Deletion of the TPM1 and MDM20 Genes Affect the Mechanical and Structural Properties of Yeast Cells

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Abstract: Many diseases including cancer are associated with a disorganised cytoskeleton. The process of characterising how cytoskeletal disorganisation affects the mechanical properties of cells offers the potential to develop new drugs and treatment regimes that may exploit mechanical weakness in cells and tissues. This work investigated the role of actin associated proteins, namely tropomyosin 1 (tpm1p) and mitochondrial distribution and morphology protein 20 (mdm20p), on the mechanical and morphological properties of yeast cells. For the first time it was shown that deletion of both the *TPM1* and *MDM20* genes resulted in a decrease in Young's modulus when compared to the wild-type cells. The deletion strains appeared to have aberrant cell walls when compared to the wild-type strain and also appeared to have lost the characteristic elliptical morphology that is normally exhibited by yeast. Deletion of the *TPM1* gene resulted in a significant increase in mean conjugate cell diameter when compared to the wild-type cells, however deletion of the *MDM20* gene did not have any significant effect upon the mean conjugate diameter of the yeast cells.

Keywords: Yeast, Actin, Tpm1p, Mdm20p, Mechanics, Mechanomics.

1   Introduction

There is great potential for advancing areas of biomedical sciences and bioengineering that may be achieved through developing a deeper understanding of how

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biological systems behave and function in response to mechanical stimuli. Little is known about how the mechanical properties of cells change with age and disease, nor about how cells and tissues respond to mechanical forces. Recent work has shown that cancer cells are mechanically weaker than their normal counterparts (Murphy, et al 2012; Cross, et al., 2009; Li et al., 2008). The causes of such mechanical weaknesses however remains unclear. It is thought that cytoskeleton disorganization, which has been associated with many diseases including cancer (reviewed in Ramaekers and Bosman 2004), might play a major role in this respect (Li et al., 2008; Suresh 2007). The cytoskeleton has a crucial role to play in a wide range of cellular functions and connects the cell both physically and biochemically to its external environment. In order to achieve such diverse and complex functions, the cytoskeleton relies on a plethora of proteins that help build, stabilize and actively remodel the cytoskeleton (Fletcher and Mullins 2010). Actin is a major component of the eukaryotic cytoskeleton. Normal organization and functioning of actin relies on both direct and indirect interaction with specific actin-associated proteins. Two such proteins that have been shown to be crucial for actin organization are tropomyosin alpha-1 protein (Tpm1p) and mitochondrial distribution and morphology protein 20 (Mdm20p).

Tropomyosins have been found in many eukaryotic cells (Liu and Bretscher 1992) and have been shown to be implicated in the assembly and stabilization of filamentous actin (F-actin) by binding along the length of the actin filaments (Ayscough 1998). Early studies into Tpm1p highlighted its structural importance in terms of actin stabilisation, where it was found that cell transformation resulted in down-regulation of tropomyosins and consequently a disruption of actin stress fibers (Leonardi et al., 1982). Similarly, Liu and Bretscher (1989) found that disruption of the *TPM1* gene in yeast resulted in the loss of F-actin cables. In contrast, Mdm20p is not thought to directly interact with F-actin, but has still been shown to have an effect on the organization of F-actin (Singer and Shaw, 2003). It is thought that Mdm20p, which is involved in mitochondrial inheritance in yeast, is important in post-translational modifications of Tpm1p. Mutations in the *MDM20* gene have been shown to result in F-actin cable defects that are similar to those which are seen in the case of *TPM1* mutations (Hermann et al., 1997). It has therefore been suggested that both Mdm20p and Tpm1p may act in the same, or in parallel, pathways so as to control the assembly, or the stabilization of actin cables (Singer et al., 2000).

The authors were therefore interested to understand whether deletions of certain genes, whose products function to help organize and stabilize the cells' internal architecture, would ultimately affect the mechanical and morphological properties of cells. Atomic force microscopy (AFM) (Binnig and Quate, 1986) has been success-

fully used to investigate the mechanical properties of cells, through the generation of force-displacement curves (Arften et al., 2010; Radmacher 2002; Lekka, et al 1999; Wu et al, 1998). However to date very little research has been conducted to investigate whether disruption to the actin cytoskeleton would affect the mechanical properties of cells. The role of Tpm1p and Mdm20p in providing cells with mechanical integrity has never been investigated. Therefore, by using a yeast model we set out to investigate whether deletion of the *TPM1 and MDM20* genes would affect the mechanical and structural properties of *S. cerevisiae* cells.

