“This is a post-peer-review, pre-copyedit version of an article published in Nature Materials. The final authenticated version is available online at: <http://dx.doi.org/10.1038/s41563-020-0685-9>”.

Tracking intracellular forces and changes to mechanical properties in mouse one-cell embryo development

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Key words: Mechanobiology, bioengineering, nanomaterials, biomaterials, silicon chip, intracellular, mammalian development, cell division.

Cells comprise mechanically active matter that governs their functionality, but intracellular mechanics are difficult to study directly and are poorly understood. However, novel injected nanodevices open new opportunities to analyze intracellular mechanobiology. Here, we identify a program of forces and changes to the cytoplasmic mechanical properties required for mouse embryo development from fertilization to the first cell division. Injected, fully internalized nanodevices responded to sperm decondensation and recondensation, and subsequent device behavior suggested a model for pronuclear convergence based on a gradient of effective cytoplasmic stiffness. The nanodevices reported reduced cytoplasmic mechanical activity during chromosome alignment and indicated that cytoplasmic stiffening occurred during embryo elongation, followed by rapid cytoplasmic softening during cytokinesis (cell division). Forces greater than those inside muscle cells were detected within embryos. These results suggest that intracellular forces are part of a concerted program that is necessary for development at the origin of a new embryonic life.

Intracellular mechanics is a key determinant of cell biology. Each cell is mechanically stabilized by a filamentous cytoskeleton that controls relative stiffness¹. Active mechanical behavior generates intracellular pulling and pushing forces and drives stochastic force fluctuation to enable cytoplasmic remodeling². Such dynamic mechanical intracellular behavior provides a tier of regulation that may be as critical to developmental processes as regulation by gene expression^{3,4} and there is interplay between the two: force and stiffness changes control transcriptional programs involved in cell differentiation⁵⁻⁷.

Successful and comprehensive models of cell mechanics will require information on different hierarchical levels to relate local interactions in the cytoskeleton to the macroscopic mechanical behavior of cells^{1,8,9}. Broadly, there are two approaches to study cell mechanics: top-down (systems-level) and bottom-up (reductionist)^{1,10}. Top-down models are based on generic principles that are not obviously dependent on lower levels in the structure. These models are derived from extracellular devices that do not make direct intracellular measurements^{1,9,11-14}. By contrast, bottom-up methods derive system properties from those of their constituents (*e.g.* molecular assemblies including the cytoskeleton)^{1,8,9,11,15}.

Bottom-up approaches^{16,17,18} can be based on local intracellular measurements but the descriptions of constituent functions cannot necessarily be extrapolated to higher-order structures (*e.g.* the cell) due to their complex heterogeneity¹. Thus, improved models of cell mechanical behavior will be required that meet the considerable challenges of devising top-down direct intracellular models. Tools to complete the internalized top-down picture were not available until recent advances in silicon-based nanodevices that can be reproducibly manufactured, are versatile, and have the potential to be placed precisely and completely inside cells^{19,20}.

In the context of embryogenesis, externally-induced stiffness in mouse embryonic stem cells influences the expression of pluripotency factors such as Oct4 to drive differentiation^{21,22}. In addition, endogenous mechanical transitions play a critical role in preimplantation development of mouse embryos after several days²³, but almost nothing is known about whether similar processes are important for the gross changes that accompany development immediately after fertilization.

We accordingly sought a top-down approach to study the cytoplasmic mechanics of mammalian one-cell embryos (formed by fertilization and continuing until the first mitotic cell division), which are relatively large: ~170 pl compared to ~1 pl for other mammalian cells^{24,25}. This large cellular volume has relevant biological consequences. The distances involved pose considerable challenges for intracellular transport; cytoplasmic cargoes travel tens of microns to mediate key events including chromatin remodeling, pronuclear convergence and cell division²⁶. This suggests that cell mechanics and, in particular, intracellular forces and changes to the mechanical properties of the cytoplasm play a crucial role, yet no mechanical model accounts for them. We now detail direct mechanical readouts obtained following nanodevice injection and integrate them with molecular and gross morphological analyses. Results directly show an active program of forces and mechanical property changes that drive early embryo development (Supplementary Fig. 1).

Mass production of intracellular force-detecting nanodevices

Measuring internal (intracellular) embryo mechanics in this work required the scalable, high-fidelity production of force-change-sensitive chips that can be entirely internalized within mouse metaphase II (mII; mature fertilizable) oocytes. Such devices needed to be large enough to average out random (local) molecular perturbations so that their intracellular behavior would reflect force fluctuations and changes in cytoplasmic stiffness. For a top-

down model, relatively large 2D devices are required (22 μm , is similar to the diameter of the pronucleus, pn). This precludes the use of spherical 3D tools of similar diameter, including some types of bead, or oil droplets²⁷, whose large volumes could perturb cytoplasmic dynamics. Device thickness also had to be on the nanometer scale, akin to cytoskeletal structures (Fig. 1a), giving them an extraordinarily small volume and a high mechanical sensitivity.

