

Mitochondrial displacements in response to nanomechanical forces

Yaron R. Silberberg^a, Andrew E. Pelling^a, Gleb E. Yakubov^b, William R. Crum^c, David J. Hawkes^c and Mike A. Horton^{a*}

Mechanical stress affects and regulates many aspects of the cell, including morphology, growth, differentiation, gene expression and apoptosis. In this study we show how mechanical stress perturbs the intracellular structures of the cell and induces mechanical responses. In order to correlate mechanical perturbations to cellular responses, we used a combined fluorescence-atomic force microscope (AFM) to produce well defined nanomechanical perturbations of 10 nN while simultaneously tracking the real-time motion of fluorescently labelled mitochondria in live cells. The spatial displacement of the organelles in response to applied loads demonstrates the highly dynamic mechanical response of mitochondria in fibroblast cells. The average displacement of all mitochondrial structures analysed showed an increase of ~40%, post-perturbation (~160 nm in comparison to basal displacements of ~110 nm). These results show that local forces can produce organelle displacements at locations far from the initial point of contact (up to ~40 μm). In order to examine the role of the cytoskeleton in force transmission and its effect on mitochondrial displacements, both the actin and microtubule cytoskeleton were disrupted using Cytochalasin D and Nocodazole, respectively. Our results show that there is no significant change in mitochondrial displacement following indentation after such treatments. These results demonstrate the role of the cytoskeleton in force transmission through the cell and on mitochondrial displacements. In addition, it is suggested that care must be taken when performing mechanical experiments on living cells with the AFM, as these local mechanical perturbations may have significant structural and even biochemical effects on the cell. Copyright © 2008 John Wiley & Sons, Ltd.

Keywords: Atomic Force Microscopy; Fluorescence Microscopy; mitochondria; nanomechanics; mechanotransduction; particle tracking

INTRODUCTION

The Atomic Force Microscope (AFM) (Binnig *et al.*, 1986) has become an invaluable tool for investigating biological systems. The ability to study living cells in fluid under physiological conditions has facilitated both nano-scale imaging (Putman *et al.*, 1994) and the measurement of various mechanical and material properties of living cells, such as viscoelasticity (Radmacher *et al.*, 1992; Charras *et al.*, 2001) and mechanical dynamics (Rotsch *et al.*, 1999; Pelling *et al.*, 2004). Recent technical developments have integrated traditional microscopy methods, such as fluorescence and laser scanning confocal microscopies into AFM systems (Lehenkari *et al.*, 2000; Charras and Horton, 2002b; Haupt *et al.*, 2006). This has enabled the simultaneous measurement of material properties of living cells and their biological responses and signalling pathways to be made.

Mechanical stress, in addition to other environmental signals, can induce morphological changes and affect various signalling pathways in the cell (Ingber, 1997). Examples include cytoskeletal stiffening (Wang *et al.*, 1993), phosphorylation of kinases (Takahashi *et al.*, 1997), and activation of growth factors (Sadoshima and Izumo, 1997). The AFM has become an attractive tool for investigating mechanical and material properties of biological samples in their native conditions. These include the investigation of cellular strain distribution and cytoskeleton disruption in response to stress (Charras and Horton, 2002a; Charras and Horton, 2002b), and the extraction of Young's

modulus and determination of elasticity properties (Vinckier and Semenza, 1998; Wu *et al.*, 1998).

The use of AFM to measure local material properties traditionally requires the measurement of force curves, in which the tip is indented into the cell. Even at low forces, these indentations may affect the intracellular components of the cell. In many cases one would expect that organelles directly beneath the indenting tip would be mechanically displaced. However, it is not clear if these locally applied forces will affect the displacement and arrangement of organelles further from the tip, such as those located at the cell

* London Centre for Nanotechnology, University College London, 17-19 Gordon Street, London, WC1H 0AH, UK.
E-mail: m.horton@ucl.ac.uk

a Y. R. Silberberg, A. E. Pelling, M. A. Horton
The London Centre for Nanotechnology and Centre for NanoMedicine,
University College London, 17-19 Gordon Street, London, WC1H 0AH, UK

b G. E. Yakubov
Unilever Corporate Research, Colworth Park, Sharnbrook, Bedfordshire, MK44
1LQ, UK

c W. R. Crum, D. J. Hawkes
Centre for Medical Image Computing (CMIC), Malet Place Engineering Building
University College London, Gower Street, London WC1E 6BT, UK

Abbreviations used: AFM, Atomic Force Microscopy; PI, propidium iodide; CytD, cytochalasin D.

edge, where active processes such as membrane extension and cell migration are regulated. As well, it is not fully understood in what way, and to what extent, these nanomechanical forces and indentations affect the rearrangement of organelles such as the cytoskeleton, nucleus and other structures, leading to more general phenotypic effects. In this study, we use simultaneous fluorescence-AFM and semi-automated image structure-tracking algorithms to investigate the spatial rearrangements of internal cell organelles (mitochondria) in response to locally applied loads of 10 nN.