2   Materials & Methods

2.1   Yeast Culture

The yeast strains that were used in this study have been described previously (Gross and Kinzy, 2007). *Saccharomyces cerevisiae* yeast strains were grown in either yeast extract-peptone-dextrose [YEPD; 2% (wt/vol) bacto yeast extract, 2% (wt/vol) peptone, 2% (wt/vol) dextrose], or defined synthetic complete medium (C) supplemented with 2% (wt/vol) dextrose as a carbon source, at 30°C, 250rpm overnight.

2.2   Determination of Yeast Cell Size by Scanning Electron Microscopy

In order to ensure cell viability, *S. cerevisiae* cells were grown, as described above, to a cell density with an A600 value of 0.5. Next, the yeast cells were diluted at a ratio of 1:10 with YEPD medium and centrifuged at $664 \times g$ for 5 minutes. The supernatant was then discarded and the pellet washed ($\times 5$) in dH_2O by centrifugation at $664 \times g$ for 5 minutes. The yeast cells were then fixed in 2.5% glutaraldehyde in a shaking incubator at 30°C, 250 rpm for 90 minutes. Following fixation, $100\mu l$ of yeast cell suspension cells was deposited onto a 13mm alloy stub and left to air dry for 2 hours. For imaging, cells were gold sputter-coated for 2.5 minutes at 25mA using an EMITECH sputter-coater. All images and measurements were obtained using an Inspect S (FEI, USA) scanning electron microscope at 12.5kV. To determine the mean conjugate diameter all measurements were taken along the minor axis of the yeast cells (see Figure 1).

2.3   Analysis of Yeast Cell Mechanics using AFM

To ensure cell viability, *S. cerevisiae* were grown, as described previously, to an A600 of 0.5. Next the cells were centrifuged at $1500 \times g$ for 5 minutes and resuspended in dH_2O. Immobilisation of the yeast cells was achieved by attachment to polyethylenemide (PEI) coated glass slides, as described previously (Arfsten et al., 2010). Briefly, glass slides were first acid washed for 15 minutes in 1M HCL, then
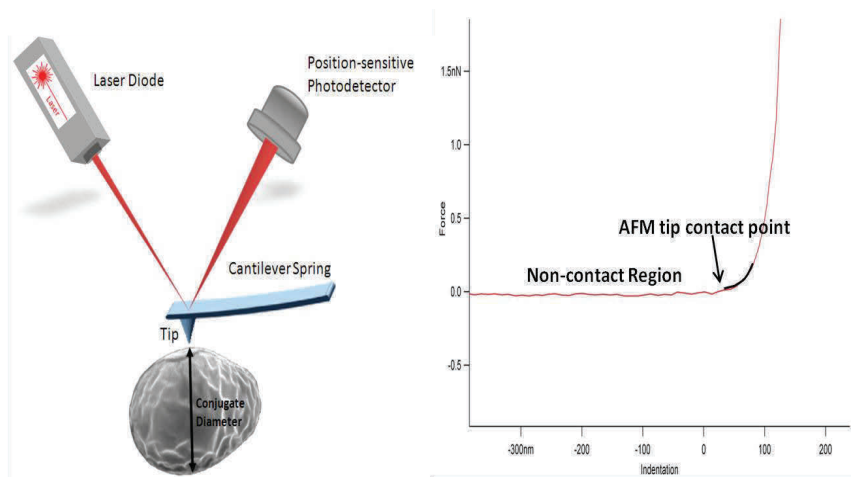
Figure 1: Schematic of AFM showing where the force measurements were made upon the individual yeasts cells (SEM image) and an example force-indentation curve showing the tip-sample contact point. The conjugate diameter along the minor axis that was used for yeast cell size analysis is also highlighted.

washed with dH_2O and allowed to air-dry at room temperature, before being covered by 10% PEI. The slides were left to incubate at $4°C$ for 24 hours and then washed thoroughly with dH_2O. Next, $500\mu l$ of the cell suspension was deposited onto a PEI-coated glass slide and this was left to incubate at room temperature for 1 hour to allow the cells to adhere. Non-attached, or weakly attached, cells were removed by rinsing the slide with dH_2O.