To achieve these device dimensions, we employed silicon chip technology that should be readily achievable by MEMS laboratories. This technology permits a high degree of control and reproducibility over geometrical dimensions (Supplementary Fig. 2), allowing us to mass-produce complex 3D 'H-comb' nanodevices comprising eight cantilever prongs, with a total width of 10.5 μm (Fig. 1b). Silicon oxide served as a sacrificial layer onto which was deposited a polysilicon structural layer (Fig. 1c, Supplementary Fig. 3 and Supplementary Methods). Photolithography combined with polysilicon etching delineated the device shape, followed by etching to remove the sacrificial layer. We produced $>1.5 \times 10^7$ device copies per 100 mm-diameter silicon wafer (Fig. 1d). A large device width solved the uncertainty of the maximum displacement, δ , determined by angular orientation (Supplementary Fig. 4 and 5).

The cell interior has reduced inertial forces²⁸, so although it is impracticable to determine the exact load condition applied to free-floating intracellular nanodevices, as a first approximation we modeled bending based on two representative simulations: a uniform pressure, P applied to the device surface, or a force, F applied at the center of the device, such that forces acting further from the center that produced a given degree of bending would be larger (Fig. 1e and Supplementary Fig. 6, Supplementary Methods). Although the device ends are free to rotate and translate in the axes of the beam, constraints in the bending direction (perpendicular plane) acting at the ends of the device cantilevers are required to

cause bending (perpendicular plane) at the device centre. As the nanodevices are free-floating, exhibit limited stiffness, and their dimensions are small, their mechanical sensitivity was calculated by dimensional models, which are not restricted to simple structures or simple boundary conditions and loads. These methods were based on accurate experimental
5 determination of the dimensions (Supplementary Fig. 2 and Supplementary Methods) and Young's modulus of the nanodevice polysilicon layer (Supplementary Fig. 7 and 8 and Supplementary Methods). Simulations predicted extremely low stiffness, with sensitivity to minute load states of $K_P=35 \text{ Pa} \cdot \mu\text{m}^{-1}$ and $K_F=3.39 \text{ nN} \cdot \mu\text{m}^{-1}$ (Fig. 1f) and revealed that the initial curvature of the devices due to fabrication did not affect mechanical sensitivity
10 (Supplementary Fig. 9 and Supplementary Methods).

Sensitive mechanical nanodevices within mouse one-cell embryos

There are few, if any, reports of cells harboring entirely internal exogenous artifacts of $>10 \mu\text{m}$ and it was unclear whether mII oocytes would survive injection or tolerate the presence of such large (if extremely thin) structures. We evaluated different H-comb device lengths
15 (22 and 42 μm) and thicknesses (25 to 500 nm); all were 10.5 μm wide. Microinjection precisely delivered fully-internalized nanodevices into the cytoplasm of mII oocytes by membrane puncture (Supplementary Fig. 10a,b). Nanodevice volumes were small compared to the volume of mII oocytes (with volume ratios of $\leq 3.4 \times 10^{-4}$); oocytes could survive injection with devices of all thicknesses (Supplementary Fig. 10a).

20 Co-injecting mII oocytes with $22.0 \times 10.5 \times 0.025 \mu\text{m}$ H-comb nanodevices and sperm heads permitted nanodevice delivery and fertilization in the same procedure so that force changes could be captured from the earliest moments of development in the resulting embryo (Supplementary Fig. 10c,d). One-cell embryos containing H-comb nanodevices expressed genes at control levels ($p>0.25$ for five different transcripts; $n=4$ per transcript;

Supplementary Fig. 10e), consistent with normal development. Oocytes coinjected with sperm plus nanodevices or multiple $3.0 \times 3.0 \times 0.025 \mu\text{m}$ control nanosquares produced embryos that developed at high rates ($90.0 \pm 10.0\%$) to form morphologically normal, expanded blastocysts expressing the pluripotency marker, Oct4 in the control range ($n=8$; 5 Supplementary Fig. 10f-j), indicative of healthy development and lineage specification after nanodevice injection.

We were also interested in whether nanodevices (and microspheres; see below) interacted with the cytoskeleton. We found no evidence, by F-actin or α -tubulin staining, for direct interaction between intracellular nanodevices ($n=20$) or microspheres ($n=20$) and 10 microfilaments or microtubules throughout the first embryonic cell cycle (Supplementary Fig. 11a,b), even though microtubules were readily shown to interact with control, DNA-labeled latex beads (Supplementary Fig. 11c). Because the nanodevices were injected, thereby breaking the membrane, they were not internalized by microfilaments¹⁷.