Mitochondria are semi-autonomous and highly dynamic organelles, which have the ability to change their shape and their location inside the living cell (Bereiterhahn and Voth, 1994). Localization and rearrangement of mitochondria in higher Eukaryotes is known to be dependant on the microtubule cytoskeleton (Heggeness *et al.*, 1978; Brady *et al.*, 1982). Recent research suggests that actin filaments have an important role as well, such as by facilitating mitochondrial organization in yeast and vertebrate neurons (Drubin *et al.*, 1993; Morris and Hollenbeck, 1995), and controlling mitochondrial movement and morphology (Rudiger Suelmann, 2000). Therefore, given the strong association of mitochondria with the cytoskeleton, it is predicted that forces locally applied via the AFM tip would affect their arrangement if the cytoskeleton is capable of physical mechanotransduction (Wang *et al.*, 1993; Alenghat and Ingber, 2002; Blumenfeld, 2006).

Previous work has elegantly revealed how externally loads applied using magnetic beads can result in distinct mitochondrial and cytoskeletal displacements (Hu *et al.*, 2003). Pulling of integrin adhesion receptors by bound microbeads or micropipettes was also found to result in cytoskeletal rearrangements (Maniotis *et al.*, 1997). Previously we have shown that nuclei and cytoskeleton deformations were observed following local AFM indentation (Pelling *et al.*, 2007b). In this study, we investigate the effect of instantaneous displacement of marked mitochondria upon the static application of force with the AFM. We then proceed to quantify the displacement of a large number of mitochondria per cell and also compare the change in motion following indentation with the naturally occurring mitochondrial movement. Local forces applied directly above the nucleus were shown to cause significant rearrangements of mitochondria near cell edges that were often up to $\sim 40 \mu\text{m}$ away from the point of contact. Displacements of mitochondria were calculated using ParticleTracker, an ImageJ plug-in for multiple particle detection and tracking (Sbalzarini and Koumoutsakos, 2005). Using this method, we quantified the mean displacement of selected mitochondria before and after the application of local force. The results show a statistically significant increase of $\sim 40\%$ in the average mitochondrial displacement upon application of force. Clearly, locally applied forces via the AFM tip have significant effects on the structural arrangement of organelles but it remains to be seen what, if any, biological effects these perturbations have on the cell. However, it is likely that such perturbations will have significant consequences and will drive future studies.

MATERIALS AND METHODS

Cell culture

NIH-3T3 Fibroblasts were cultured in DMEM GlutaMAX I media (Invitrogen) supplemented with 10% fetal bovine serum (Sigma)

and 100 IU/ml penicillin and 100 $\mu\text{g/ml}$ streptomycin (Sigma), at 37°C in 5% CO₂ atmosphere. Cells were plated into 60 mm plastic culture dishes (Orange Scientific) 1 day prior to the experiment (3 ml of media at cell concentration of $\sim 10^4$ cells/ml).

Fluorescence and Atomic Force Microscopy

Cells were stained and then placed in a combined AFM-fluorescence microscope (Olympus IX71 inverted optical microscope and JPK NanoWizard[®] AFM). The temperature-controlled stage was maintained at 37°C for the duration of the experiment. MSCT-AUWH tips (Veeco) were calibrated and the spring constant was experimentally measured to be $0.05 \pm 0.01 \text{ N/m}$ (Levy and Maaloum, 2002). Single interphase cells were chosen optically and the tip was positioned above the nucleus (Pelling *et al.*, 2007a). At this point, fluorescence imaging was initiated and images were acquired at 1 Hz using a Hamamatsu ORCA-ER camera.

Fluorescent markers

Cells were incubated with MitoTracker Red[®] (Invitrogen) at concentration of 200 nM, for a period of 15 min. This is a mitochondrion-selective dye that contains a thiol-reactive chloromethyl moiety, which reacts with accessible thiol groups on peptides in active mitochondria. After incubation, the media was replaced and equilibrated for at least 1 h prior to the experiment at 37°C and 5% CO₂. 10 mM HEPES buffer was added to the culture media for 30 min before the experiment to stabilize pH changes. Cells were used in the AFM under ambient conditions for <1 h. In some cases the cell impermeable dye propidium iodide (PI) (Invitrogen) was introduced into the culture medium at a 1:500 dilution to check for membrane damage. As a positive control 200 μl 0.5% Triton-X100 was added to the culture medium to damage the cell membrane, allowing PI to localize in the nucleus.

Image analysis and motion tracking

The displacements of mitochondria were determined using ParticleTracker, a feature point-tracking tool for detection and analysis of particle trajectories (Sbalzarini and Koumoutsakos, 2005). In order to quantify mitochondrial displacements, tracking analysis was conducted on three sequential frames with the cell at rest in the first two frames and perturbed using the AFM tip between the second and third frame. Pre-perturbation mitochondrial motion (i.e. natural motion) was calculated between frames 1 and 2, and post-perturbation motion between frames 2 and 3. The mean displacements of each mitochondrial structure in the selected regions were then calculated for both pre- and post-perturbation trajectories. Typically, 2 regions were selected for each cell with a total number of between 10–30 mitochondrial structures per cell. Regions selected for analysis were further away from the nucleus, towards the edge of the cell, where the cell is relatively flat and mitochondrial movement is assumed to mainly have a two-dimensional component. Statistical significance of the data was determined with a paired Student's *t*-test.

