intracellular mechanical challenges

The highly complex and dynamic organization of the cytoplasm comes with the challenge of avoiding potential negative consequences of clashes and entanglements between organelles. Because there are limited cytoskeletal 'highways', organelles and other cargos inevitably meet and perhaps even compete at times. Motor-based transports generate forces on the membrane surface. In addition, many organelles also bind to different motors that travel in opposite directions (reviewed in Hancock, 2014), which can lead to mechanical conflict. Besides motor-mediated transport, cytoskeletal components can also directly exert forces on organelles as they undergo polymerization and de-polymerization events (reviewed in Gurel et al., 2014; Svitkina, 2018). Furthermore, organelle contact sites may stably – albeit transiently in many cases – tether two organelles to enable the exchange of structural or signaling molecules. A given organelle probably also simultaneously engages in large numbers of interorganellar contacts at different locations in the cytoplasm. Such tethering behavior introduces another dimension of mechanical strain on these dynamic structures. Thus, organelles are constantly exposed to both tensile forces and compressive forces, and must cope with the mechanical stresses that they entail. This can be exemplified by the nucleus and mitochondria.

The nucleus is not at all a ball randomly positioned more or less in the center of a cell. By contrast, nuclear positioning is precisely controlled and requires dynamic movement of this organelle during many processes, such as cell division (in eukaryotes that undergo a closed mitosis), polarity establishment, differentiation, fertilization and migration (reviewed in Gundersen and Worman, 2013). The velocity of intracellular nuclear movement varies between 0.1 and 1 µm per min, but can reach up to more than 10 µm/min in extreme cases (Gundersen and Worman, 2013). Additionally, the shape of nuclei must also adapt to the (local) cell geometry and frequently requires compression of the organelle. This is exemplified to an extreme extent during white blood cell extravasation and cancer cell metastasis, where cells deform significantly to pass through tight junctions of tissue cells and dense extracellular matrix (Fig. 1A) (Barzilai et al., 2017; Karreman et al., 2016). This is not an easy task as the nucleus is rather rigid, largely due to the presence of the nuclear lamina, a nucleoskeleton lining the inner side of the nuclear envelope and consisting mainly of lamin proteins. Thus, the nucleus must be mechanically regulated.

Mitochondria are another example of large and highly dynamic organelles. In animal cells, mitochondria form extended networks of tubules, which can travel long distances across the cytoplasm at high speeds (up to 20 to 60 µm/min in neurons; reviewed in Sheng, 2014). In addition, the mitochondrial network constantly changes shape due to fusion and fission events (reviewed in van der Bliek et al., 2013). In axons and dendrites, mitochondria occupy a very limited space and share it with large bundles of microtubules. A recent cryotomography study shows that, in primary neurons, mitochondria are under extreme confinement; while their resting diameter is in the range of 300 to 500 nm, mitochondria are oftentimes threaded through thin sections of axons, which reduces their diameter to ∼20 nm (Fig. 1B; Fischer et al., 2018). In some cases, the OMM uncouples from the inner mitochondrial membrane (IMM) and is pulled inside a narrow axonal section, while the bulk of the organelle remains in the wider varicosity (Fig. 1B; Fischer et al., 2018).

Another special environment for mitochondria is also highly relevant for this discussion, namely the periphery of adherent cultured cells. Adherent cultured cells are the model of choice for mammalian cell biology, and they are particularly useful for the imaging of subcellular structures by light microscopy. The common features of these cells is their shape – comparable to a sunny-side-up egg, with the yolk represented by the nucleus, and large flat peripheral extensions representing the egg white. The periphery is particularly suited for light microscopy studies because most microscopes have a poorer resolution in the z dimension than in the x and y dimensions, and the flatness in these areas allows the resolution of complex structures in the z dimension. For instance, individual ER tubules cannot be adequately resolved in the vicinity of the nucleus, as, indeed, the ER here fills the entire volume. By contrast, in the cell periphery, the ER flattens into a thin bi-dimensional network and individual tubules can be resolved. Therefore, the cellular periphery has become *de facto* the place of choice to study organelle dynamics. However, what has remained underestimated for a long time, but recently gained attention, are the mechanical constraints associated with the periphery of most adherent cells, and especially of the cell types favored by microscopists. Super-resolution microscopy estimates indicate that the actin cortices that line up the 'ventral' and 'dorsal' plasma membranes are spaced such that the available intracellular space in these areas is merely 100 nm wide (Xu et al., 2012). While this might not represent a challenge for the ER tubules, which on average have a diameter of 70 nm, how can mitochondria, which are typically 300 to 500 nm in diameter even fit there? The answer again comes from super-resolution microscopy. 3D-STORM and 4Pi single-molecule switching nanoscopy shows that mitochondria in the periphery of the cell do not have a tubular cross-section, but are flattened like pancakes (Huang et al., 2008, 2016). However, they do not flatten entirely, and can bulge slightly from the surface of the cell. This phenomenon has been recently documented using a novel electron microscopy approach that utilizes a graphene monolayer to coat the surface of the cells (Fig. 1C; Wojcik et al., 2015). Furthermore, mitochondria bulging can be observed in live cells by using atomic force microscopy (AFM). By scanning the surface of a cell using a sharp AFM tip, it appears that mitochondria bulge out by as much as 50 nm from the surface of the periphery of the cell (Yoshida et al., 2015; Fig. 1D), illustrating that they are indeed in a very confined environment, sandwiched between the 'ventral' and 'dorsal' actin cortices of the cell.