We investigated nanodevice behavior in five nominal embryo phases: sperm 15 decondensation, recondensation and pronucleus formation (SDR, corresponding to meiotic exit), pronuclear migration (PM, corresponding to G1- and S-phases), pronuclear envelope breakdown and chromosome mingling (PEB), embryo elongation just prior to division (EL, presumptively initiating in G2-phase) and the first (1- to 2-cell) mitotic division (DIV) (Fig. 1g,h). Nanodevices register intracellular mechanics of relatively large regions of cytoplasm 20 (unlike small-scale measurements of individual random perturbations²): displacement, rotation and translation, contained information about the cytoplasmic reorganization, and nanodevice deformation revealed force magnitudes (Fig. 2a-e, Supplementary Fig. 12, 13 and Movie 1). To process this information, we propose two *ad hoc* theoretical parameters (Fig. 2f): $\zeta_k = F_{\text{max}}/Rot_{\text{max}}$, related to resistance to cytoplasmic reorganization (F_{max} and

Rot_{max} were respectively maximum force and rotation detected by the nanodevice), and $\xi_{mact} = (\Delta\delta_{av} \times Rot_{av})/time$, related to transitions in time-averaged mechanical activity (where *time* was the phase duration and $\Delta\delta_{av}$ and Rot_{av} are respectively averages of measured nanodevice bending and rotation during that time) (Fig. 2g). These parameters revealed that early mouse embryos exhibited programmed transitions in intracellular resistance to cytoplasmic reorganization (ξ_k) and a range of several orders of magnitude in mechanical activity (ξ_{mact}) (Fig. 2f).

Major mechanical activity near the sperm head during paternal genome reprogramming

Within 20 min of injection into mII oocytes, sperm heads started decondensing and increased ~7-fold in length (Fig. 2h-j and Supplementary Fig. 14) before recondensing and forming a visible pronuclear envelope^{29,30}; these changes corresponded to the SDR phase, lasting 168 ± 27 min ($n=4$). Nanodevices were typically near the sperm head (9.5 ± 4.5 μm ; $n=3$; Fig. 2h) and rotated up to $44 \pm 1^\circ$, with elastic deformations of 1.5 ± 0.8 μm ($n=4$; Fig. 2c,d,i). Maximal nanodevice deformation corresponded to a pressure (P) of 56.2 ± 28.7 Pa (1 Pa = 1 pN. μm^{-2}) and force (F) of 5.3 ± 2.7 nN (Fig. 2e). These results reveal major mechanical activity (ξ_{mact}) and thus cytoplasmic reorganization near the decondensing and recondensing paternal genome during chromatin remodeling (Fig. 2f,j). Although direct force comparisons between studies are difficult because different methods are used, forces near to the sperm head were of the order of 50 times larger than those inside aortic muscle cells measured with silicon nanowires (with peaks of 116 pN)¹⁷ and three orders of magnitude greater than typical forces exerted by single cytoskeletal motors (<10 pN)³¹.

A cytoplasmic gradient of effective stiffness, GES, enhances pronuclear convergence

Following their appearance 4.0~4.5 h after fertilization, mouse pronuclei converged on the embryo center until membrane breakdown 812 ± 195 min after sperm injection ($n=7$; Fig. 2g)^{30,32}. We refer to this convergence as the pronuclear migration (PM) phase (Fig. 1g, h and Supplementary Fig. 15a). On small spatiotemporal scales, PM was directionally stochastic ($n=13$; Fig. 3a) and accompanied by random ruffling of up to $2 \mu\text{m}$ at the embryo surface ($n=13$; Fig. 3b; Supplementary Fig. 15b and Supplementary Movie 2); nanodevices also exhibited $\sim 2 \mu\text{m}$ random displacements even when close to the center (Fig. 3c). Pronuclear and nanodevice random displacements reflected random kinetic activity and were concurrent with centralizing directional displacements (Fig. 3a,d). Nanodevices deformed by $1.3 \pm 0.1 \mu\text{m}$ ($n=13$; Fig. 2d), corresponding to loads of 47.9 ± 6.1 Pa and 4.5 ± 0.5 nN ($n=13$; Fig. 2e) and rotated $47.0 \pm 6.7^\circ$ ($n=13$; Fig. 2c), evidencing a program of gradual (over several h) long-range cytoplasmic reorganization required for pronuclear convergence at the center.

Pronuclear centering has previously been attributed to an intracellular pressure gradient, predicting higher migration speeds for larger objects³³. To probe this model, we generated embryos containing microspheres that resembled pronuclei in shape and density (polyethylene microspheres of $1.00 \pm 0.01 \text{ g.ml}^{-1}$)³⁴ but with a range of sizes (9.9 - $18.8 \mu\text{m}$ in diameter). Microspheres were injected with sperm, or without sperm followed by activation to produce haploid parthenogenotes. Most pronuclei were larger than the microspheres, with average diameters of $13.1 \pm 1.7 \mu\text{m}$ (maternal; $n=8$) and $19.8 \pm 0.3 \mu\text{m}$ (paternal; $n=8$) or $18.1 \pm 2.4 \mu\text{m}$ in parthenogenotes ($n=8$). In contrast to pronuclei and nanodevices within embryos, microspheres exhibited a range of trajectories that tended to avoid the center ($7 \leq n \leq 21$; Fig. 3d and Supplementary Movie 3) and we found no evidence of a correlation between microsphere maximum velocity and size (Fig. 3e). The observations that

nanodevices near the center underwent similar random displacements to those at the cortex (Fig. 3c), and that microspheres tended to avoid the center (Fig. 3d; discussed below), argued against the involvement of an intracellular pressure gradient alone³³.