In order to validate the tracking analysis, a second image registration method was used. The displacement of mitochondria was estimated using a semi-automatic image registration

technique (Crum *et al.*, 2005). This method determines the sub-pixel displacements that transform one image into the space of another. The registration maximizes a measure of image-similarity so that a perfect registration results in two structurally identical images with the relative motion between the image features encoded in the displacement field. There are many possible algorithms which could be adopted (Zitova and Flusser, 2003). We used a fluid registration technique (Christensen *et al.*, 1996) where the displacements are obtained by modelling the flow of a viscous fluid. It was confirmed that this second registration method provided comparable results to those obtained with the ParticleTracker tool.

RESULTS

Local forces produce structural perturbations

Mitochondria form dense three-dimensional networks around the nucleus and become flattened and more sparsely distributed at the edges of the cell (Figure 1A). Here, we have examined how locally applied forces above the nucleus are physically transmitted over long distances to the cell edge (Figure 1B). Typically, it proved difficult, using current far-field optical imaging, to distinguish and separate two-dimensional versus three-dimensional movement of mitochondria around the nucleus in response to applied force from the AFM tip; therefore, we limited our analysis to the cell edge (Figure 1C,D). In these regions, the cell is very flat, as little as 200 nm thick, and mitochondria are assumed to move perpendicular to the normal force delivered by the AFM tip over the nucleus, enabling accurate measurement of physical mechanotransduction of force

from the AFM tip. Furthermore, individual mitochondria can be resolved much more clearly in these regions, allowing a more accurate image registration and tracking analysis to be performed. Consequently, we are able to examine how local forces applied over the nucleus are translated to the furthest reaches of the cell, which is of particular interest.

Mitochondria are dynamic structures, which display basal movements driven by the cytoskeleton. Thus, in order to measure and distinguish baseline displacements from displacements caused by the AFM tip, we designed the following experiment which included a built-in control for each cell measured. Prior to image capture, the AFM tip was first optically positioned $\sim 2 \mu\text{m}$ above the cell and the time to contact was approximately 250 ms (Pelling *et al.*, 2007a) (Figure 1B). Then image capture was started and after collecting several images of basal movement of mitochondria, the tip was brought into contact with the cell at an applied force of 10 nN. The contact time of the tip was ~ 3 s and the total imaging time was typically 10 s.

By creating two fluorescence image overlays (images 1 + 2, prior to the perturbation and images 2 + 3, after perturbation) we are able to qualitatively observe that the AFM tip does indeed produce increased displacements of the mitochondria (Figure 2A–D). The magnitude of displacement is also visible in the stress maps (Figure 2E,F), where besides the obvious displacement around the centre of the cell, displacements further away towards the cell edge are also visible.

In order to produce a quantitative displacement analysis, we used the ParticleTracker software (as described in the section 'Materials and Methods'). For each cell measured ($n = 21$), displacements were calculated for the average basal displacements in addition to the average perturbed displacements of individual mitochondrial structures ($n = 323$, for all cells).