An Asylum Research Molecular Force Probe (MFP-3D) atomic force microscope with V-shaped silicon nitride cantilevers (spring constant 0.02N/m, OMCL-TR400 PSA-1, Olympus) were used for all measurements. Prior to taking any measurements, the cantilever was first calibrated in order to accurately determine its spring constant (k), which is a software driven procedure for the MFP-3D. All measurements were taken on the centre of the cells, as determined microscopically (see schematic shown in Figure 1), with the proviso that any yeast cells which appeared to be budding were not chosen for measurement. All force measurements were carried out using a scan velocity of $2\mu m/s$.

2.4 *Analysis of AFM Force-Displacement Curves*

All force data were analysed using the Hertz model, which has been successfully used to determine the Young's modulus for a range of biological cells (Lekka et al.,

1999; Radmacher, 2002; Wu et al., 1998). Classical Hertzian contact theory (Hertz, 1882) tells us that the relationship between the applied force, F, which is applied by a conical indenter to a surface and the resulting deformation of that surface, δ, is given by:

$$F = \frac{2}{\pi} \frac{E}{(1-\upsilon^2)} \tan \alpha \delta^2$$

Where E is the Young's Modulus of the surface material, ν is the Poisson's ratio of the material (assumed to be 0.5 for living cells) and α is the half-angle of the conical indenter. The Hertz model was fitted to the data over the first 50nm of indentation using software routines written in Matlab. The AFM tip-sample contact point was determined using a hierarchical Bayesian change-point analysis as described by Rudoy et al., (2010).

2.5   *Statistical Analysis*

Statistical analysis was performed using Minitab$^{\circledR}$ Version 16.1.1. For data which was normally distributed, an F-test was performed to determine the variance of the data. A t-test corresponding to either equal, or unequal, variance was performed with a $p<0.01$, or $p<0.05$, significance as indicated for the individual results. Data which was not normally distributed was transformed using natural logarithm (ln) and a Mann-Whitney test was performed with $p<0.05$ significance as indicated in the individual results.

3   **Results**

3.1   *The effects of TPM1 and MDM20 deletions on S. cerevisiae yeast cell size and morphology*

In order to determine whether deletion of the *TPM1* and *MDM20* genes have any effects on the morphology of *S. cerevisiae*, scanning electron microscopy (SEM) studies were carried out to measure the mean conjugate diameter (μm) of the cells. Figure 2 shows the mean conjugate diameter (μm) of *S. cerevisiae* strains with gene deletions for *TPM1* or *MDM20*, versus the wild-type strain MC214. As can be seen from Figure 2, there was a 5.6 % increase in the mean conjugate diameter (μm) for the *S. cerevisiae* strain that lacked the *TPM1* gene (*tpm1Δ*), when compared to the wild-type strain MC214 ($P<0.01$). In contrast, there was found to be no significant difference in the mean conjugate diameter (μm) between the wild-type (MC214) and the strain lacking the *MDM20* gene (*mdm20Δ*).

The actin cytoskeleton and some actin associated proteins are known to provide cells with both structural and morphological integrity (Stricker et al., 2010). We
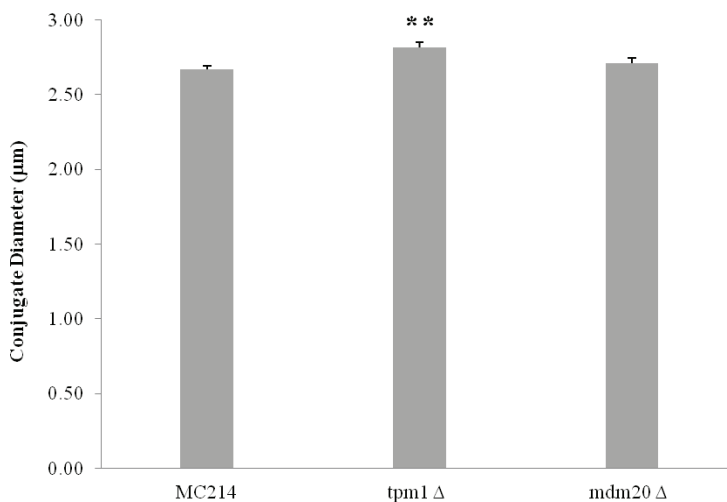
Figure 2: Mean conjugate diameter (n=100) of wild-type (MC214) and mutant (TPM1 & MDM20) *S. cerevisiae* strains (** p<0.01). Error bars represent the standard error of the mean (StEM).