These examples raise several interesting questions. What are the forces being applied to intracellular organelles by cytoskeletal movement, by confinement in thin cellular processes or by encounter with other dynamic structures? What are the consequences for the organelle function? Are there any control mechanisms to ensure that excessive forces do not damage these organelles? In the following sections, we will highlight a few known examples of mechano-responses of intracellular organelles, particularly the nucleus and mitochondria.

How do organelles deal with mechanical challenges?

The nucleus

Nuclear positioning and shape are dynamic and are finely regulated. Much of the regulation is achieved by close interactions with cytoskeletal components that involve both the microtubule and actin networks (Gundersen and Worman, 2013). In this context, specialized protein complexes – e.g. the linker of nucleoskeleton and cytoskeleton (LINC) complexes – are particularly important (Fig. 2A, left). LINC complexes comprise both the inner nuclear membrane SUN proteins, which bind to the nuclear lamina and chromatin, and the outer nuclear membrane nesprin proteins, which link the SUN proteins to the cytoplasmic cytoskeleton (reviewed in

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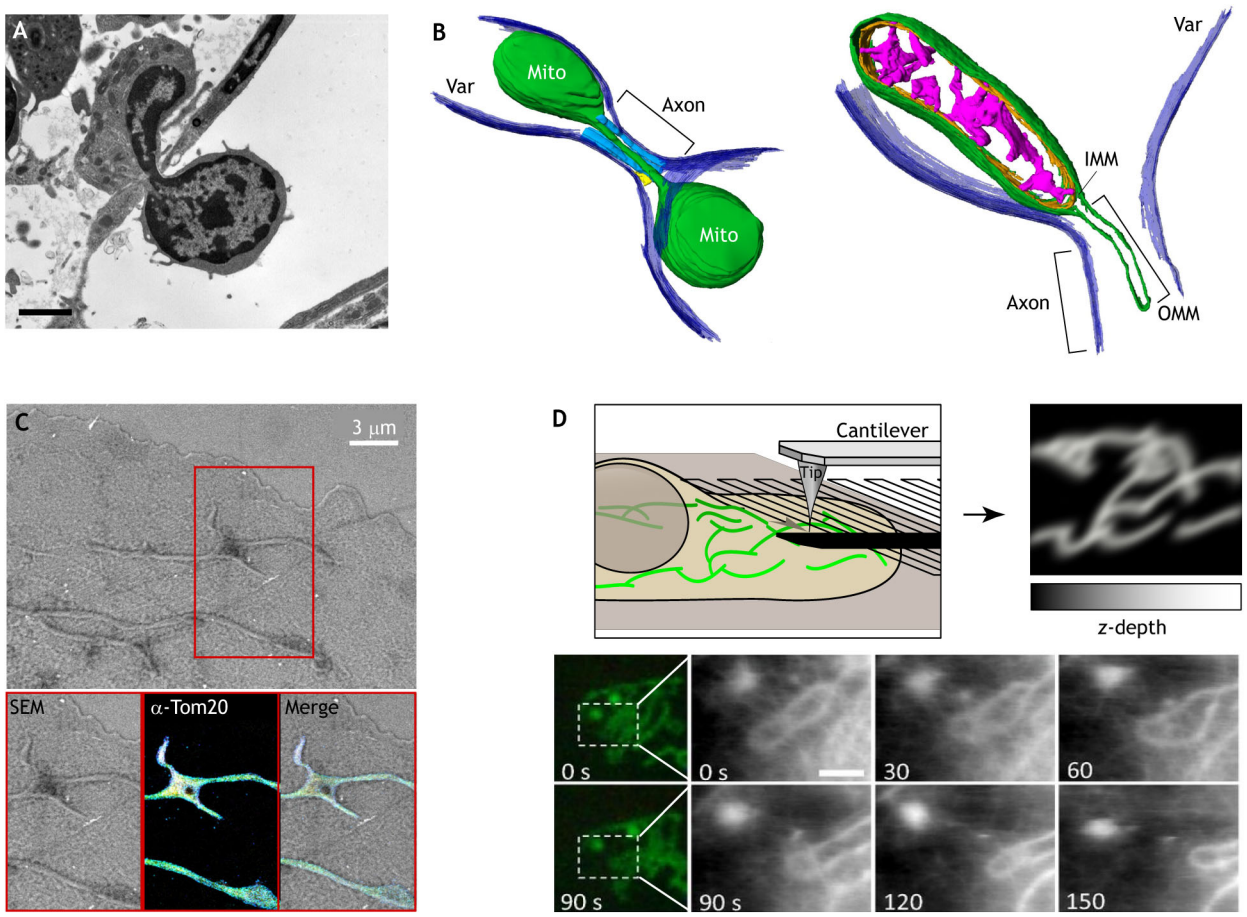