The mechanical properties of mouse one-cell embryo cytoplasm can be modelled in terms of elastic and viscous elements using a combination of springs and dashpots - approximations that have commonly been used to fit cell mechanics data³⁵. However, the role played by conditions at the embryo periphery (a stiff cortical system) to determine interior effective cytoplasmic mechanical properties, and their consequences for the movement of large organelles, have not been elucidated. The cytoplasm exhibits background random fluctuating forces that cover elastic and viscous regimes^{2,35}. Consistent with this, we found that elastic and viscous simulations (Fig. 3f) supported a model in which the embryo cytoplasm exhibits a gradient of effective stiffness (GES). In the GES model, the effective elastic constant and viscosity are increased by the relatively stiff cortical system in a manner that is also a function of the internal particle size. In agreement with this, the processive random migration of endogenous cytoplasmic particles is typically smaller the closer the particles are to the cortex (Supplementary Fig. 16 and Movie 4).

Although inherent intraembryonic heterogeneity limits the GES model, the model predicts that an increase in pronuclear size during the PM-phase would favor movement towards the embryo center, where the effective stiffness is smaller (Fig. 3g). This prediction was validated by real-time videomicroscopy revealing an increase in mouse pronuclear size during the PM-phase (Fig. 3h) and pronuclear growth during human one-cell embryo development (Supplementary Fig. 16). Pronuclear growth may accordingly contribute to centralization.

In addition, assuming that the elastic contribution is greater in the direction of the displacement, the GES model predicts that for any given position inside the embryo, the elastic constant for centralization is smaller than that for displacement to the periphery (Fig. 3i,j and Supplementary Fig. 15c-i). The primary consequence of this is that random forces induce centering of relatively large intracellular objects. To test this, we co-injected sperm and microspheres, but inhibited pronuclear formation with wheat germ agglutinin³⁶. In the absence of larger objects such as pronuclei, the GES model predicts that microspheres migrate to the embryo center by random displacements (even without increasing their size), which they did ($n=5$; Fig. 3k). Furthermore, in the absence of pronuclei, relatively large endogenous particles tend to locate away from the periphery (Supplementary Fig. 16).

The GES model also accounted for the slowing of pronuclei as they approached the embryo center, where the effective elastic constant and viscosity difference in any direction are small (Fig. 3f,j). Random displacement did not account for this behavior (Fig. 3c). In addition, GES explains the increment of mechanical loads detected by nanodevices during this phase (Fig. 2e); this could also contribute to pronuclear centering and links nanodevice behavior to the GES model.

Given that beads in the presence of typically larger pronuclei are excluded from the center (Fig. 3d) and travel with speed profiles that overlap those of pronuclei (Fig. 3l), it is possible that large objects such as pronuclei dictate cytoplasmic rearrangements on large intracellular scales. This effect could be augmented by the previously-reported^{33,37,38} existence of an F-actin cloud surrounding pronuclei and extending their effective diameter, which we corroborated by imaging of the F-actin-binding protein, Utrophin fused to mCherry (Utr-mCherry; $n=13$; Fig. 4a,b and Supplementary Movies 5 and 6).

Weak cytoplasmic mechanical activity consistent with spindle formation and chromosome alignment

The pronuclear envelope breakdown (PEB) phase (lasting 113.0 ± 30.8 min; $n=12$) (Fig. 2g) immediately followed the PM-phase and included spindle formation and chromosome alignment prior to the onset of embryo elongation. During the PEB-phase, nanodevices reported cytoplasmic mechanical load changes at the lower threshold of detection and the smallest of the entire embryo cell cycle (8.6 ± 1.7 Pa, 0.8 ± 0.2 nN) (Fig. 2e). When PEB occurred, embryo surface membrane ruffling stopped simultaneously and abruptly (Supplementary Movies 5 and 6) in agreement with the minimal mechanical activity (ζ_{mact}) reported by the nanodevices (Fig. 2f, right). This coincided with a marked increment of cortical F-actin, reflecting actin redistribution from the embryo interior to cortex (Fig. 4a-d). A reduction of cytoplasmic forces during the PEB-phase could facilitate spindle assembly and chromosome alignment.

