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学 位 論 文 内 容 の 要 旨

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学 位 論 文 題 名

Study on Microtubule Dynamics Regulated by Exogenous Factors
（外性因子により制御される微小管ダイナミクスに関する研究）

Living cells rely on a highly dynamic scaffold, the cytoskeleton, for maintaining their functional and morphological organization. In all eukaryotic cells, the cytoskeletal network consists of three different types of filamentous protein polymers namely intermediate filaments, actin filaments, and microtubules (MTs). MTs are assembled from α, β-tubulin heterodimers that arrange head to tail into linear protofilaments, which in turn associate laterally and form filamentous MTs. In cells, MTs play significant roles in a wide range of cellular activities including cell division, cell morphogenesis and cell motility. To accomplish these cellular functions, MTs depend on an intrinsic non-equilibrium dynamic behavior called ‘ dynamic instability ’, where the two ends of MT abruptly switch between phases of growth and shrinkage. In cells, MT dynamic instability is tightly regulated by multiple factors. Recently, accumulating evidences have just started to shed light on the role of some biochemical species and MT interacting proteins including other cytoskeletal components in regulating MT dynamics inside cells. Moreover, MTs are confined in the dense cytoskeletal networks and experience strong geometrical constraints due to the finite size of cells, which is also known to significantly influence the dynamics of MTs in cells. In recent years, in vitro studies on MTs have been promising in exploring different fundamental aspects of MTs, which were found helpful for understanding the intrinsic dynamic properties of MTs in cells. Thus in this dissertation, by performing in vitro investigations, I have studied the effect of reactive oxygen species (ROS) and actin filaments in modulating MT dynamic instability. In order to investigate the influence of confinement on MT dynamic behavior, I have developed a system for the purpose of observing MT dynamic instability in vitro under geometrical confinement in a cell-like environment that was also described in this dissertation.

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

In chapter 2, the findings of the comprehensive investigation on the regulatory effect of ROS on MT dynamic instability have been summarized. To evaluate the effect ROS on MT dynamics, MT dynamic instability was studied in both ROS and ROS-free environments. By comparing the growth and shrinkage rate, rescue and catastrophe frequency of MTs, it was found that ROS can noticeably affect the dynamic instability of MTs. The MTs showed enhanced dynamics in the ROS free environment. The growth and shrinkage rate of MTs in the ROS free environment were found to be much higher compared to that in the presence of ROS. Moreover, the rescue and catastrophe frequency were also found to be largely affected by the ROS. In addition to these results, ROS-free environment ensured a prolonged observation of the dynamic instability of MTs compared to that observed in the presence of ROS. The findings in this work pinpoints the importance of maintaining a ROS free environment in the in vitro study of dynamic instability of MTs in order to obtain a more realistic scenario of the dynamic behavior of MTs.

In chapter 3, the regulatory effect of actin filaments on the dynamic instability of MTs was discussed. I studied MT dynamic instability both in the presence and absence of actin filaments in vitro. Among the four characteristic parameters of MT dynamic instability, the catastrophe frequency of MTs was increased in the presence of actin filaments, although other parameters, the growth and shrinkage rate and the rescue frequency of MTs were found almost the same in the presence and absence of actin filaments. The results in this work can be a strong evidence about the regulation of MT dynamics by actin filaments that would help in understanding the regulation of MT dynamics by actin filaments in cells.

In chapter 4, I have described a system for the purpose of observing MT dynamic instability under geometrical confinement in a cell-like environment. In this system, glass substrate was patterned through photolithography method to make hydrophobic and hydrophilic space on the substrate. MTs have been confined on the hydrophilic space of the patterned surface using an oil-water interface in the presence of lipid layer. This system is capable of creating precise confinement conditions mimicking the typical cellular environments. Using this system, I have successfully confined the MTs on the patterned surface and observed the dynamic instability of MTs. Therefore, the system could be used for the detailed investigations of the influence of confinement on the dynamic instability of MTs.

In chapter 5, all the important findings and future prospects of this research work have been summarized. This dissertation describes the findings on how the dynamic instability of MTs is influenced by ROS and actin filaments in vitro. In cells, MT dynamic instability is tightly regulated by multiple factors, which ultimately results in the well-functioning of cells. Therefore, the results obtained in this study will deliver a deeper understanding about the regulation of MT dynamics as well as the crosstalk between dynamic MTs and actin networks to understand a wide range of essential cellular processes. I expect that the results might be a basic understanding to further explore the mechanistic detail behind the regulatory effect of different biochemical species on MT dynamics and to reveal the cytoskeleton self-regulation by the interaction between dynamic MTs and actin filaments. In addition to this, the reported experimental system for studying MT dynamic instability under geometrical confinement in a cell-like environment could be extended to study more complex system of MT dynamics, which would help acquire profound insight about the regulation of MT dynamics.