Fig. 1. Cellular confinement affects cell and organelle shape and organization. (A) Severe blood cell deformation. Transmission electron microscope image of a thin section cut through an area of bone marrow near the cartilage–bone interface in a mouse kneecap. Image shows a small opening in the thin endothelium of the vascular sinus wall, where a blood cell is crossing the vascular sinus wall and into the sinus lumen. It can be easily appreciated how severely deformed the blood cell is at the opening of the vascular sinus wall. Image provided by Louisa Howard and Roy Fava, Dartmouth College (http://remf.dartmouth.edu/images/mammalianKneecapTEM/source/3.html). Scale bar: 2 µm. (B) Two examples of 3D segmented reconstructions of cryo-electron tomographs of thin axon segments and varicosities (Var) in cultured neurons. Plasma membrane (dark blue), microtubules (light blue), mitochondrial outer membrane (dark green), mitochondrial inner boundary membrane (orange), cristae (pink), endoplasmic reticulum (yellow). Left, a mitochondrion extending from one varicosity, through the thin axonal process, to another varicosity is shown. The portion of the mitochondrion inside the axonal process is severely constricted, making the whole mitochondrion a dumbbell shape. The diameter of the mitochondrion has been measured as 200 to 300 nm in the viscosities and 19 nm in the axon segment (Fischer et al., 2018). Right, another mitochondrion that is mostly located inside a varicosity, but its outer membrane is pulled away from the inner membrane, into the thin axon segment. These tomographs show the extent of natural mitochondrial deformation in biological specimens. These images have been adapted from Fischer et al. (2018), where they was published under a CC-BY license (https://creativecommons.org/licenses/by/4.0/). (C) Mitochondria bulging out of the surface of the cell as imaged by graphene scanning electron microscopy (SEM). A wet, fixed COS-7 cell was imaged by correlated graphene-SEM and 3D-STORM upon immunolabeling for TOM20. Top, SEM image of the periphery of the cell, showing the surface topology of the sample. Bottom left, inset of the SEM image from the area marked by the red box. Bottom middle, 3D-STORM image from the same area upon immunostaining for TOM20, a mitochondrial outer membrane marker. Bottom right, merged image from the two insets on the left. Mitochondria in these areas, as identified by TOM20 staining, are clearly visible as bulges on the surface of the cell by SEM. These images have been adapted from Wojcik et al. (2015) where they was published under a CC-BY license (https://creativecommons.org/licenses/by/4.0/). (D) Mitochondria bulging out of the cytoplasm as imaged by high-speed atomic force microscopy (HS-AFM). Simultaneous fluorescent microscopy and tip-scan HS-AFM images of a live COS7 cell. The AFM tip scans the surface of the cell and records the height of the specimen at each *x-y* coordinate (top left). This information is then plotted as a grayscale on top of an *x-y* coordinate map with white being the highest and black the lowest (top right). Bottom, sequential images of mitochondria labeled with CellLight mitochondria–GFP (green) and AFM images from the indicated area (dotted box). Only fluorescence images from time 0 and 90 s are shown. Scale bar: 1 µm. Mitochondria in these areas, as identified by the CellLight signal, clearly protrude out of the cytoplasmic surface of the cell, indicating that they are confined under the cell surface. The bottom panels are reproduced from Yoshida et al. (2015) with permission from John Wiley and Sons.

Meier, 2016). Some LINC complexes are crucial for keeping the nucleus in place, while others are involved in moving it in the course of dynamic cellular processes. The outer hair cells (OHCs) in the cochlea are a great example where nuclear positioning is crucial for cellular function and survival. OHCs – the sound amplifiers in the inner ear – contract and relax at sonic frequencies (Fig. 2A, upper right; reviewed in Ashmore, 2008), and thus are subjected to a high load of vibration-induced mechanical stress. A specialized LINC complex that is formed by nesprin 4 (Nesp4, also known as SYNE4) and SUN1 holds the nucleus in place at the base of the cell under normal conditions. Deficiency in either protein causes the nucleus to detach from the cell base (Fig. 2A, top right; Horn et al., 2013), potentially destabilizing this organelle in the vibrating OHC. Mice deficient in Nesp4 or SUN1 are born with morphologically normal

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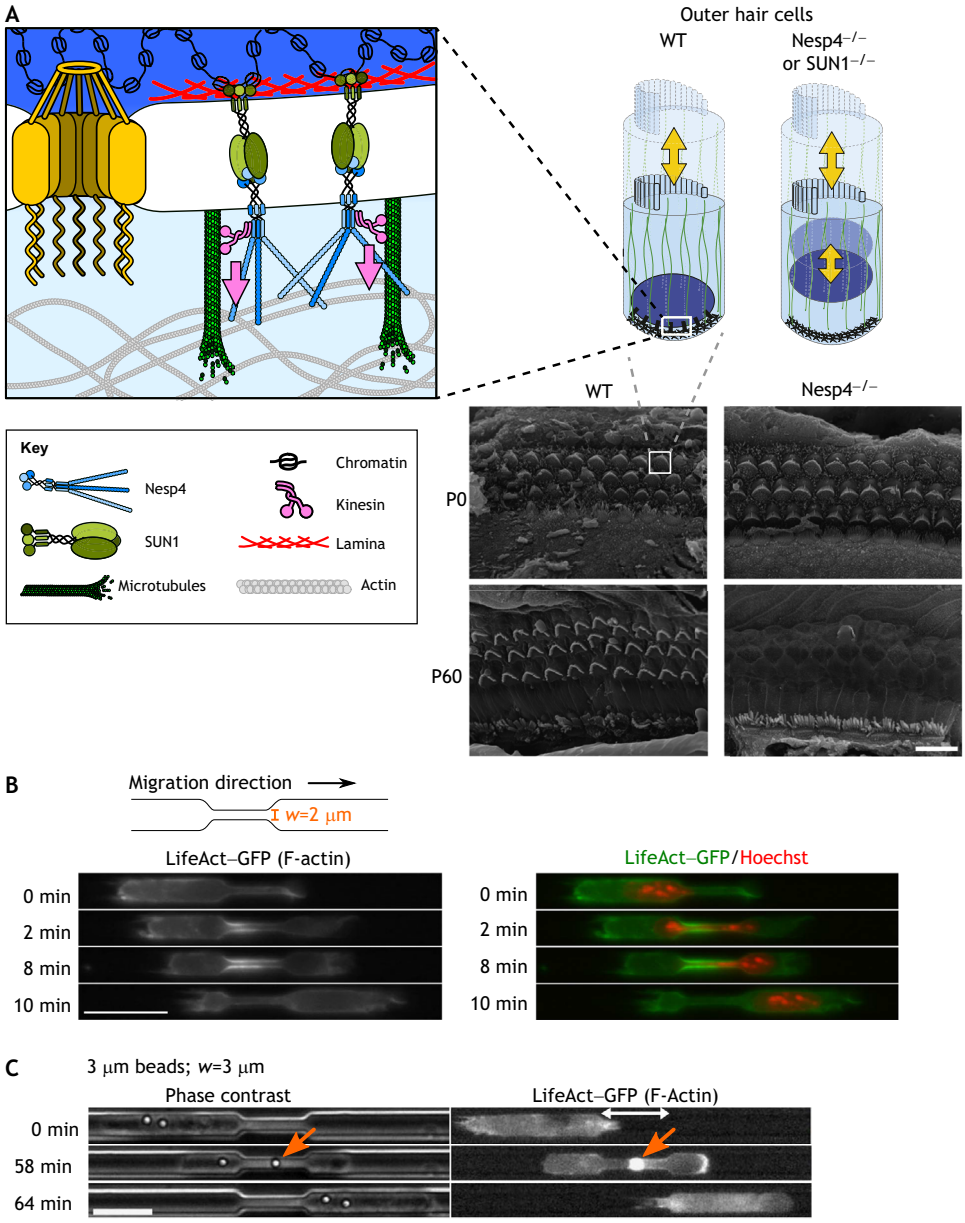

