博士の専攻分野の名称　博士（理学）　氏名　アフリン タンジナ

学　位　論　文　題　名

In Vitro Study on Mechanical Stress Induced Microtubule Deformation and its Effect on Motor Protein-based Cargo Transportation

（力学刺激が誘起する微小管変形現象とモータータンパク質による積荷輸送への影響に関する研究）

CHAPTER 1: General Introduction

The sensing and response of living cells to external cues e.g. mechanical forces, and physical microenvironments are crucial for their survival[1-3] and for control of many cellular activities, including growth, motility, intracellular transportation, mechanotransduction etc.[4-9] Whilst cells can sense and process the external forces, they are also able to exert their own internal forces as a means of 'active mechanosensing' that involves structural rearrangements of the cytoskeleton[10]. The cytoskeleton plays the key role in the correct execution of the developmental program at cellular, tissue and whole organism levels through the mechanosensing[11,12]. As the major structural component of the cytoskeleton, microtubules (MTs) are drawing much attention for their responsiveness to the external mechanical stimuli exhibited by deformation, which influence their mechanical property and thereby help MTs regulate the cellular activities[13-17]. However, the mechanism of sensing the mechanical stimuli by the cytoskeletal component, MT and conversion of it into biochemical signals for intracellular signal transduction is still not clear. Furthermore, it has been suspected that mechanical stress modulates the functionality of MTs although any solid evidence is lacking yet. Therefore, in this dissertation, I have studied the mechano-responsiveness of MTs under compressive stress and functionality of the mechanically deformed MTs systematically in an *in vitro* condition. It is suspected that in cell the surrounding elastic media or the MT-associated proteins play the key role in the buckling behavior of MTs under compression. In this dissertation, I have investigated the role of a MT-associated protein, kinesin in determining the buckling behavior of MT. I have also verified theoretical predictions of "elastic foundation model" (EFM) in explaining the buckling behavior of MTs and its dependence on kinesin spacing. For precise control of the experimental conditions and quantitative analysis, the effect of variable mode of interaction of MT with the elastic medium in the buckling behavior of MT has been studied by computer simulation and the predictions of the EFM have been compared. Most importantly, to study the functionality of the mechanically deformed MT, biomolecular motor protein driven cargo transportation has been monitored along the compressive stress-induced buckled MT in a quasi-cellular condition which has been described in detail in this dissertation.

CHAPTER 2: Buckling of Microtubules on a 2D Elastic Medium

In this chapter, compression stress induced mechanical deformation of microtubules (MTs) have been demonstrated on a two-dimensional (2D) elastic medium and the role of compression strain, strain rate, and a MT-associated protein, kinesin has been investigated in the deformation of MTs. MT is the key structural component of the cytoskeleton. It is a dynamic polymer of two heterodimer called α-tubulin and β-tubulin[18-20]. It plays significant roles in cell shape determination, cell division, cell motility and intracellular transportation[13-17]. If I look at the mechanical properties, I find that MT is the most rigid component of cytoskeleton and the bending rigidity of MTs is about 100 times larger than that of actin filaments[15]. This mechanical integrity of MT is inevitable for ensuring the cellular processes. Despite the superior mechanical integrity, MTs are also found highly curved in cells in response to internally generated force, interaction with motor proteins or external mechanical impact[16,17,21-25]. Such as buckling of MT is observed in the beating heart, in cell contractility, in the periphery of epithelial cell etc. However, it was reported that free MTs in vitro undergo classical Eulerian buckling, with a large wavelength at a small critical buckling force. In contrast in cell MTs shows short wavelength multiwave buckling surrounded by a reinforcing cytoskeleton which leads to a larger critical force. Therefore, it is necessary to consider the medium condition surrounding the MT. To understand in detail the role of surrounding elastic medium in the buckling of MT, elastic foundation model (EFM) has been proposed. In this model, the role of intermediate filaments has been explained which provide the elastic support to the MT in cell for which MT can support a compressive load which is 4 order of magnitude greater, before it buckles. For the buckling of MTs supported by a continuum elastic foundation the buckling wavelength could be expressed by this equation.