Cytoplasmic stiffening governs embryo elongation

The embryo elongation phase, EL, lasted 14 ± 2 min ($n=12$) and was characterized by spindle separation and embryonic axial elongation of $\sim 10\%$ (~ 8 μm) (Fig. 5a and Supplementary Movie 7). After the embryo poles contacted the zona pellucida (ZP), embryo elongation continued at the same rate, ~ 1.25 $\mu\text{m} \cdot \text{min}^{-1}$ (Fig. 5a, right). Motors driving spindle elongation thus generate forces that are sufficient to maintain over-all cellular elongation at a constant rate with or without resistance from the ZP. Signature plots for rates of axial elongation and equatorial contraction were conserved in mouse haploid parthenogenotes ($n=4$) and most of the human embryos ($n=17$) produced by intracytoplasmic sperm injection (ICSI) that we examined (Fig. 5b), suggesting that the underlying mechanics are shared.

These plots incorporated changes that reflected mechanical transitions that we designated (in temporal order), EL1, EL2 and DIV (Fig. 5a, right [inset]).

Immunofluorescence images of embryos revealed changes in microtubule organization that apparently coincided with EL1, EL2 and DIV (Fig. 5c). EL1 microtubules shape the spindle and localize cortically with F-actin, augmenting cortical stiffness. At EL2 (just before cleavage furrow formation at the center of the embryo), a tubulin meshwork³⁹ appeared in the equatorial region (Fig. 5c; $n=13$). Radial microtubules then increasingly emanated from spindle poles, first towards their proximal overlying equatorial cortex and subsequently (during DIV), towards polar cortical regions ($n=9$; Fig. 5c), when elongation practically stopped.

Overall, EL-phase embryos experienced the largest global deformation, but nanodevices counter-intuitively reported the smallest rotations (Fig. 2c), indicating minimal cytoplasmic reorganization. Consistent with their decreased rotation, nanodevices detected the greatest cytoplasmic resistance to reorganization, ξ_k , at EL (Fig. 2f, left). The fact that the embryo normally elongated even though radial microtubules were absent (Fig. 5c,d) suggests that the mechanical load generated by the elongating spindle is transmitted through the bulk cytoplasm to the axial cortex. Immunofluorescence images of actin and myosin II distribution showed an increment of myosin II from EL1 to EL2 (Fig. 5c,d), in agreement with previously suggested myosin-mediated cytoplasmic stiffening. Thus, we have directly demonstrated that the cytoplasmic stiffness of mouse embryos increases during the EL-phase, accompanied by an increase in myosin II activity. This stiffening would enhance spindle force transmission to the embryo cortex during elongation. Coarse spindle centering is facilitated by spindle elongation (given the stiffer cortex), in agreement with the GES model.

Cytoplasmic softening and the largest forces occur during division

During the brief division-phase, DIV (lasting 5.5 ± 1.6 min; $n=12$), nanodevices near the cleavage plane underwent rotations of up to $137 \pm 2^\circ$ and deformations of 4.2 ± 0.3 μm (Fig. 2c,d and Fig. 5e,f), corresponding to a pressure of 233 ± 20 Pa (17.7 ± 1.5 nN) (Fig. 2e).

5 Maximal mechanical activity (ξ_{mact}) was approximately two orders of magnitude higher than at any other time in the embryo (Fig. 2f, right). Combined with the rapidity of nanodevice bending and relaxation (Fig. 5e,f and Supplementary Movie 8) this suggested a decrease in cytoplasmic resistance to reorganization (ξ_k) during DIV, as detected by the devices (Fig. 2f, left); that is, there was active softening of the cytoplasm. The changes in cytoplasmic
10 stiffness correlated with cytoplasmic myosin II dynamics (Fig. 5g) in accordance with a role for myosin II in the control of cytoplasmic stiffness. Near the time of DIV completion, spindle and radial microtubules underwent a collective motion similar to an umbrella folding as each of the two chromosome sets moved towards their respective cortical pole (Fig. 5h,i). The fact that chromosome sets are located close to the embryo poles combined with the large
15 mechanical activity (ξ_{mact}) revealed by the nanodevices, suggested that the chromosomes are localized further from the division plane, where cytoplasmic reorganization is greater; this could help guarantee chromosome segregation to respective daughter cells. Radial microtubule disassembly (Fig. 5h,i) as the DIV-phase ended resembled recently-described microtubule behavior⁴⁰. Rapid cleavage plane progression, cytoplasmic reorganization and
20 chromosome positioning away from the division plane would be facilitated by the low cytoplasmic stiffness reported by the nanodevices.