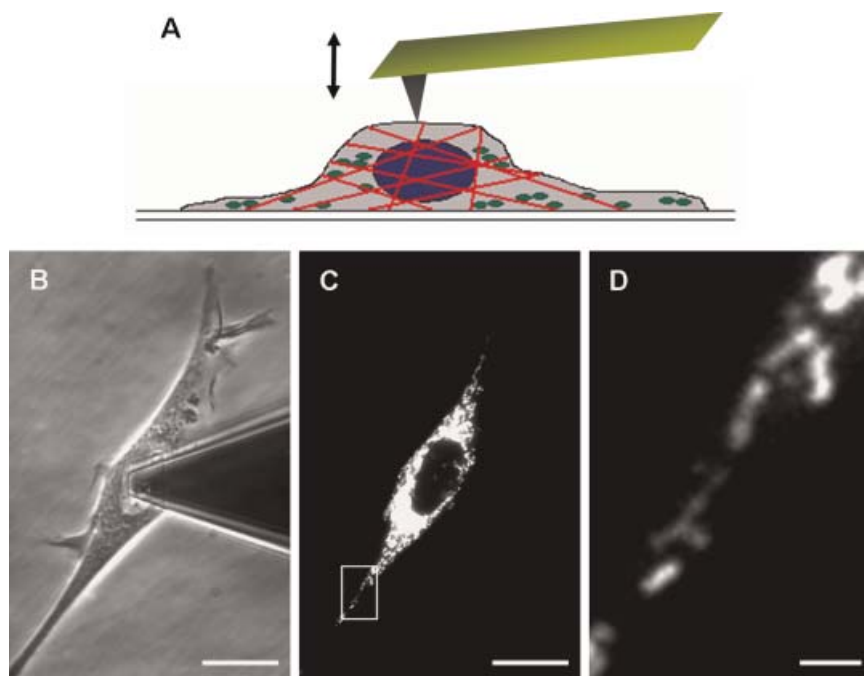


Figure 1. (A) Schematic illustration of an AFM tip above a living cell (mitochondria are indicated in green, cytoskeletal elements in brown, and the nucleus in blue). (B) Phase-contrast image of an AFM tip in contact with a living cell. (C) Fluorescent image of mitochondria. The cell was stained using MitoTracker (Invitrogen) mitochondria live-cell marker. (D) Magnified section showing distinguishable mitochondrial structures. Scale bars: B–C: 20 μm ; D: 2 μm .

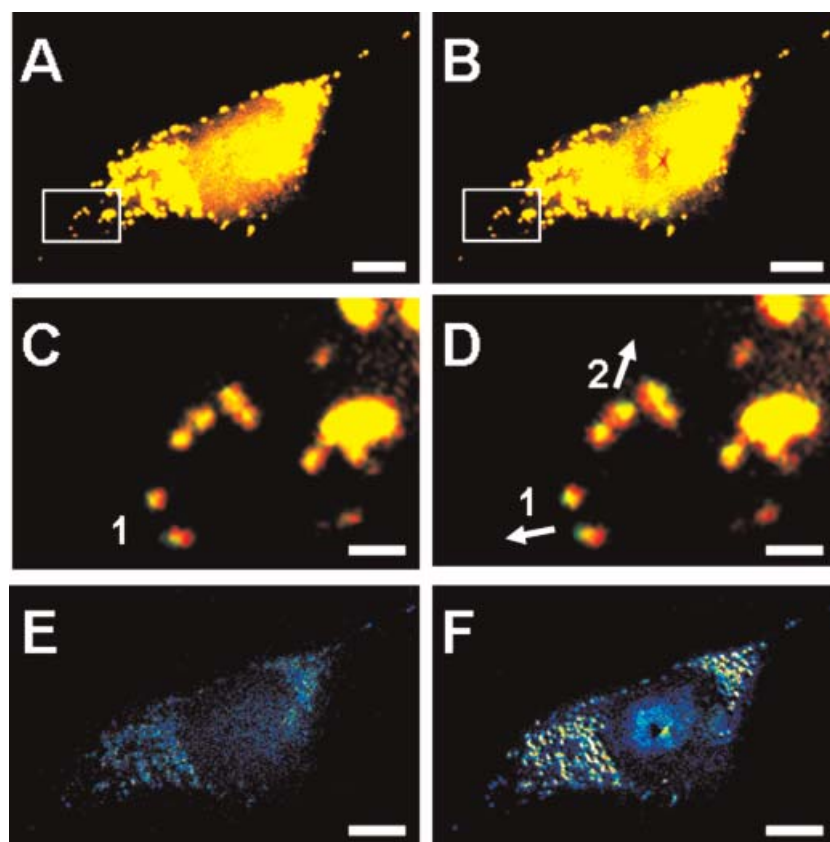


Figure 2. Comparison between control (left column) and post-perturbation (right column) images. (A) Overlay of consecutive fluorescent images 1 (red) and 2 (green), both prior to AFM perturbation. (B) Overlay of consecutive images 2 (red, before AFM perturbation) and 3 (green, after perturbation). The yellow colour results from the red-green overlay and appears denser around the nucleus, as discussed earlier. The reflection image of the perturbing AFM tip can be seen in the centre of the nucleus. (C,D) Magnified sections of the cell where differential motion of mitochondria can be visually observed. Arrows show direction of displacement of different mitochondrial structures (D1, 2; the green colour shows the post-perturbation image and thus the direction of displacement). Although some natural displacements are evident in the control image (C1), the displacement in the post-perturbation image is clearly higher and includes a larger number of organelles (D1, D2). (E,F) Stress maps of the cell, showing the magnitude of displacements before and after perturbation with the AFM tip. Scale bars are: A–B: 10 μm ; C–D: 2 μm ; E–F: 10 μm .

Nanomechanical force induces significant mitochondrial rearrangements

The results reveal that $\sim 80\%$ of cells displayed an increase in mitochondrial displacement over the basal movements within each cell, whereas $\sim 20\%$ of the cells displayed an interesting decrease in displacement, where the magnitude of displacement was smaller than basal levels. This behaviour could reflect the dynamic relationship of mitochondria with the cytoskeleton, for example naturally or mechanically induced dissociation of the mitochondria from the cytoskeleton. Extending the analysis to the total number of mitochondria analyzed ($n = 323$), we found that the average basal displacement of mitochondria was $114 \pm 6 \text{ nm}$. However, after pushing with the AFM tip, the average displacement increased to $160 \pm 10 \text{ nm}$ ($P < 8 \times 10^{-7}$, $\alpha = 0.05$) (Figure 3A). Therefore, locally applied forces over the nucleus induced a statistically significant rearrangement of mitochondria at the cell edges, increasing 39.7% following indentation at an average distance of $\sim 26 \mu\text{m}$ from the point of contact. In our analysis it is clear that the mitochondria around the nucleus also moves in response to the tip; however, it is difficult to separate the 2D and 3D components of the motion

using standard fluorescence microscopy and we leave that analysis for a future study with quantitative confocal microscopy techniques under current development.