Fig. 2. Mechano-response of the nucleus. (A) Linker of nucleoskeleton and cytoskeleton (LINC) complexes hold nuclei at the bottom of outer hair cells (OHCs) and are essential for cellular survival. Left, schematic representation of the LINC complexes in OHCs. LINC complexes comprise a SUN protein at the inner nuclear membrane, which binds to nuclear lamina and chromatin, and a nesprin (Nesp) protein at the outer nuclear membrane, which links the SUN protein to cytoplasmic cytoskeleton. By forming a physical bridge between the nucleus and the cytoplasmic cytoskeleton, LINC complexes are important for nuclear positioning and movement. In OHCs of the inner ear, a LINC complex formed by SUN1 and Nesp4 (top left) keeps the nucleus at the base of the cylindrical cell (diagrams on top right). Because OHCs change cell length at sonic frequencies – in order to amplify sound – it is hypothesized that stably keeping the nucleus at the base prevents this organelle from bouncing around while the OHCs vibrate. Bottom right, scanning electron microscopy images of organs of Corti of wild-type (WT) or nesprin 4 knockout (Nesp4$^{-/-}$) mice at the indicated age (P, post-partum). Mice lacking Nesp4 gradually lose their OHCs and, consequently, hearing after birth (Horn et al., 2013). OHCs (white box) are recognizable as the ordered three rows of cells in wild-type conditions. Scale bar: 10 µm. The SEM images have been reproduced from Horn et al., (2013) with permission from the American Society for Clinical Investigation. (B) Actin polymerization around the nucleus deforms this organelle during cell migration through constrictions. Top, schematic of a microfabricated channel containing a constriction site of 2 µm wide. Bottom, time-lapse fluorescence microscopy of a dendritic cell migrating through such a microfabricated constriction. DNA and actin are visualized using Hoechst (red) and LifeAct–GFP (green), respectively. Intense actin polymerization is observed where the nucleus is the most constricted. Scale bar: 20 µm. (C) Actin assembly inside constrictions is not specific to the nucleus and can be induced by confinement of rigid particles. A dendritic cell is allowed to internalize 3 µm diameter beads and then migrates through a microfabricated channel with a constriction that is 3 µm wide (similar in to B). When the internalized bead is trapped inside the constriction, intense actin polymerization is observed around the bead (red arrows), while there is no actin polymerization around beads outside of the constriction. Thus, the actin polymerization observed results from mechanical stimulation and not biochemical properties of the nucleus. Scale bar: 20 µm. The images in B and C have been adapted from Thiam et al. (2016) where they was published under a CC-BY license (https://creativecommons.org/licenses/by/4.0/).

OHCs, which rapidly degenerate after birth (Fig. 2A, bottom right), causing gradual hearing loss (Horn et al., 2013). It can be speculated that postnatal sound exposure increases mechanical stress on the OHCs, and failure to stabilize the nuclei at the correct position in these cells leads to physical damage to this organelle and potentially other intracellular contents, eventually resulting in OHC

degeneration. Other LINC complexes, such as those composed of nesprin 2 and SUN2, are part of the so-called transmembrane actin-associated nuclear (TAN) lines that facilitate retrograde nuclear movement in polarizing fibroblasts (Luxton et al., 2010). Besides regulating nuclear position, LINC complexes also regulate nuclear shape. A dome-like actin cap composed of contractile actomyocin filaments covers the top of nuclei in fibroblasts and is thought to regulate nuclear shape by connecting to the LINC complex (Khatau et al., 2009; Kim et al., 2013).