Experimental perturbation of the mechanical program

This work predicts a program of mechanical and force changes within embryos that we next evaluated *via* perturbation experiments. We reasoned that this might be achieved by the

actomyosin motor inhibitor, blebbistatin, to reduce force generation⁴¹ and cytoplasmic stiffness⁴² within the embryo, both of which are likely to involve the blebbistatin target, Myosin II. Accordingly, we found that embryos incubated in the presence of blebbistatin underwent a reduction in cortical ruffling and pronuclear convergence during the PM-phase relative to untreated controls (Fig. 6a,b; Supplementary Movie 9). In controls, pronuclei and other relatively large cytoplasmic structures moved comparatively little, but with blebbistatin treatment they exhibited greater random movement (Supplementary Movie 9), in agreement with a reduction of cytoplasmic stiffness⁴². Embryo exposure to blebbistatin did not prevent cell division (Fig. 6c and Supplementary Fig. 17) but it did induce division to occur asymmetrically (Fig. 6d).

Compared to unexposed controls (Fig. 2c-e), nanodevices within blebbistatin-treated embryos exhibited a marked reduction in rotation (related to cytoplasmic reorganization), and cytoplasmic mechanical loads throughout much of the one-cell stage (Fig. 6e-g). These reductions were consistent with a functional role for the actomyosin complex in force generation. In addition, the reduced forces and increased random pronuclear movement in blebbistatin-treated embryos provided evidence for a reduction in effective stiffness caused by myosin inhibition⁴².

Although blebbistatin-treated PM-phase embryos experienced a two-fold cytoplasmic mechanical load reduction (Fig. 2e and Fig. 6g), pronuclear centring was reduced by a factor of ~10, suggesting that cytoplasmic forces alone are not sufficient to account for pronuclear centring (Fig. 6b). The reduction in cytoplasmic stiffness caused by blebbistatin would also reduce spindle force transmission to the cortex, leading to aberrant spindle centring followed by asymmetric division, as observed (Fig. 6d).

Discussion

This work adopts a top-down approach that identifies a program of intracellular force and mechanical property changes in the first cell cycle of mouse development following fertilization. We quantify these changes and show how they relate to cytoplasmic rearrangements at different stages of the cell cycle. Intracellular nanodevices responded stereotypically throughout one-cell embryo development, revealing a program of cytoplasmic force and stiffness changes that map to developmental progression. Nanodevice deflections during the SDR-phase revealed major mechanical activity that coincided with dynamic paternal chromatin remodeling²⁹. This is possibly the first direct force measurement associated with any intracellular genome reprogramming, with relevance to chromatin remodeling including the formation of chromatin remodeling territories^{43,44}. Although the GES model is descriptive in nature, it explains why pronuclear displacement is larger in the direction of center of the one-cell embryo during the PM-phase, predicts the contribution of pronuclear growth to centring and is compatible with force gradient models. Simple elastic, viscous and viscoelastic models that do not consider effects on cytoplasmic effective mechanical properties due to boundary conditions may be limited when describing the mechanical behaviour of the cytoplasm, since the stiffness or effective viscosity of the cytoplasm can depend on how close it is to relatively rigid parts of the cell. We sought to accommodate this limitation in the GES model, which accounts for the contribution made by the relatively stiff cortex. However, even though the model is predictive (eg of increase in pronuclear diameter during the PM-phase) and has been successfully tested by experimental perturbation, there is a lack of account given to the molecular nature of some of the force generators and it must be interpreted with caution given unknown roles played by known and unknown physical parameters, including cytoplasmic heterogeneity. Spindle alignment is

facilitated by reducing cytoplasmic mechanical activity and could act as a mechanism to avoid chromosome mis-sorting during cell division.

During the embryo elongation (EL) phase, nanodevices reported high cytoplasmic resistance to reorganization (akin to cytoplasmic stiffening), which counterintuitively occurred in the absence of radial microtubules. Such cytoplasmic stiffening would enhance spindle centering and the transmission to the cortex of mechanical loads exerted by spindle elongation. Acute softening of the cytoplasm would be required for a rapid cleavage plane progression and extensive cytoplasmic reorganization⁴⁰ during cell scission (the DIV-phase). This work suggests that cytoplasmic forces larger than 10 nN operate and may indeed be necessary during completion of the first cell cycle in mouse embryonic development. These forces are 100 times larger than forces reported for muscle cells¹⁷; our devices measure average forces that drive cytoplasmic reorganization rather than intracellular point forces. We show that actomyosin complex perturbation (by blebbistatin) affected the mechanical program of mouse one-cell embryos (Fig. 6h), in agreement with consequential reduction in pronuclear centring and an increase in the frequency of asymmetric division. Intracellular nanodevices allowed us track large average reductions in cytoplasmic mechanical loads and cytoplasmic redistribution (device rotation).

Our findings are consistent with fundamental roles for intracellular forces and cytoplasmic mechanical properties in early mammalian development; EL- and DIV-phases exhibited deformation kinetics that were similar in mouse and human embryos. The findings could be applicable generally to cell division, which remains a substantial unsolved issue in cell mechanobiology. We accordingly anticipate that this work will open a new window onto intracellular physics and provide complementary information to existing techniques for cell mechanics.