Anti-cytoskeletal drug experiments

In order to investigate the role of the cytoskeleton in transmitting force, we used the anti-cytoskeletal drugs cytochalasin D (CytD) and Nocodazole to disrupt both the actin and microtubule networks, respectively (Charras and Horton, 2002b). Cells were incubated for 30 min with each of the drugs (10 μM Nocodazole ($n = 13$ cells), 5 μM CytD ($n = 17$ cells)), prior to experimentation. Over 300 trajectories of separate mitochondrial structures were analyzed in each case. Figure 3 shows the average displacement before and after indentation for each set of conditions. We found that the average natural displacement of mitochondria in cells treated with CytD was $56 \pm 3 \text{ nm}$ and $58 \pm 3 \text{ nm}$ ($P > 0.6$) after perturbation with the AFM tip. For Nocodazole-treated cells, the average natural displacement was $57 \pm 2 \text{ nm}$ and $54 \pm 2 \text{ nm}$ ($P > 0.3$) after perturbation. Therefore, the results show no statistically significant difference between the pre- and post-perturbation displacements, in both cases. These results clearly

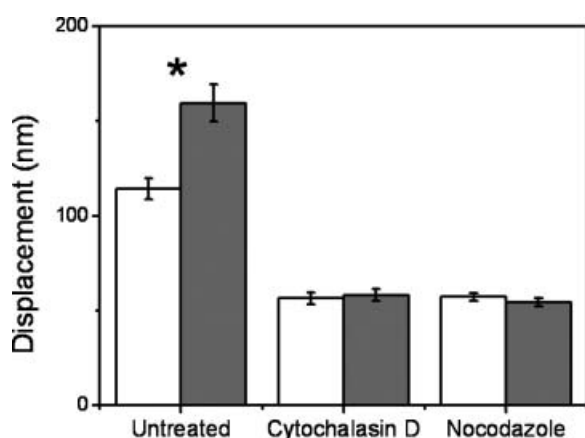


Figure 3. Comparison of the difference in mean average displacement (in nm) of mitochondria between the control (white bars) and the post-perturbation (grey bars) images for untreated cells and for cells treated with the anti-cytoskeletal drugs CytD and Nocodazole. ($n = 323$, $*P < 8E-7$). The average displacement of the mitochondria analyzed in untreated cells increased in $\sim 40\%$ in response to perturbation with the AFM tip, from 114 ± 6 nm to 160 ± 10 nm post-perturbation (mean \pm SE) ($n = 323$, $P < 8E-7$). The average natural displacement of mitochondria in cells treated with CytD was 56 ± 3 nm and 58 ± 3 nm (mean \pm SE) after perturbation with the AFM tip. For Nocodazole-treated cells, the average natural displacement was 57 ± 2 nm and 54 ± 2 nm after perturbation. Paired Student's *t*-tests ($P > 0.6$ for CytD, $P > 0.3$ for Nocodazole) demonstrated for both drugs that there is no statistically significant difference between the pre- and post-perturbation displacements.

show that mitochondrial displacements following a locally applied force are completely dependent on an intact actin and microtubule cytoskeletal network. However, the natural displacements of the mitochondria in cells pre-treated with CytD and Nocodazole are significantly different ($P < E-20$) compared to untreated cells. The average natural displacement was $\sim 50\%$ lower in cells treated with either one of the two drugs, in comparison with the natural displacement in untreated cells. These data suggest that the cytoskeletal network also has an important role to play in governing natural motions of mitochondria within living cells. It shows that natural mitochondrial motion is strongly dependent on both intact actin filaments and the microtubule network, confirming prior findings on the cytoskeleton's role in mitochondrial transport (Heggeness *et al.*, 1978; Brady *et al.*, 1982; Rudiger Suelmann, 2000).