$$\lambda = 2\pi\left(\frac{EI}{k}\right)^{1/4} \cdot \left(L_d\right)^{1/4} \qquad \text{Equation (1)}$$

In this chapter, my purposes were development of an experimental setup for the buckling of MTs on a 2D elastic medium, demonstrating buckling on a 2D elastic medium and investigation of the role of elastic medium in the buckling of MTs. To demonstrate deformation of MT, an experimental set up has been designed. Here, MTs were adsorbed on kinesin

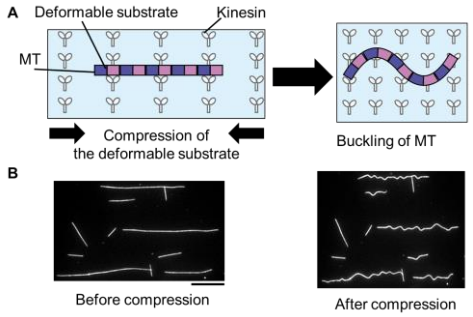

Fig. 1 (A) an experimental setup for the buckling of MTs on a 2D elastic medium. (B) Multiwave Buckling of MTs could be produced under 25% compressive stress successfully on a 2D elastic medium. Kinesin: 50 nM; Scale bar: 10 μm.

coated soft substrate, polydimethylsiloxane (PDMS) as shown in Fig. 1 (A). Two DC motors were installed to control stretch strain and strain rate using a computer program. This experimental strategy could successfully produce buckling of MTs under compressive stress on a 2D elastic medium. Moreover, short wavelength multiwave buckling is also reproduced in the laboratory as shown in Fig. 1 (B).

Next, to understand the role of kinesin, the effect of kinesin spacing on MT buckling has been investigated. Kinesin spacing is found to determine the buckling mode of MTs where a transition from long wavelength Euler type to short wavelength multiwave buckling was observed (Fig. 2A). Next at different kinesin spacing, I investigated the effect of compressive strain on buckling wavelength of MT. Compressive strain strongly affects the buckling of MTs where nonlinear response is observed at two different strain regions (Fig. 2B and 2C). This means with increasing compressive strain, a transition from elastic to plastic deformation mode takes place.

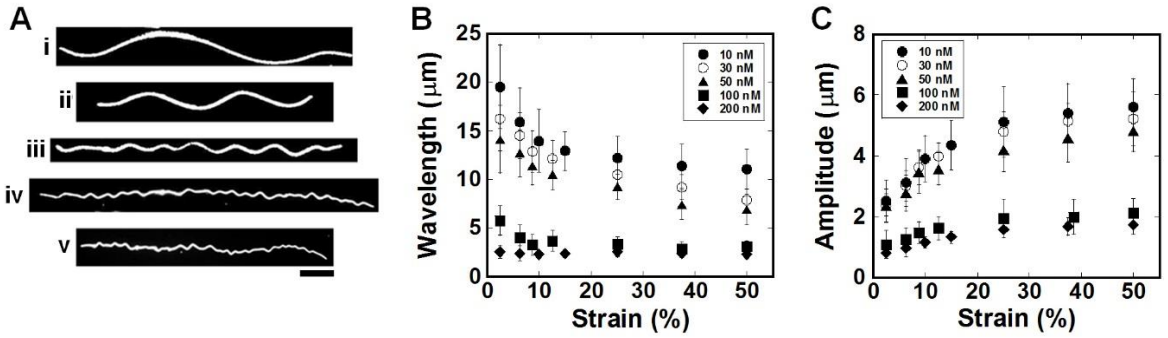

Fig. 2 (A) Representative fluorescence microscopy images show the effect of kinesin concentration on the buckling of MTs. Images were captured at 12.5% strain and the kinesin concentrations were: i) 10, ii) 30, iii) 50, iv) 100 and v) 200 nM that resulted in kinesin spacing of 95, 51, 36, 23, and 18 nm respectively. Compression stress was applied at a rate of 0.42% s^{-1}. (B) Change of buckling wavelength and (C) buckling amplitude with the change of applied compression strain at different kinesin concentrations. Error bar: standard deviation; scale bar: 5 μm.