Actin structures have also been reported to form around the nucleus in a mechano-responsive manner, through mechanisms that are mediated by both formins and the Arp2/3 complex. Formins are involved in actin polymerization and associate with the fast-growing (barbed) ends of actin filaments, whereas Arp2/3 promotes actin growth by inducing branching of actin filaments (reviewed in Pollard, 2007). Mechanical stimulation of the periphery of fibroblasts, away from the nucleus, induces formation of perinuclear actin rims (Shao et al., 2015). This response appears to be triggered by an increase of intracellular Ca^{2+} level in response to mechano-stimulation, and depends specifically on inverted formin 2 (INF2), and not on Arp2/3 (Shao et al., 2015; Wales et al., 2016). Whether actin rim formation protects nuclei from mechanical damage remains to be demonstrated.

Interestingly, another report describes an Arp2/3-dependent and formin-independent perinuclear actin response during amoeboid cell migration (Thiam et al., 2016). Immune and cancer cells are known to squeeze through extremely narrow spaces between endothelial cells during extravasation and metastasis, respectively. This behavior can be mimicked by letting cells migrate in microfabricated chambers with narrow (1–2 µm wide) openings. When cells squeeze through such chambers, the nucleus, being the bulkiest and most rigid part of the cell, has to deform significantly. This deformation is so severe that it frequently leads to a transient loss of nuclear envelope integrity, resulting in DNA damage that must be repaired after release of the mechanical pressure (Denais et al., 2016; Raab et al., 2016). The initial nuclear deformation requires significant force and is achieved by an intense polymerization of actin around the nucleus, which can be visualized using live actin probes (Fig. 2B). This leads to a temporary rupture of the lamina nucleoskeleton, allowing nuclear passage (Thiam et al., 2016). This so-called confinement-induced actin network (CiAN) is also mechano-responsive. In fact, a tight compression of the nucleus is necessary and sufficient for CiAN formation. Depletion of lamin proteins and, therefore, reduction of nuclear stiffness, abolishes CiAN formation when cells migrate through constrictions of the same size. Moreover, internalized polystyrene beads also induce CiAN formation when they become trapped in constrictions during cell migration (Fig. 2C), indicating that only mechanical rigidity and bulkiness is necessary to elicit CiAN, and not a specific property of the nucleus (Thiam et al., 2016). However, whether mechano-sensing of the CiAN also occurs in a Ca^{2+}-dependent manner, as reported for the perinuclear actin rims mentioned above (Shao et al., 2015), remains to be clarified.

Formin- and Arp2/3-mediated actin polymerization has also been reported around other organelles (see below). Whether these two distinct pathways represent different responses to different (severities of) mechanical cues or in different cell types remains to be elucidated.

Mitochondria

Mitochondria form an extensive and highly dynamic network of tubules throughout the cell. Mitochondrial dynamics is not restricted to their movement, but also includes events of fusion and fission, both of which are catalyzed by large dynamin-related GTPases. Fusion depends on the OMM mitofusin proteins (MFN1 and MFN2) and the IMM protein Opa1 (reviewed in van der Bliek et al., 2017). Fission relies on the recruitment of dynamin-related protein 1 (DRP1; also known as DNM1L) by several OMM-integral adaptor proteins, including mitochondrial fission factor (MFF) and mitochondrial dynamic proteins (Mid49 and Mid51, also known as MIEF2 and MIEF1, respectively) (van der Bliek et al., 2017). DRP1 uses the energy of GTP hydrolysis to constrict mitochondrial tubules down to a diameter of 50 to 60 nm (Francy et al., 2015), following which dynamin 2 completes the final membrane constriction, leading to fission of the tubule (Lee et al., 2016). Nevertheless, the determinants of the timing and place of fusion and fission events are only partially understood. It has been shown that ER–mitochondrial contact sites dictate future fission sites (Friedman et al., 2011). Such ER-marked fission sites also coincide with active mitochondrial DNA (mtDNA) replication sites (Lewis et al., 2016), suggesting that mitochondrial fission could be a means to equally distribute mitochondrial genomes. However, it remains a mystery how such a signal is transduced from the nucleoids in the matrix, where mtDNA replication occurs, across two membranes to the cytoplasmic side of the OMM, where the fission machineries and the ER tubules are located.

How does this motile, dynamic and network-shaped organelle deal with intracellular collisions and entanglements? Intrigued by this question, our group recently set out to investigate how mitochondria behave upon mechanical challenge (Helle et al., 2017). To artificially induce intracellular collisions, we took advantage of the motile intracellular bacterium *Shigella flexineri*. In order to spread from one host cell to another without being exposed to the extracellular host defense mechanisms, *Shigella* mobilizes itself by polymerizing host actin at its surface, forming so-called actin comet tails (Ireton, 2013). As a result, motile bacteria ‘thrust’ through the host cytoplasm, colliding into every structure on their path, including mitochondria (Fig. 3A). We found that bacterium-induced collisions cause local constriction on the mitochondrial tubule, where DRP1 oligomers subsequently assemble, eventually leading to fission at the constricted site (Helle et al., 2017). Similar observations were made when individual mitochondrial tubules were simply mechanically compressed by an atomic force microscope (Fig. 3B), indicating that force alone is sufficient to induce DRP1-mediated fission. The amount of force required is entirely physiological, as already the mere spreading of a cell across a patterned surface caused sufficient endogenous constrictive force to trigger mechanically induced mitochondrial fission (Fig. 3C). Our further investigations showed that the OMM adaptor protein MFF preferentially localizes to mechanically constricted sites, both in the presence or absence of DRP1, placing it upstream of DRP1 recruitment, and suggesting that it acts as a mechano-sensor (Helle et al., 2017).