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Acknowledgements

The authors are extremely grateful to Drs. Ben Nichols, Juan Campos, Teresa Suárez and Cheryll Tickle for their constructive comments during manuscript preparation and thank Animal Facility support staff for ensuring the welfare of animals used in this work. We acknowledge support to A.C.F.P. from the Medical Research Council, UK (G1000839, MR/N000080/1 and MR/N020294/1) and the Biology and Biological Science Research Council, UK (BB/P009506/1) and to J.A.P from the Spanish Government, grant TEC2014-51940-C2 and TEC2017-85059-C3 with Feder funding. The authors also thank the clean room staff of IMB-CNM for assistance with chip fabrication. A.C.F.P. and J.A.P. conceived core experiments. M.D. and R.G.-M. performed nanodevice fabrication, T.S. and M.A. performed microinjection and embryo production and M.A. and M.D.V. undertook image collection. Data analysis and modeling were by N.T., M.A., M.I.A and J.A.P. A.C.F.P. and J.A.P. wrote most of the manuscript. The authors declare no competing financial interests.

FIGURE LEGENDS

Figure 1. Fabricated nanodevices as intracellular sensors. **a**, Schematic of microtubules (mt) and actin microfilaments (mf). **b**, Geometry of fabricated 'H-comb' nanodevices. **c**, Schematic representation of nanodevice fabrication technology. **d**, Scanning electron micrograph (SEM) image of a single nanodevice (top) and reproducible nanodevice batch fabrication. Scale bars, 10 μm . **e**, Device load state and simulated normalized vertical displacement by FEM for uniform distributed pressure load (P , top) and force loads (F). **f**, Simulated maximum vertical displacement versus applied pressure and force. **g**, Schematic representation of different stages in the mouse 1-cell embryo (zygote) following fertilization, showing key structures in embryo mechanics and embryo stages. **h**, Vidcaps of mouse embryos at different stages, each containing a microinjected nanodevice. Scale bar, 20 μm .

Figure 2. 'H-comb' nanodevices detect mechanical loads inside mouse zygotes. **a**, Representative plots showing rotations [$\text{Rot}(w)$, $\text{Rot}(l)$, $\text{Rot}(z)$, top] and nanodevice deformation ($\Delta\delta$, bottom). Each background colour corresponds to one of the different embryonic stages of Figure 1g,h. **b**, As per (a), showing the 2D nanodevice trajectory during different embryo stages. **c**, Maximum rotations [$\text{Rot}(w)$, $\text{Rot}(l)$, $\text{Rot}(z)$] and (**d**) $\Delta\delta$ for each stage, with the range of lower confidence indicated in grey. **e**, Simulation of the pressure or force on the nanodevice necessary to obtain given values of $\Delta\delta_{\text{max}}$. Error bars depict measurement uncertainty. **f**, Transitions of ζ_k and ζ_{mact} . **g**, Average duration of each nominal stage of 1-cell embryo (zygote) development. **h**, Fluorescence image of autofluorescing nanodevice (green), showing labelled oocyte- (top) or sperm-derived (bottom) chromatin (red). **i**, Rotation [$\text{Rot}(w)$, $\text{Rot}(l)$, $\text{Rot}(z)$, top left] and $\Delta\delta$ (lower left) and images (right) at (25, 90, 120 and 150 min, as indicated). Red and yellow circles respectively indicate the

positions of female and male chromatin. **j**, Schematic showing the displacement of the devices associated with paternal (sperm-derived) genome remodelling.

Figure 3. Mechanics during the PM-phase. **a**, Two-dimensional (2D) pronuclear translations around the embryo center. **b**, Membrane ruffling showing (upper) schematic of external embryo morphology for different assigned phases, and (lower) images of half of an embryo during the PM-phase (left), schematically rendered to show membrane ruffling. **c**, Membrane topography changes at two opposite points and movement of the nanodevice (located near the center) with time. **d**, Initial and final distances from the nanodevice, pronuclei and microinjected microspheres respectively to the embryo center following sperm injection and in haploid parthenogenetic embryos. **e**, Maximum reported speeds versus sphere diameters. **f**, Qualitative simulated normalized effective elastic constant (left) and viscosity (right) for the displacement of spherical objects of diameters 1-20 μm inside a sphere of 70 μm diameter with a stiff solid shell (*i.e.* an embryo) versus the position of the object to the embryo center (EC). Dotted lines show theoretical values (valid for objects near the border). **g**, Schematic representations of the effective stiffness model for spherical object centring with increasing size. **h**, Pronuclear expansion during PM phase determined experimentally by videomicroscopy analysis. **i**, Schematic showing the difference of effective stiffness outwards or inwards for each pronucleus (top), embryo ruffling (bottom, left) and the corresponding effective stiffness. **j**, Qualitative, normalized effective elastic constants for the displacement of spherical objects of diameters 1-20 μm inside a sphere of 70 μm diameter with a stiff solid shell (*i.e.* an embryo) versus the position of the object to the embryo center (EC) for (i) **k**, Representative micrographs of oocytes injected with spheres plus sperm with (left) or without the inhibition of ensuing pronucleus membrane formation by wheat germ agglutinin (WGA). The displacement tendency of microspheres is represented schematically in each case. **l**,

Relative speeds of male pronuclei, female pronuclei and microspheres with time (0 = fertilization +4 h).