Membrane damage control experiments

Cells used in this study were typically $5\text{--}8\ \mu\text{m}$ in height and an applied force of 10 nN will produce a large indentation into the cell of $\sim 2\ \mu\text{m}$ (or 25–40% of cell height). It was important to ensure that the applied force was not damaging the cell membrane, thus indirectly influencing mitochondrial behaviour. Force curves were measured over the nucleus under conditions similar to our experiment (1 Hz, with a maximum applied force of 10 nN). It is clear from the measured force curve (Figure 4A) that no discontinuities or significant adhesion events occurred before or after the application of 10 nN force, in agreement with previous work in which the membrane was purposely damaged

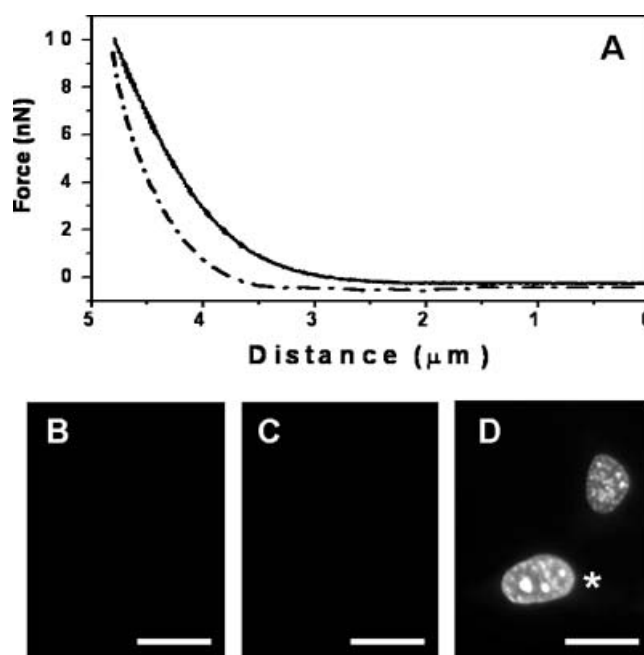


Figure 4. Membrane damage control experiment. (A) A typical force-distance curve measured on a live cell with a maximum force of 10 nN (straight line = approach; dashed line = retract). No discontinuities were observed in the approach portion of the force curve and no significant adhesion events were observed in the retract portion of the curve. This suggests that there was no penetration of the AFM tip through the membrane. (B–D) In a second control experiment, the membrane-impermeable nuclear dye propidium iodide (PI) was added to the medium prior to perturbation at 10 nN force. Fluorescent images were obtained 1 min (B), and 20 min (C) after perturbation. As a positive control Triton X-100 was added to the medium and an image obtained 20 s afterwards (D). In comparison to AFM (B,C), PI was observed to quickly localize in the nucleus after Triton X-100 exposure, producing a strong, well-defined, fluorescent signal (* shows the nucleus of the cell that was perturbed with the AFM tip). Scale bars: $20\ \mu\text{m}$.

(Obataya *et al.*, 2005). As a second control, PI was introduced into the medium during the experiment. PI is a membrane impermeable DNA intercalating fluorescent dye which is routinely used to check for cell membrane damage or breakage during apoptosis. If membrane damage occurs, PI enters the cell and will quickly localize in the nucleus and become fluorescent; otherwise, PI remains outside of the cell and does not fluoresce. With PI in the medium, fluorescence imaging before and after pushing will reveal any damage to the cell membrane. In our study, we observed no evidence of membrane breakage and PI fluorescence immediately after perturbation and up to 20 min later (Figure 4B,C). As a positive control, we also added 0.5% Triton-X100 to the medium to purposefully damage the membrane. Upon addition of Triton-X100, PI immediately localized in the nucleus producing bright fluorescence, which can be seen as a well defined image of round and intact nuclei (Figure 4D). Taken together, these results confirm that the perturbation by the AFM tip does not damage the cell membrane of NIH3T3 fibroblasts or induce any longer term damage within the timeframe or conditions of our experiments. Therefore, any observed rearrangement of mitochondria in this study is due to the local perturbation by the AFM tip.

DISCUSSION AND CONCLUSIONS

In this study, we investigated the effect of mechanical forces on cells, by tracking the displacement of mitochondria in response to applied force. Mitochondria form tubular networks that are continuously changing their shape and move throughout the cell, also over long distances. The natural motion of the mitochondria is facilitated by the cytoskeleton which acts very much like a rail-track (Bereiterhahn and Voth, 1994). By following mitochondrial displacements in response to applied force, we can gain insights into the association of mitochondria with the cytoskeleton, and enhance our fundamental understanding of the intracellular architecture and physical mechanotransduction pathways.

Here, we have shown that application of force induces significant mitochondrial rearrangements. A statistically significant increase in the displacement magnitude was revealed after indentation with the AFM tip. On average, the displacement of mitochondria increased in ~40% relative to the basal displacements after the local perturbation. In preliminary work, we have also observed a lower threshold of applied force that affects mitochondria at the edges of the cell. At applied forces of ~1 nN, no significant effects were observed at the edge of the cell (data not shown). However, even though we have not yet examined the effect on mitochondria closer to the point of contact, these results suggest that the physical distance over which transduction of force along the cytoskeleton operates is dependent on applied force (Blumenfeld, 2006).