However, it has been known for some time that not all mitochondrial constrictions become fission sites (Friedman et al., 2011; Lewis et al., 2016). This was recently explained by a new model that takes tangential tension into consideration. Tangential tension on the mitochondrial membrane likely originates from pulling forces of motor proteins. By using time-lapse super-resolution microscopy, Carlini et al. have measured mitochondrial constrictions down to 100 nm and observed that 34% of the constricted mitochondria recover, instead of undergoing fission. Here, fission frequency correlated with the local membrane-bending energy, indicating that the total bending energy resulting from both

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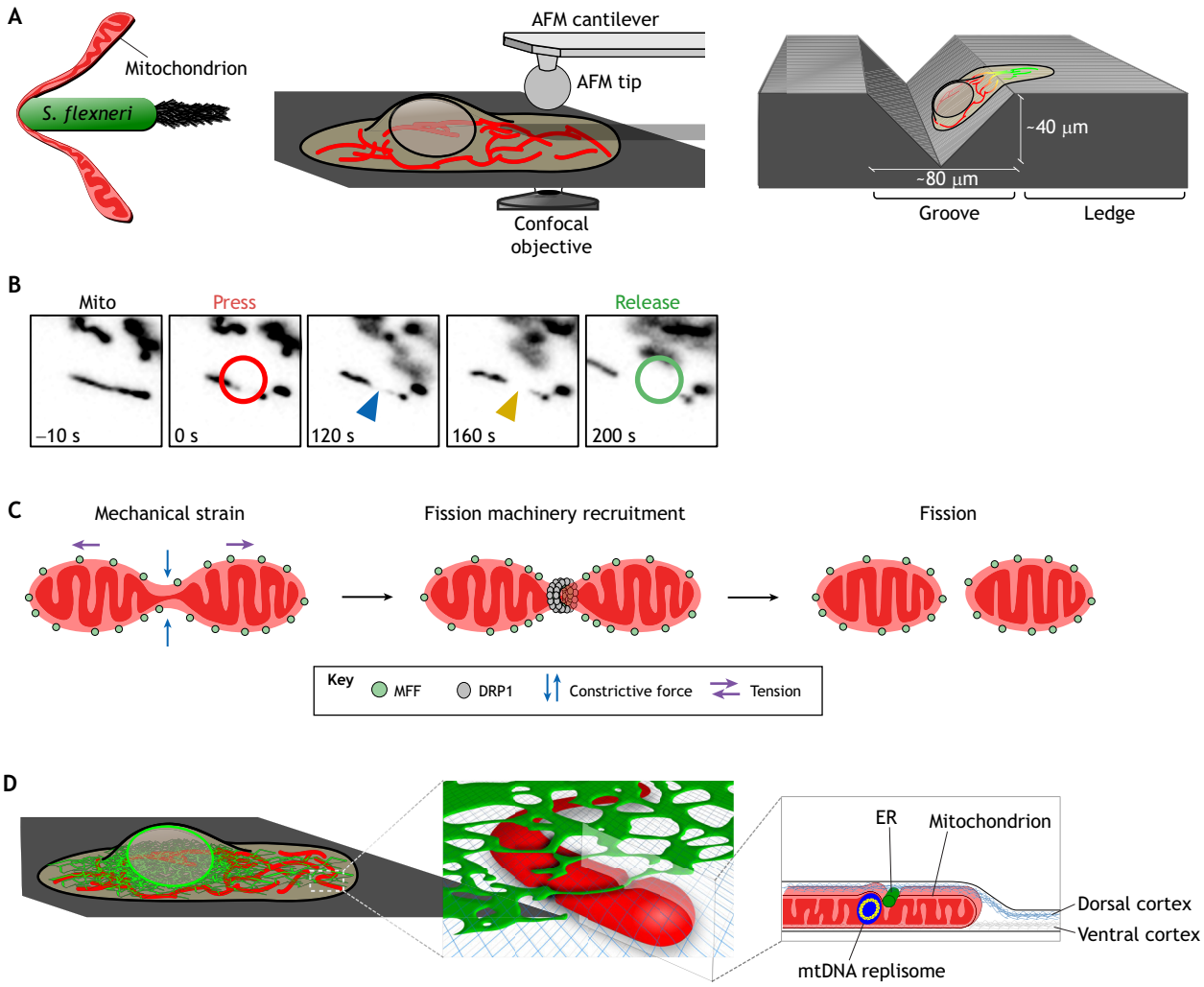