Then the elastic foundation model is compared with the experimental results on the buckling of MTs obtained in this work. At the long kinesin spacing region the slope of the straight line drawn following our experimental data points was in an excellent agreement with that predicted theoretically by the elastic foundation model. On the other hand, a considerable deviation of the experimental results from the theoretical prediction was noticed at relatively short kinesin spacing region. This result suggests that, at least another factor might be involved in this regard, which was not taken into account by this

model so far. From previous study, I found how the spacing of kinesin affects the elastic modulus of MT where increased interaction with kinesin was found to decrease the Young's modulus of MTs[26]. To reproduce the dependence of Young's modulus of MT on kinesin spacing, here I assume a fitting equation (2).

$$\log E = \log E_0 - \beta\left(\frac{\log L_d}{\log L_{dc}}\right)^{\alpha} \qquad \text{Equation (2)}$$

Here, E_0 is the Young's modulus of a bare MT, L_{dc} is the critical spacing between kinesins. Then in the light of equation (2), modification in the EFM was brought by taking into consideration the role of kinesin in altering the mechanical property of MTs that could be expressed by equation (3).

$$\log \lambda = \log 2\pi + \frac{1}{4}\log\left(\frac{I}{k}\right) + \frac{1}{4}\left[\log E_0 - \beta\left(\frac{\log L_d}{\log L_{dc}}\right)^{\alpha}\right] + \frac{1}{4}\log L_d \qquad \text{Equation (3)}$$

The modified EFM model is shown in Fig. 3 where the solid line in the main panel (slope = 1/4) is drawn to show the relationship between kinesin spacing and buckling wavelength as theoretically predicted by the elastic foundation model. The dashed line is the fitting curve according to the modified elastic foundation model based on the present work that takes into account the change in Young's modulus of MT by kinesins. Therefore change in mechanical property of MTs by kinesin must be taken into account.

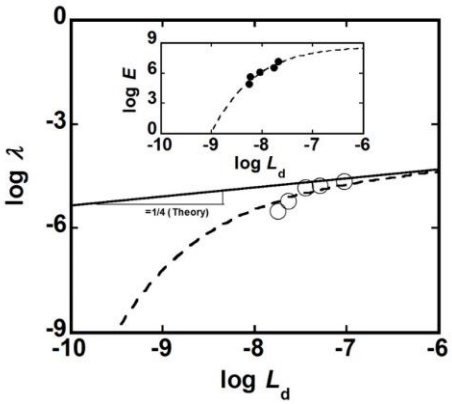

Fig. 3 The theory (EFM) is verified using the experimental results on the buckling of MTs obtained in this work. Inset shows the curve fitting of the dependence of MT's Young's modulus (E) on kinesin spacing (L_d).

In conclusion, the first ever compression stress induced buckling of MTs has been demonstrated on a 2D elastic medium by using a newly developed 'micro-stretcher'[27]. Extent of stress and interaction with surrounding medium determine the buckling mode of MTs. Comparison of the results with the predictions by the EFM suggests that any change in mechanical property of MTs by a MT-associated protein is required to consider for successfully predicting the buckling behavior of MTs. This work will help understand the role of surrounding medium and mechanical strain in the deformation of MT, which consequently will aid in obtaining a meticulous scenario of the compression stress induced deformation of MT in cells.

CHAPTER 3: In Silico Buckling of Microtubules on Elastic Media via Breakable Bonds

In the previous chapter I could understand that the elastic medium influences the buckling behavior of microtubule (MT) that elastic foundation model (EFM) could not explain clearly. Therefore it is required to investigate in detail the importance of the interaction of MT with the elastic medium. In this chapter, I have studied the importance of mode of interaction between MT and surrounding medium in buckling behavior of MT that often found in cell.