were therefore interested to see whether deletion of the *TPM1* or *MDM20* genes had any noticeable effects on gross cell morphology. Imaging by scanning electron microscopy revealed a difference in the cell surface morphology between the wild-type strain MC214 and the gene deletion strains. In particular, the surface of the wild-type *S. cerevisiae* (MC214) strain appeared smoother than was the case for both the deletion strains (*tpm1Δ* and *mdm20Δ*, respectively), which both appear to exhibit aberrant cell walls (as shown in Figure 3). This is particularly true for t*pm1Δ* cells, whose cell walls appear to be more aberrant than those of the *mdm20Δ* cells (see Figure 3). It can also be seen from Figure 3 that the overall cell morphology is somewhat different between the three strains, with the wild-type strain (MC214) seemingly having the characteristic elliptical yeast morphology, whilst the *tpm1Δ* and *mdm20Δ* strains both had a more irregular morphology.

3.2 The effects of TPM1 and MDM20 deletions on the mechanical properties of S. cerevisiae

In order to determine if deletions of TPM1 and MDM20 have any effects on the mechanical properties of *S. cerevisiae* cells, AFM was used to carry out force-indentation measurements (n=30) on the respective cells (as shown in Figure 1). The Hertz model was fit to the force-indentation curve in order to determine the
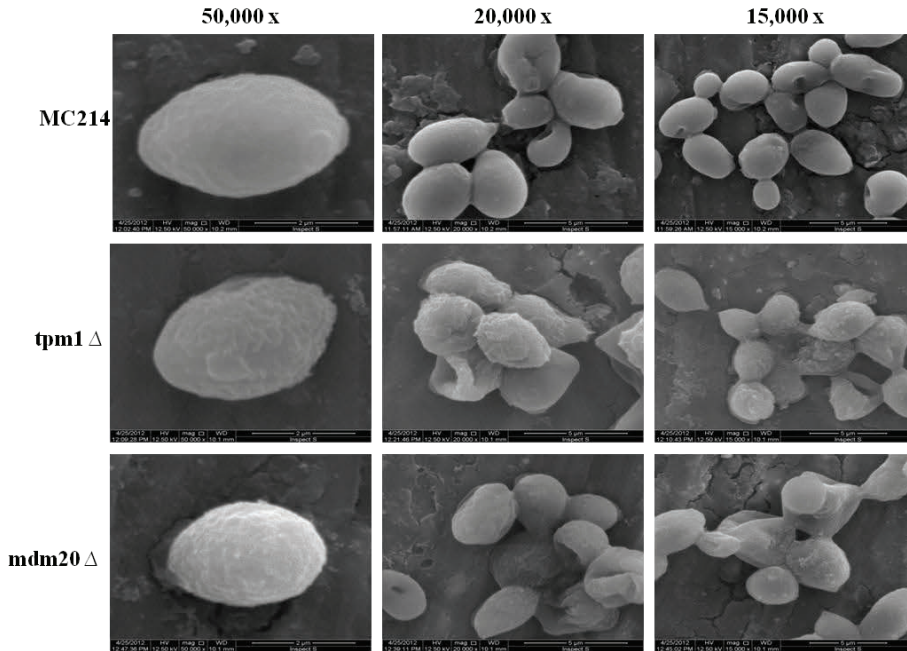
Figure 3: Scanning electron micrographs of wild-type (MC214), TPM1Δ and MDM20Δ *S.cerevisiae* strains.

Young's modulus (E). We analysed the fit of the Hertz model over a range of indentation depths on the yeast cells and found that the model fitted the data well up to indentations of approximately 50-100nm (data not shown). We therefore, for consistency and to ensure a good fit to the force curves, fitted the Hertz model to only the first 50nm of indentation for all our force curves. Figure 4 shows that the mean Young's modulus for the wild-type yeast strain (1.57×10^5 N/m^2) is greater than that of both the *tpm1Δ* and *mdm20Δ* deletion yeast strains (with Young's modulus values of 9.29×10^4 and 6.39×10^4 N/m^2, respectively). Interestingly, it was found that deletion of the *MDM20* gene, which is not known to directly associate with actin, resulted in a greater decrease in the mean Young's modulus values than that of yeast cells with *TPM1* gene deletion.