Fig. 3. Mechano-response of mitochondria. (A) Illustrated here are three different experimental systems to mechanically challenge mitochondria in live cells. Left, *Shigella flexneri* (green) is an intracellular bacteria that polymerizes host actin upon entry into the cytoplasm. The resulting so-called actin comet tail (black) pushes the bacterium forward, enabling intracellular motility. While exploring the host cytoplasm, the motile bacteria bump into organelles, including mitochondria (red), thereupon exerting mechanical stress. Middle, an atomic force microscope contains a controllable cantilever that can transmit minute forces to the sample. By using a round tip, this system can be used to apply pressure on mitochondria (red) in live cells, without penetrating the plasma membrane. Cellular responses can be observed using a conventional inverted confocal microscope. Right, cells can be grown on uneven surfaces, specifically vinyl disks, where the intrinsic cell spreading across the sharp edge causes a constriction of the cytoplasm, and thereby of the mitochondria (color) within. Color coding of the mitochondrial network indicates the *z* position, with green being high up on the flat ledge area and red being down in the groove. (B) Example of AFM-induced mitochondrial fission. Mitochondria in U2OS cells have been labeled using a fluorescent protein targeted to the matrix (Mito). At *t*=0 s, the cantilever of the AFM approaches the cell in Contact mode, with a force set at 15 nN, at the position of the red ring. The green ring marks the time and area of tip retraction. The blue arrowhead indicates a mitochondrion that is visibly thinned by the pressure, but has not yet undergone fission. The fission event is indicated by an orange arrowhead. (C) Schematic illustration of MFF-mediated force-induced mitochondrial fission. Mechanical stresses, including constrictive force (blue arrows) and tension (purple arrows) can cause local constrictions on mitochondrial tubules and increase the membrane tension at the constriction sites. MFF (green), an OMM protein, has an intrinsic preference for thinner tubules and, thus, concentrates at the constricted sections, effectively making MFF a mechano-sensor. Mechano-sensing is then translated to fission due to interactions between MFF and DRP1 (gray), the fission dynamin-related protein, which further constricts the tubule, eventually leading to membrane scission. (D) Model of ER-induced mechanical strain on mitochondria at the flattened cell periphery. The periphery of some adherent cultured cells is very flat, sometimes measuring a mere 100 nm in height (Xu et al., 2012). Mitochondrial tubules (red), which are 300–500 nm thick normally, are flattened by cortical actin networks (pale blue and gray), and even bulge out, sometimes by as much as 50 nm (Yoshida et al., 2015). At such confined places, an overlaying structure such as the ER (green) will cause a further constriction on the mitochondrial tubule. When such constrictions occur in the vicinity of a rigid mtDNA replisome (dark blue) the mitochondrial membranes sustain an even higher tension. The constrictive force and tension together may be sufficient to cause mechanically induced mitochondrial fission. This model provides an alternative explanation for the observed increase in mitochondrial fission frequency close to mtDNA replication centers and at ER-mitochondrial contact sites at the periphery of cultured cells. The images in A (middle and right) and B have been adapted from Helle et al. (2017) where they were published under a CC-BY license (https://creativecommons.org/licenses/by/4.0/).

the constrictive force and membrane tension eventually determined the fate of mitochondria (Carlini et al., 2018 preprint; Fig. 3C).

Taken together, it thus appears that mitochondria can resolve mechanical stress by undergoing fission at strained sites. Being able to sense mechanical strain and undergoing controlled division upon mechanical stress may help to prevent overstretching and physical breakage of mitochondrial tubules, which could affect the integrity of the entire organelle and result in the release of apoptotic factors and other deleterious molecules. Considering the complexity of the intracellular environment, mitochondria are likely confronted

with different types and severity of mechanical stresses. Future research will reveal additional mechano-responses of this crucial, dynamic organelle.

The implications of the mechano-sensitivity of mitochondria

The notion that mitochondria are mechano-sensitive and that both tensile and compressive mechanical forces play an important role in fission sheds a new light on this process. As mentioned above, it has been known for several years that ER–mitochondrial contact sites mark future mitochondrial fission sites (Friedman et al., 2011), a conclusion largely based on microscopic data obtained at the periphery of cultured mammalian cells, and that mitochondria at these regions are mechanically compressed as pancakes. Additional structures such as ER tubules located on top of, or below, mitochondria will inevitably cause further mechanical strain on the organelle; this might provide an alternative explanation as to why ER-mitochondria contact sites imaged in these areas tend to coincide with mitochondrial constriction sites (Fig. 3F; Friedman et al., 2011). But, how much force can be delivered by an overlapping ER tubule? It is probably only small if the ER is considered as a highly flexible membrane tube. But more likely, the true situation is more complicated. The nucleus and endosomes (see below), can be coated by actin shells that are both mechano-sensitive and -responsive. It is tempting to speculate that the same might be true for mitochondria and the ER. Indeed, it has been observed that actin filaments transiently form around mitochondria, and influence fusion and fission behaviors of this organelle (Li et al., 2015; Moore et al., 2016). Moreover, several actin regulators have been reported to directly contribute to ER-mediated mitochondrial fission, including the ER-localized formin INF2 (Korobova et al., 2013), the actin-nucleating protein Spire 1C (Manor et al., 2015), and myosin II (Korobova et al., 2014).

Thus, it is conceivable that actin filaments might constantly survey the surface of undisturbed mitochondria, and possibly also the ER though direct evidence of this is yet lacking. Upon compression, actin filaments, owing to being mechano-sensitive themselves, could self-amplify, producing increased actin polymerization and thereby, membrane constriction. MFF, being able to sense constricted sites on mitochondria (see above) would then enrich at such mechanically compressed sites and subsequently recruit DRP1 to initiate fission.

Curiously, INF2-mediated actin filament formation around mitochondria is also Ca^{2+}-activated (Chakrabarti et al., 2018), similar to what has been observed for the perinuclear actin rims (Shao et al., 2015). Whether Ca^{2+} plays a general messenger role in communicating mechanical stresses between organelles requires comprehensive future investigation.

This mechano-fission model could also explain why, at the periphery of cultured mammalian cells, ER-marked mitochondrial fission sites coincide with active mtDNA replication sites (Lewis et al., 2016). As stated above, it is unclear how mtDNA replication sites in the matrix of mitochondria could signal to the OMM to trigger fission. In light of the considerations above, it is tempting to speculate that the signaling cascade might not be biochemical, but mechanical. The mtDNA replisome is composed of a number of large protein complexes – the DNA polymerase POLG1, the accessory POLG2, the Twinkle helicase, the single-strand binding protein mtSSB, and the transcription and DNA maintenance factor TFAM (reviewed in Ricchetti, 2018) – and may constitute relatively rigid areas (‘islands’) within mitochondrial tubules. When already flattened mitochondria are overlaid with an ER tubule, the presence of rigid mtDNA replication islands might result in a higher local membrane-bending energy, thus making fission in their vicinity more probable (Fig. 3D).