Since it appears challenging to control the experimental condition precisely. The following approaches has been taken such as: In silico buckling of MT on a 2D elastic medium, investigation of buckling of MT considering breakable interaction between kinesin and MT and permanent interaction of MT with hypothetical linker. The simulation strategy is similar to the previous experimental work where MT is attached on a two-dimensional (2D) elastic medium using kinesin as anchor. Here, the changes in shape of MTs were simulated with brownian dynamics considering fixed segment length (0.50 μm). The contraction of the substrate was simulated by changing positions of kinesin or the linker tails. Using this strategy, the simulation also reproduced short wavelength multiwave buckling successfully similar to experiment as shown in Fig 4.

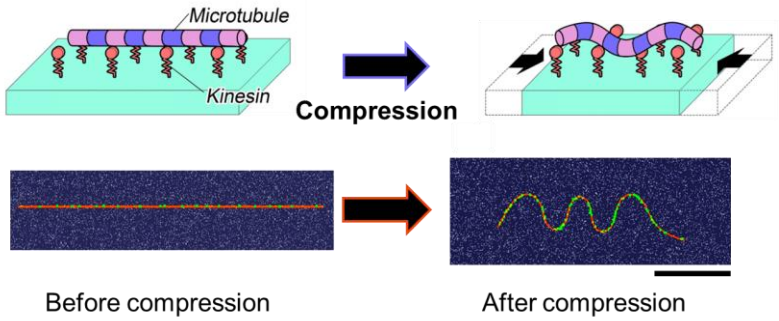

Fig. 4 In silico buckling of MT with two different interactions with kinesin on a 2D elastic medium. Here, the white dots represent kinesins and the green dots represent kinesins bound to the MT; scale bar: 5 μm, compressive strain: 50%; strain rate: $5\% \cdot s^{-1}$.

By using computer simulations, the buckling of reconstructed MT with two different interaction with kinesin has been observed after applying compressive stress. In breakable interaction, a kinesin bound to a MT was assumed to detach when the pulling force exceeded the detachment force of 7 pN. On the other hand, in case of permanent interaction: The bound kinesin remains attached to MT. Spontaneous dissociations of bound kinesin were neglected. In both cases, mode of interaction affects the buckling extent of MTs.

At both types of interactions between MT and kinesin, I investigated the change in the buckling extent of MT with the change of kinesin density. I observed that buckling behavior is different considering different mode of interactions. Therefore, kinesin density also affects buckling extent of MTs for both of the interaction modes. So, I focused on the change in buckling wavelength of buckled MT in both conditions. Considering force-induced breakable interaction between MT and kinesin the change in buckling wavelength is higher compared to permanent interaction between MT and hypothetical linkers.

Then I verified the simulation results with elastic foundation model. With kinesins, I found that buckling behavior of the simulated MTs deviated from the predictions of the elastic foundation model and consequently, the simulation reproduced the previous experimental results that were considered for low kinesin density only[27]. In contrast, considering permanent interaction of the hypothetical linker to buckled MT, the simulation result showed well agreement with the elastic foundation model. Therefore it can be said that force-induced detachment of kinesin from MT is important in buckling of MT which was not considered in the theory for which the experimental results were also deviated from the theory.

