学 位 論 文 内 容 の 要 旨

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）

The sensing and response of living cells to external cues e.g. mechanical forces, and physical microenvironments are crucial for their survival and for control of many cellular activities, including growth, motility, intracellular transportation, mechanotransduction etc. 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. The cytoskeleton plays the key role in the correct execution of the developmental program at cellular, tissue and whole organism levels through the mechanosensing. 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. 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" 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 elastic foundation model 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.

In chapter 1, the purpose of this dissertation and background of this study have been described.

In chapter 2, by using a newly developed device named as 'mechanical chamber', mechano-responsiveness of MTs to compressive stress has been investigated in a quasi-cellular system. It has been observed that MTs undergo mechanical deformation manifested by buckling under mechanical stress (Fig. 1). The role of compressive strain, strain rate, and most importantly the role of kinesin in determining buckling mode of MTs has been studied. Extent of applied stress and spacing of kinesin along a MT determine the buckling extent and buckling mode of the MT respectively while strain rate had no effect on the MT buckling. Comparison of the obtained results with the predictions by elastic foundation model suggests that any change in mechanical property of MTs by a MT-associated protein is required to consider for better understanding of the deformation of MTs. This work will help

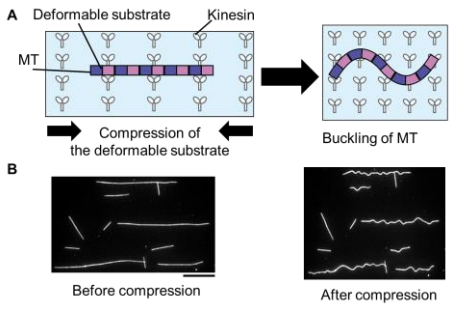

Fig. 1 (A) an experimental setup for the buckling of MTs on a 2D elastic medium. (B) Multiwave Buckling of MTs could be produced under 25% compressive stress successfully on a 2D elastic medium. Kinesin: 50 nM; Scale bar: 10 μm.

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 MTs in cells.

In chapter 3, I have studied the importance of mode of interaction between MT and surrounding medium in buckling behavior of MT that often found in cell. By using computer simulations, I have studied the buckling of reconstructed MTs on 2D elastic medium, which complement experimental studies (Fig. 2). More specifically, while forced-induced detachment of kinesins from MTs is considered, the simulation reproduces the previous experimental results and shows deviations from predictions of the elastic foundation model. On the contrary, with hypothetical linkers permanently bound to MTs, the simulation demonstrates the predictions of the elastic foundation model. This work clearly reveals the importance of mode of interaction of MT with the medium in determining the buckling behavior of MT.

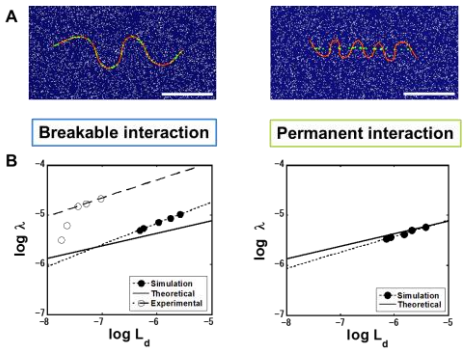

Fig. 2 (A) *In silico* buckling of MT with two different interactions with kinesin on a 2D elastic medium. (B) Different mode of interaction between MT and elastic medium is responsible for the deviation between experiment and theory.

In chapter 4, by employing a simple quasi-cellular system I have investigated the response of MTs to compression stress and its consequent impact on their functionalities in the motor protein-driven transportation dynamics (Fig. 3). The results show 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, corroborates that MTs are able to work as mechanotransducer. The affinity of the two motor proteins for MTs was altered, but to an unequal extent, upon buckling of MTs. The observed dualistic modulation of transportation dynamics of the motor proteins 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.

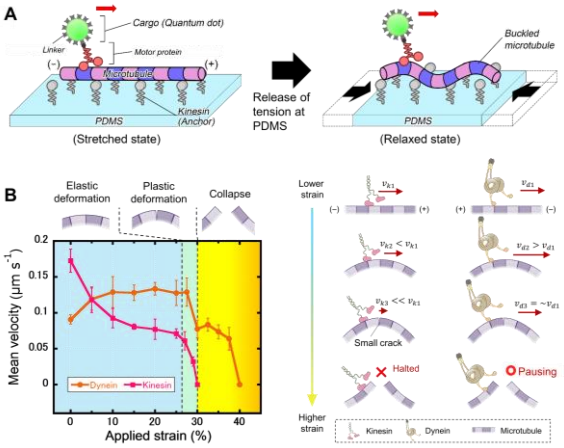

Fig. 3 (A) Experimental design of motor protein driven Qdot transportation along buckled MT on a 2D elastic medium. (B) Velocity-applied strain plots for kinesins and dyneins clearly reveals that movement of kinesin is much dependent on the condition of MTs compared to the dyneins. Error bar: standard error.

In chapter 5, 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 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. This work will also help develop rational design principle for a new mechanoresponsive functional materials inspired from nature, which should have broad impact over a wide range of material science and engineering.