Taken together, mechano-induced mitochondrial fission might provide a mechanistic explanation for important yet unexplained phenomena in mitochondrial biology, for instance, the correlation between mitochondrial fission and the presence of ER tubules, and the increased fission frequency found close to replicating mtDNA nucleoids. Verifying these hypotheses will require the simultaneous imaging of replicating nucleoids and AFM-probing of the mechanical properties of mitochondria in areas where they bulge out of the plasma membrane.

Concluding remarks

Mechanobiology is a booming field (reviewed in Moeendarbary and Harris, 2014; Petridou et al., 2017). Traction force can be sensed by integrin proteins that connect the substrate to the cytoskeleton, and/or by cadherins that connect the cytoskeletons of neighboring cells. How the cytoskeleton, at the same time, can be mechano-transducing and mechano-receptive is an intense matter of study. Force sensing by the plasma membrane is performed by mechano-sensitive ion channels, as well as curvature-sensing proteins (reviewed in Beedle et al., 2015; Diz-Muñoz et al., 2013). For instance, caveolae are regular folds in the plasma membrane. These folds collapse when tension is applied to the plasma membrane, allowing an immediate supply of membrane surface to ease the tension. This unfolding can also lead to signaling. For instance, in yeast, unfolding of eisosomes, which are plasma membrane folds functionally related to caveolae, leads to the desequestering of the regulators Slm1 and Slm2. Slm1 and Slm2, in turn, stimulate lipid synthesis in a TORC2-dependent manner to compensate for the missing membrane surface (Berchtold et al., 2012). Overall, while force sensing by the cytoskeleton and the plasma membrane is well studied, much less is known about the mechanical properties of internal organelles.

Here, we have mainly discussed the effects of mechanical forces on the nucleus and mitochondria. However, these are unlikely to be the only mechano-sensitive organelles. As mentioned above, CiAN formation also occurs around internalized polystyrene beads when they are under sufficient confinement during cell squeezing (Thiam et al., 2016). These internalized beads presumably reside in endosomes or other endocytic compartments, suggesting that mechano-sensitive CiAN formation is not organelle specific. Interestingly, ER contacts have also been reported to determine endosomal constriction and fission sites, as also based on a microscopy study performed at the thin periphery of cultured cells (Rowland et al., 2014). It remains to be investigated whether ER-mediated endosomal fission occurs through CiAN or other actin polymerization pathways. Furthermore, it also needs to be investigated whether other membranous organelles are also mechano-sensitive. For instance, peroxisomes also utilize MFF, the mechano-sensor on mitochondria, to carry out fission mediated by DRP1 recruitment (Gandre-Babbe and van der Bliek, 2008). Because peroxisomes are much smaller than mitochondria, only up to 100 nm in diameter (Lodhi and Semenkovich, 2014), the dynamics of MFF recruitment and thus its mechano-sensitivity, may be quite different for this organelle. Lysosomes have also been reported to tubulate under starvation, antigen challenge or constitutively in various cell types (Li et al., 2016; Mrakovic et al., 2012; Yu et al., 2010). While the tubular morphology of this organelle has been known for decades (Swanson et al., 1987), we still know little about the biomechanical implications of lysosomal tubulation. Interestingly, an alternatively spliced DRP1 isoform,

named DRP1ABCD, has been found on lysosomes, late endosomes and the plasma membrane, in addition to mitochondria (Itoh et al., 2018), certainly opening up new interesting areas of research.

The mechano-response of organelles might not just be limited to fission events. For instance, mitochondria have been shown to be captured in actin cages prior to mitophagy (Kruppa and Buss, 2018), calling out the question of whether this important process is also, to some extent, influenced by mechanical forces. Furthermore, organelles that are not known to undergo fission may also respond to mechanical strain. As we have outlined above, the nucleus is mechanically deformed to allow cells to migrate through extremely narrow openings (Denais et al., 2016; Raab et al., 2016; Thiam et al., 2016). Nonetheless, the mechano-responsiveness of many other membranous organelles is, to date, largely unknown. For instance, the size and surface of Golgi cisternae exposes them to clashes and collisions with other cellular structures. Indeed, the pushing of internalized latex beads against the Golgi in retinal pigment epithelial (RPE-1) cells causes a defect in trafficking, presumably by inhibiting the abscission of vesicles (Guet et al., 2014). This raises the interesting question of how the Golgi deals with forces applied by cellular structures. With regard to the ER, no fission mechanism for this organelle has been described. Does it respond to mechanical strain itself or does it always resolve any clashes and entanglement by inducing fission of the organelle it is in contact with?

In conclusion, the intracellular content is constantly subjected to mechanical stresses. Organelles need to cope with the crowdedness of the intracellular space and the dynamic trafficking activities therein. This is aggravated during development (e.g. neuron differentiation and neural crest cell migration), white blood cell extravasation and tissue invasion, as well as tumor metastasis. It will be crucial to understand the mechano-sensing and -responding strategies of individual organelles. Together, this will help us to generate a mechano-interaction map of cellular content and to better understand this additional dimension of organelle communication.

Competing interests
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