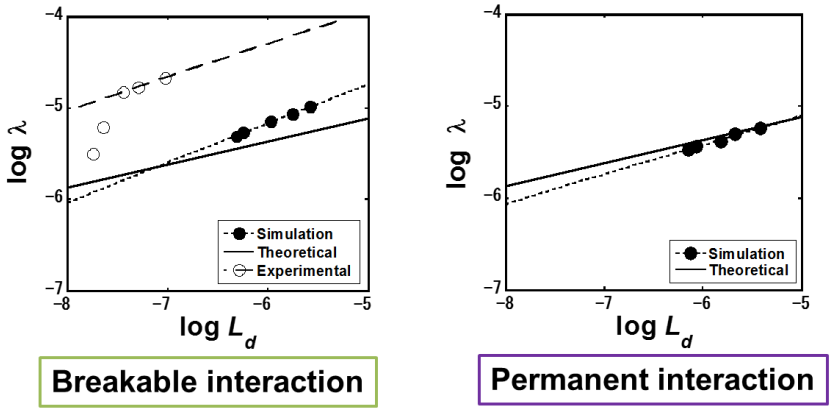

Fig. 5 Comparison among the breakable and permanent interactions of MTs to elastic media. (A) Buckling of MTs with permanent interactions to elastic media. (B) Buckling of MTs at interacting with kinesins from MTs. The solid lines (slope = 0.25) show the relationship between kinesin spacing and buckling wavelength as predicted by the theory. The dotted lines show the regressions of the simulation results.

The findings indicate that the force-induced detachments of kinesins from MTs play a significant role in the buckling behavior of MT that was not considered in the EFM[28]. This work clearly reveals the importance of mode of interaction of MT with the medium in determining the buckling behavior of MT.

CHAPTER 4: Mechanical Deformation of Microtubules Dualistically Regulates the Functions of Biomolecular Motors

Microtubule (MT), the most rigid cytoskeletal component, plays significant roles in mechanoregulation of cellular processes, although any concrete evidence is awaiting yet. In this chapter, I have investigated the response of MTs to compression stress and its consequent impact on their functionalities in the motor protein-driven transportation dynamics by employing a simple quasi-cellular system. I have found that MTs posses mechano-functional property by virtue of which they can dualistically regulate the dynamics of motor protein driven transportation. Kinesin driven Qdot transportation is significantly retarded whereas dynein based transportation is accelerated due to the buckling of MTs, indicates that MTs are able to work as mechanotransducer. The affinity of the two motor proteins for MTs was found to be altered, but to an unequal extent, upon buckling of MTs. The observed dualistic modulation of motor proteins driven transportation dynamics by buckled MTs seems related to different extent of alteration in their affinity for the buckled MTs. Collectively the results suggest that mechano-kinetic preference of associated proteins for mechanically deformed MTs may form the basis for mechanoregulation of cellular processes by MTs.

CHAPTER 5: Concluding Remarks

In this chapter, all the important findings and future prospects of this research work have been summarized. This dissertation describes how mechanical stress induces deformation of MTs and also how the deformation of the MTs modulate their biochemical interaction with an associated motor protein leading to altered dynamics of motor driven transportation along MTs. This study provides the first definite evidence that mechanical stress at the cellular cytoskeleton i.e., MTs significantly affects the dynamics of associated motor proteins. This knowledge would help our current understanding on how mechanical stress modulates the motor protein based intracellular transportation in cells, particularly in neurons where mechanical stress induced modulation of intracellular transportation is involved in neuron functionality such as presynaptic clustering, neurotransmission etc. This study provides deeper insights about the cellular mechanotransduction where the mechanical stress is transduced to modulate functionality of the cytoskeleton, which would mark it as a significant advancement in material science as well as cellular biochemistry.