Interestingly, in some cases, we could visually identify differential motion of close-by mitochondria in response to the applied force. Here, mitochondrial displacements were observed to increase in response to force; however, they moved in opposite directions relative to one another. Given that mitochondria are closely associated with the cytoskeleton (Heggeness *et al.*, 1978), these results allow us to tentatively suggest that the cytoskeleton of the cell can facilitate the mechanical transduction of local point forces over long distances (Alenghat and Ingber, 2002). This may have significant implications for the development of new theoretical models of cell mechanics. Recently, it has been proposed that a cell may be 'universally' described as a soft glassy material (Trepatt *et al.*, 2007), which is clearly inconsistent with our data, and it has recently been shown that the soft glassy rheology phenomenon in living cells is also time-scale-dependent (Stamenovic *et al.*, 2007). We hypothesize that

this behaviour is also likely to be dependent on cell type, the mechanical environment and physiological conditions, making generalizations difficult. Future theoretical treatments should take into account the dynamic spatial, and perhaps temporal, mechanical behaviour of these complex systems (Pelling *et al.*, 2007a).

Anti-cytoskeletal drug experiments show the dependency of mitochondrial displacements on both the actin network and the microtubules. Disrupting either of the two resulted in a drastic decrease in the natural motion of mitochondria. Furthermore, in cells where either the actin or microtubule networks were disrupted, there was no significant change in mitochondrial motion following perturbation with the AFM. These results allow us to tentatively suggest that the cell requires an intact actin and microtubule cytoskeleton to translate applied forces into increased mitochondria movements. However, it is clear that further work is required utilizing fluorescent fusion proteins to directly show the transduction of force along cytoskeletal filaments resulting in mitochondrial displacements.

In summary, we have shown in this study that forces applied to the cell with an AFM tip produce significant spatial rearrangements of internal cell organelles. In particular, local forces can produce organelle displacements at the edge of the cell far from the initial point of contact. These results also suggest that care must be taken when performing mechanical experiments on living cells with the AFM, as these local mechanical perturbations may have significant structural and even biochemical effects on the cell.

Acknowledgements

Y.R.S. would like to thank the BBSRC and Unilever for a CASE studentship. He also wishes to thank A.E.P. for all the help and advice in preparing this paper. A.E.P. and M.A.H. would like to acknowledge the Interdisciplinary Research Collaboration (IRC) in Nanotechnology (Cambridge, EPSRC UK) for financial support through an IRC Exploratory Research Grant and gratefully acknowledge the 'Dr. Mortimer and Mrs. Theresa Sackler Trust'. M.A.H. thanks the Wellcome Trust for programme grant support. W.R.C. and D.J.H. would like to thank Medical Images and Signals IRC (EPSRC GR/N14248/01 and UK Medical Research Council Grant no. D2025/31) for funding.

REFERENCES

- Alenghat FJ, Ingber DE. 2002. Mechanotransduction: All signals point to cytoskeleton, matrix, and integrins. *Science STKE* **119**: 6–9.
- Bereiterhahn J, Voth M. 1994. Dynamics of mitochondria in living cells—shape changes, dislocations, fusion, and fission of mitochondria. *Microsc. Res. Tech.* **27**: 198–219.
- Binnig G, Quate CF, Gerber C. 1986. Atomic force microscope. *Phys. Rev. Lett.* **56**: 930–933.
- Blumenfeld R. 2006. Isostaticity and controlled force transmission in the cytoskeleton: a model awaiting experimental evidence. *Biophys. J.* **91**: 1970–1983.
- Brady S, Lasek R, Allen R. 1982. Fast axonal transport in extruded axoplasm from squid giant axon. *Science* **218**: 1129–1131.
- Charras GT, Horton MA. 2002a. Determination of cellular strains by combined atomic force microscopy and finite element modeling. *Biophys. J.* **83**: 858–879.
- Charras GT, Horton MA. 2002b. Single cell mechanotransduction and its modulation analyzed by atomic force microscope indentation. *Biophys. J.* **82**: 2970–2981.
- Charras GT, Lehenkari PP, Horton MA. 2001. Atomic force microscopy can be used to mechanically stimulate osteoblasts and evaluate cellular strain distributions. *Ultramicroscopy* **86**: 85–95.
- Christensen GE, Rabbitt RD, Miller MI. 1996. Deformable templates using large deformation kinematics. *IEEE Trans. Image Process.* **5**: 1435–1447.
- Crum WR, Tanner C, Hawkes DJ. 2005. Anisotropic multi-scale fluid registration: evaluation in magnetic resonance breast imaging. *Phys. Med. Biol.* **50**: 5153–5174.
- Drubin D, Jones H, Wertman K. 1993. Actin structure and function: roles in mitochondrial organization and morphogenesis in budding yeast and identification of the phalloidin-binding site. *Mol. Biol. Cell* **4**: 1277–1294.