4 Discussion

Many diseases including cancer are associated with a disruption to the normal organisation of the cytoskeleton (Magin et al., 2004). However, to date little work has been done to try and link cytoskeleton disorganisation and cell mechanics. This pa-
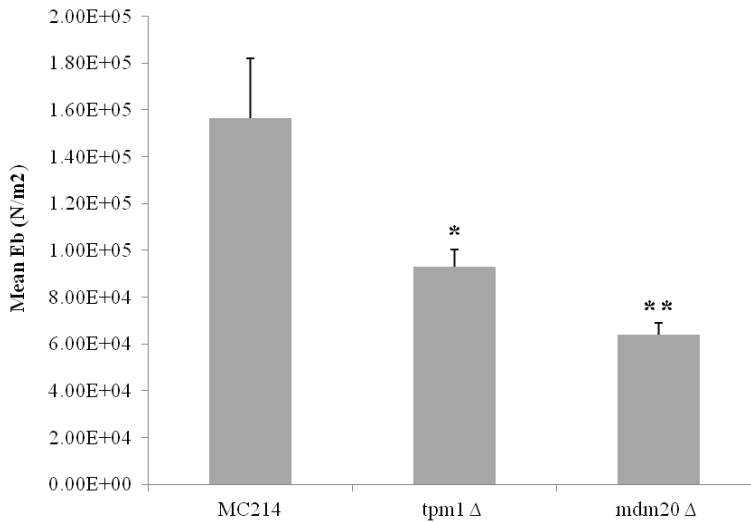
Figure 4: Mean Young's modulus (E) of wild-type (MC214, n=30) and mutant *S. cerevisiae* strains (TPM1Δ n=30 and MDM20Δ n=30) ($p < 0.01$ **, $p < 0.05$ *). Error bars represent the standard error of the mean (StEM).

per was primarily concerned with understanding whether deletions of *TPM1* and *MDM20* genes affect the mechanical and morphological properties of yeast cells. The yeast strains used in this study have been described previously and have been shown to have a disorganised actin cytoskeleton when compared to the wild-type strain (Gross and Kinzy 2007). This is not surprising, as others have shown that Tpm1p and Mdm20p are crucial in actin organisation and stabilisation (Singer and Shaw 2003; Varga et al., 2005). Using SEM we investigated whether deletions of the *TPM1* and *MDM20* genes would have any effects on yeast cell size and morphology. We measured the mean conjugate diameter and found that deletion of the *TPM1* gene resulted in a significant increase in yeast cell size whereas deletion of *MDM20* did not result in any significant differences in yeast cell size (see Figure 2). At present we do not know why deletion of *TPM1* resulted in an increase in cell size, while deletion of *MDM20* did not. Both proteins are known to contribute to the normal organisation of the actin cytoskeleton whilst actin itself is important for maintaining the structural integrity of yeast cells (Zeng and Cai, 1999). Loss of the Mdm20p protein has been shown to result in a loss of F-actin cables (Gross and Kinzy, 2007; Liu and Bretscher, 1992). However, this reduction in F-actin cables was found to be reversible when Tpm1p plasmids were introduced into the cell, thereby suggesting that the cell can compensate for loss of Mdm20p function through

increased levels of Tpm1p (Liu and Bretscher, 1992). This may explain the differences that were observed in terms of cell size, i.e. Tpm1p may be compensating for the lack of Mdm20p.

Both deletion strains were found to be irregular in morphology and exhibited aberrant cell walls when compared to the wild-type strain (as shown in Figure 3). Again, this is not surprising, as others have shown that disruption to the cytoskeleton and to *TPM1* results in heterogeneity in cell size and alterations to the cell wall, including chitin deposition (Miroslav and Kopecká, 1995; Singer and Shaw 2003). Actin is thought to generate tension within cells (Ingber 2003), which is thought contribute to the morphological integrity of cells (McBeath et al., 2004). Therefore, loss of tension within the cells through disruption of F-actin cables may explain why those yeasts cells that were lacking in Tpm1p and Mdm20p lose their typical elliptical morphology. It could also explain why we see changes to the cell wall. For example, the cell wall of the wild-type strain has a relatively smooth appearance, suggesting that the cell wall may be under tension. In contrast, the cell walls of the *TPM1* or *MDM20* deletion strains appear to have lost this smooth appearance and have become somewhat convoluted. This is more apparent with the loss of *TPM1* and could be due to the loss of F-actin cables altering the balance of tension/compression within the cell.