REFERENCES

1. Discher, D. E.; Janmey, P.; Wang, Y. L. *Science*, **2005**, *310*, 1139–1143.
2. Giannone, G.; Sheetz, M. P. *Trends Cell Biol*. **2006**, *16*, 213–223.
3. Janmey, P. A.; McCulloch, C. A. *Annu. Rev. Biomed. Eng.* **2007**, *9*, 1–34.
4. Alberts, B. et al. *Molecular Biology of the Cell.* 5th Edition. [965–1114] (Garland Science, **2008**).
5. Fletcher, D. A.; Mullins, R. D. *Nature* **2010**, *463*, 485–492.
6. Howard, J. *Mechanics of Motor Protein and the Cytoskeleton*. [111–112] (Sinauer Associates, Inc., Sunderland, Massachusetts, **2001**).
7. Rodriguez, O. C.; Schaefer, A. W.; Mandato, C. A.; Forscher, P.; Bement, W. M, Waterman-Storer, C. M. *Nat. Cell Biol.* **2003**, *5*, 599–609.
8. Scholey, J. M.; Brust-Mascher, I.; Mogilner, A. Cell division. *Nature* **2003***, 422*, 746–752.
9. Ross, J. L.; Ali, M. Y.; Warshaw, D. M. *Curr. Opin. Cell. Biol.* **2008**, *20*, 41–47.
10. Holle, A.W.; Engler, A. J. *Curr. Opin. Biotechnol.* **2011**, *22*, 648–654.
11. Wozniak, M. A.; Chen, C. S. *Nature Rev. Mol. Cell Biol.* **2009**, *10*, 34–43.
12. Nelson, C. M.; Jean, R. P.; Tan, J. L.; Liu, W. F.; Sniadecki, N. J.; Spector, A. A.; Chen, C. S. *Proc. Natl. Acad. Sci. U S A* **2005**, *102*, 11594–11599.
13. Liu, A. P.; Fletcher, D. A. *Nature Rev. Mol. Cell Biol.* **2009**, *10*, 644–650.
14. Gittes, F.; Mickey, B.; Nettleton, J.; Howard, J.; *J. Cell Biol.* **1993**, *120*, 923–934.
15. Brangwynne, C. P.; MacKintosh, F. C.; Weitz, D. A. *Proc. Natl. Acad. Sci. U S A* **2007**, *104*, 16128–16133.
16. Brangwynne, C. P.; MacKintosh, F. C.; Kumar, S.; Geisse, N. A.; Talbot, J.; Mahadevan, L.; Parker, K. K.; Ingber, D. E.; Weitz, D. A. *J. Cell Biol.* **2006**, *173*, 733–741.
17. Odde, D. J.; Ma, L.; Briggs, A. H.; DeMarco, A.; Kirschner, M. W. *J. Cell Sci.* **1999**, *112*, 3283–3288.
18. Wang, H. W.; Nogales, E. *Nature* **2005**, *435*, 911–915.
19. VanBuren, V.; Odde, D. J.; Cassimeris, L. *Proc. Natl. Acad. Sci. U. S. A.* **2002**, *99*, 6035–6040.
20. Drabik, P.; Gusarov, S.; Kovalenko, A. *Biophys. J.* **2007**, *92*, 394–403.
21. Wang, N.; Naruse, K.; Stamenović, D.; Fredberg, J. J.; Mijailovich, S. M.; Tolić-Nørrelykke, I. M.; Polte, T.; Mannix, R.; Ingber, D. E. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 7765–7770.
22. Dogterom, M.; Kerssemakers, J. W.; Romet-Lemonne, G.; Janson, M. E. *Curr. Opin. Cell Biol.* **2005**, *17*, 67–74.
23. Guo, Y.; Liu, Y.; Tang, J. X.; Valles Jr., J. M. *Phy. Rev. Lett.* **2007**, *98*, 198103-1–198103-4.
24. Yi, L.; Chang, T.; Ru, C. *J. Appl. Phy.* **2008**, *103*, 103516-1–103516-6.
25. Gupton, S. L.; Salmon, W. C.; Waterman-Storer, C. M. *Curr. Biol.* **2002***, 12,* 1891–1899.
26. Kabir, A. M. R.; Inoue, D.; Hamano, Y.; Mayama, H.; Sada, K.; Kakugo, A. *Biomacromolecules* **2014***, 15*, 1797–1805.
27. Kabir, A. M. R.; Inoue, D.; Afrin, T.; Mayama, H.; Sada, K.; Kakugo, A. *Sci. Rep.* **2015**, *5:17222*, DOI: 10.1038/srep17222.
28. Afrin, T.; Kabir, A. M. R.; Inoue, D.; Sada, K.; Kakugo, A.; Nitta, T. *Biochem. Biophys. Res. Comm.* **2016**, *480*, 132–138.