- Haupt BJ, Pelling AE, Horton MA. 2006. Integrated confocal and scanning probe microscopy for biomedical research. *Sci. World J.* **6**: 1609–1618.
- Heggeness MH, Simon M, Singer SJ. 1978. Association of mitochondria with microtubules in cultured cells. *PNAS* **75**: 3863–3866.
- Hu SH, Chen JX, Fabry B, Numaguchi Y, Gouldstone A, Ingber DE, Fredberg JJ, Butler JP, Wang N. 2003. Intracellular stress tomography reveals stress focusing and structural anisotropy in cytoskeleton of living cells. *Am. J. Physiol. Cell Physiol.* **285**: C1082–C1090.
- Ingber DE. 1997. Tensegrity: the architectural basis of cellular mechanotransduction. *Annu. Rev. Physiol.* **59**: 575–599.
- Lehenkari PP, Charras GT, Nykanen A, Horton MA. 2000. Adapting atomic force microscopy for cell biology. *Ultramicroscopy* **82**: 289–295.
- Levy R, Maaloum M. 2002. Measuring the spring constant of atomic force microscope cantilevers: thermal fluctuations and other methods. *Nanotechnology* **13**: 33–37.
- Maniotis AJ, Chen CS, Ingber DE. 1997. Demonstration of mechanical connections between integrins cytoskeletal filaments, and nucleoplasm that stabilize nuclear structure. *Proc. Natl. Acad. Sci. USA* **94**: 849–854.
- Morris R, Hollenbeck P. 1995. Axonal transport of mitochondria along microtubules and f-actin in living vertebrate neurons. *J. Cell Biol.* **131**: 1315–1326.
- Obataya I, Nakamura C, Han S, Nakamura N, Miyake J. 2005. Nanoscale operation of a living cell using an atomic force microscope with a nanoneedle. *Nano Lett.* **5**: 27–30.
- Pelling AE, Sehati S, Gralla EB, Valentine JS, Gimzewski JK. 2004. Local nanomechanical motion of the cell wall of *saccharomyces cerevisiae*. *Science* **305**: 1147–1150.
- Pelling AE, Dawson DW, Carreon DM, Christiansen JJ, Shen RR, Teitell MA, Gimzewski JK. 2007a. Distinct contributions of microtubule subtypes to cell membrane shape and stability. *Nanomedicine* **3**: 43–52.
- Pelling AE, Nicholls BM, Silberberg YR, Horton MA. 2007b. Approaches for investigating mechanobiological dynamics in living cells with fluorescence and atomic force microscopies. In *Modern Research and Educational Topics on Microscopy*, Vol. **3**, 3–10, Méndez-Vilas A, Diaz J (eds). Badajoz: Formatex.
- Putman CA, van der Werf KO, de Groot BG, van Hulst NF, Greve J. 1994. Viscoelasticity of living cells allows high resolution imaging by tapping mode atomic force microscopy. *Biophys. J.* **67**: 1749–1753.
- Radmacher M, Tillmann RW, Fritz M, Gaub HE. 1992. From molecules to cells—imaging soft samples with the atomic force microscope. *Science* **257**: 1900–1905.
- Rotsch C, Jacobson K, Radmacher M. 1999. Dimensional and mechanical dynamics of active and stable edges in motile fibroblasts investigated by using atomic force microscopy. *Proc. Natl. Acad. Sci. USA* **96**: 921–926.
- Rudiger Suelmann RF. 2000. Mitochondrial movement and morphology depend on an intact actin cytoskeleton in *aspergillus nidulans*. *Cell Motil. Cytoskeleton* **45**: 42–50.
- Sadoshima J, Izumo S. 1997. The cellular and molecular response of cardiac myocytes to mechanical stress. *Annu. Rev. Physiol.* **59**: 551–571.
- Sbalzarini IF, Koumoutsakos P. 2005. Feature point tracking and trajectory analysis for video imaging in cell biology. *J. Struct. Biol.* **151**: 182–195.
- Stamenovic D, Rosenblatt N, Montoya-Zavala M, Matthews BD, Hu S, Suki B, Wang N, Ingber DE. 2007. Rheological behavior of living cells is timescale-dependent. *Biophys. J.* **93**: L39–L41.
- Takahashi M, Ishida T, Traub O, Corson MA, Berk BC. 1997. Mechanotransduction in endothelial cells: temporal signaling events in response to shear stress. *J. Vasc. Res.* **34**: 212–219.
- Treppe X, Deng LH, An SS, Navajas D, Tschumperlin DJ, Gerthoffer WT, Butler JP, Fredberg JJ. 2007. Universal physical responses to stretch in the living cell. *Nature* **447**: 592–595.
- Vinckier A, Semenza G. 1998. Measuring elasticity of biological materials by atomic force microscopy. *FEBS Lett.* **430**: 12–16.
- Wang N, Butler JP, Ingber DE. 1993. Mechanotransduction across the cell-surface and through the cytoskeleton. *Science* **260**: 1124–1127.
- Wu HW, Kuhn T, Moy VT. 1998. Mechanical properties of I929 cells measured by atomic force microscopy: effects of anticytoskeletal drugs and membrane crosslinking. *Scanning* **20**: 389–397.
- Zitova B, Flusser J. 2003. Image registration methods: a survey. *Image Vis. Comput.* **21**: 977–1000.