Mechanical studies using AFM found that the mean Young's modulus of the wild-type yeast cell was within a similar range to those reported in the literature (Bui et al 2008; Touhami et al 2003; and reviewed in Arfsten et al., 2010). Deletion of the *TPM1* or *MDM20* genes was found to cause a significant reduction in the Young's modulus when compared to the wild-type strain (as shown in Figure 4), thus highlighting the biophysical effects of reducing actin cable formation in yeast. F-actin stress fibers within mammalian cells (e.g. fibroblasts) have been shown to provide tension through the generation of prestress (Kidoaki et al., 2006). Disruption of these stress fibers has been shown to reduce the level of prestress in cells and to therefore result in a decrease in elasticity (Madden et al, 2007). Do actin cables within yeast cells also provide prestress in yeast cells? Could the loss of elasticity that is seen here be due to the disruption of F-actin cable formation that is associated with these deletion strains? This certainly seems plausible, however further work would need to be carried out to try and answer these questions, which is beyond the scope of this work.

Given that the deformation level that was used in this study was relatively small (i.e. an indentation depth of 50nm), then changes to the cell wall integrity would be expected to strongly influence the elastic response, particularly if tension has been lost within the cell wall. As Tpm1p is known to have a stabilising affect on F-actin (Singer and Shaw, 2003) it was thought that cells lacking Tpm1p would be me-

chanically compromised to a greater extent than would be the case for cells lacking Mdm20p. Surprisingly however it was found that deletion of the *MDM20* gene actually had a greater effect at reducing cellular elasticity than did deletion of *TPM1* (6.39×10^4 versus 9.29×10^4 N/m^{2,} respectively). It is unclear as to why deletion of *MDM20* would cause such a dramatic decrease in the Young's modulus, given that it is not known to be a structural protein and that it does not interact directly with F-actin. Mdm20p however is responsible for mitochondrial inheritance in yeast. Yeast cells have been reported to have as many as ten mitochondria (Jensen et al., 2000), which under aerobic conditions localise near to the cell membrane (Stevens 1997). Therefore, it is possible that a reduction in the number and the location of mitochondria could have contributed to the significant reduction in elasticity that is seen here for *MDM20* deletion. It has been suggested be some researchers that it is the cell wall that is responsible for providing yeast cells with mechanical integrity (Arfsten, et al., 2010). However, given the significance of the actin cytoskeleton with respect to a structural mechanical functionality in other cell types, the role of actin should not overlooked in terms of providing yeast cells with mechanical integrity. Recent literature has shown, for the first time, that the bacterial actin-homolog MreB, contributes nearly as much stiffness to *Escherichia coli* (*E. coli*) cells, than the peptidoglycan cell wall. It is thought that this is due to MreB being rigidly linked to the *E. coli.* cell wall (Wang et al., 2010). Interestingly, Jiang et al suggest that bacterial cells lacking MreB exhibit an instability that favours rounded cells and that MreB can mechanically reinforce the cell wall and prevent the onset of this instability (Jiang et al., 2010). Could this be the same for yeast? Could this account for the disruption to the elliptical yeast cell morphology that is seen here? This is certainly plausible, especially as it has recently been reported that the actin cytoskeleton is coupled, through molecular linkages, to the yeast cell during endocytosis and that this linkage transmits forces that are generated by the actin cytoskeleton to the cell wall (Skruzny et al., 2012).

5   Conclusion

This work has highlighted the importance of actin-associated proteins in providing yeast with both structural and mechanical integrity. Deletion of proteins that are known to help organise and stabilise actin can result in structural changes that affect the bulk mechanical properties of yeast cells. To our knowledge this is the first investigation into the effects of *TPM1* and *MDM20* gene deletions upon cell mechanics. AFM has become a powerful tool and offers us new approaches to study cell biology. It is hoped that this work will help to encourage further studies into the role of the cytoskeleton and its associated proteins upon the physical properties of cells. Understanding how the physical properties of cells changes with disease and

elucidating the underlying causes may help us to develop future treatments for disorders that are associated with cellular mechanical weakness by directly targeting such mechanical weaknesses.

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