Figure 4. Dynamic F-actin redistribution in mouse zygotes. **a**, Vertically-paired bright-field (BF, upper) and Utr-mCherry fluorescence (center) vidcaps and profile line-plots from live embryos at the times indicated after pronucleus formation. Asterisks indicate pronuclear localization. Scale bars, 20 μm . **b**, Three-dimensional surface plots of Utr-mCherry pixel intensity across a representative embryo of **(a)**. **c**, Schematic of the region of interest (ROI) for **(d)**. **d**, Utr-mCherry mean fluorescence intensity of representative embryos for region of interest, ROI₁ (left) (cytoplasm excluding pronuclei; $n=11$), Utr-mCherry mean, minimum and maximum intensities for a representative embryo in ROI₂ (center) (half of the embryo excluding polar bodies), and (right) maximum intensity of ROI₁ (cytoplasm) and ROI₃ (cortex).

Figure 5. Morphological and cytoskeletal changes during elongation and division. **a**, Representative images (left) of embryo elongation, showing an initial stage prior to the onset of elongation (I), elongation in which zero axial cortical curvature is achieved in the equatorial region (II) and embryo elongation after cleavage plane instatement (III). Axial (d_{ax}) and equatorial (d_{eq}) diameters of five mouse embryos (right), showing absolute values of d_{ax} and d_{eq} diameter increments (inset). Bar, 10 μm . **b**, d_{ax} and d_{eq} are compared between mouse embryos generated by ICSI (mICSI), parthenogenotes (1nP) and human embryos generated by ICSI (hICSI). **c**, Immunofluorescence images (top) of tubulin- α showing microtubules in mouse 1-cell embryos during initial spindle elongation (EL1), equatorial tubulin meshwork appearance (EL2) and after the appearance of radial microtubules and invagination (DIV). Immunofluorescence images (center) of F-actin and myosin II (bottom) showing a clear increase of myosin II after EL1 and a decrease after EL2. Genomic DNA is stained with

propidium iodide (PI) in all the images. Scale bar, 20 μm . **d**, Corresponding schematic interpretations for (c). **e**, Plots showing rotation and deformation (with corresponding forces) of intracellular nanodevices prior to and during DIV-phase. **f**, Images of a representative embryo with corresponding simulated device deflections (insets). **g**, Average cytoplasmic myosin II intensity (determined by quantitative immunofluorescence) at PEB, EL and DIV stages, showing an increase during EL phase and a posterior decrease during the DIV-phase. **h**, Schematic representation of microtubule distribution. **i**, Immunofluorescence micrographs of α -tubulin showing microtubule (green) and nuclear DNA (red) distributions near the end of the DIV-phase.

Figure 6. Tracking perturbation to the mechanical program of mouse embryos treated

with blebbistatin. **a**, Fertilized embryos were cultured with or without blebbistatin (200 μM) and time-lapse images captured during PM- to PEB-phases. Panels show video capture images of embryos immediately after pronuclear formation (Initial) and at PEB (Final) in when cultured without (control, Cont.) or with blebbistatin (B), indicating initial starting and final positions (large dots) and the trajectories of presumptive female (green) and male (red) pronuclei. **b**, Graph showing distances between pronuclei and embryo centers at initial (pronucleus formation) and final (PEB) positions following culture without (control, Cont.) or with 200 μM blebbistatin (B). Data were collected from five controls and seven blebbistatin-treated embryos. **c**, Percentage of 1-cell embryos cultured in the actomyosin inhibitor, blebbistatin (200 μM), that had cleaved to 2-cells after 24 h. **d**, Percentage of embryos undergoing asymmetric division after exposure to 200 μM blebbistatin. **e**, Maximum intracellular device rotations [Rot(w), Rot(l), Rot(z)], and **(f)** $\Delta\delta_{max}$ during each embryo stage, with the range of lower confidence indicated in grey. **g**, Simulation of the pressure and force acting on the nanodevice necessary to obtain given values of $\Delta\delta_{max}$. Error bars depict

measurement uncertainty in (e-g). **h**, Models of the mechanical program for non-perturbed, control embryos (left) and embryos perturbed by blebbistatin treatment (B). Nanodevices report an altered embryo mechanical program, with reduced cytoplasmic reorganization ($\text{Rot}(z)$) and reduced average cytoplasmic mechanical load levels ($\Delta\delta_{\text{max}}$). The vertical scale of the coloured boxes is the same for control and blebbistatin-treated embryos.

Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6