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【参考】

生命科学研究科　学位論文に係る評価基準

[博士後期課程]

生命科学に関する高度で幅広い学識、専攻分野における優れた研究能力、そして生命科学の理解・発展に寄与する新しい発見もしくは概念等が示されており、論理的かつ一貫性をもって記述されていること。

【注意】

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(Form 1)

Kyoto University	Doctor of Philosophy in Life Sciences	Name	ZHANG, Wanzhen
Thesis Title	Redox-dependent regulation of molecular crowding barrier in the nuclear pore		

(Thesis Summary)

As a primary gateway of nucleocytoplasmic transport, the nuclear pore complex (NPC) plays important roles in cellular homeostasis and regulation. The NPC is a large macromolecular assembly that contains more than 30 proteins called nucleoporins (Nups), forming a molecular crowding barrier inside the pore. Although oxidative stress is known to affect the transport/permeability through the NPC, the molecular mechanism of this adaptive control remains unknown. In this study, redox-dependent responses of nuclear transport were investigated from a structural aspect of the NPC. Molecular crowding states of individual Nups were examined with a help of a crowding-sensitive fluorescent probe. Based on the detail analyses of the obtained images, Nups were classified into three categories: DTT-sensitive, H_2O_2-sensitive and insensitive Nups. Amino acid substitutions of cysteine residues to serine in H_2O_2-sensitive Nups partly abolished the H_2O_2-dependent crowding control, demonstrating that disulfide bonds play critical roles in this regulation. Fluorescence recovery after photobleaching analyses of importin α/ß-dependent nuclear transport under oxidative stress also revealed that disulfide bonds are involved in the redox-dependent regulation of the nuclear transport. Combine with previous achievements from single molecular transport assays, a cysteine-based proximal control model was proposed to explain the adjustment of the molecular crowding barrier of the NPC under redox conditions. Taken together, this study demonstrated that redox environments can control the NPC-mediated nuclear transport via directly inducing disulfide bonds among channel-forming Nups.

(Form 2)

（Thesis Evaluation Summary）

It has been known for many years that when cells are exposed to oxidative stress, the macromolecular transport between cytoplasm and nucleoplasm is affected, which has been considered as an important cellular response to the stress. This thesis study is aimed to elucidate the molecular mechanism of this cellular response against oxidative stress.

It was previously demonstrated that reactive oxygen species generated in the cytoplasm and intracellular organelle upon oxidative stress affected the intracellular localization of nuclear transport receptors such as importin α, as well as some subunits of the nuclear pore complex (NPC), which serves as the gate of nuclear transport. In this thesis study, on the other hand, the author hypothesized that reactive oxygen species increase the formation of disulfide bonds between/among NPC subunits (nucleoporins, Nups), and change the molecular crowding state within the pore, which results in the different conductance of the pore against transporting molecules. This is very unique viewpoint and the study is different from other previous studies.

To quantify the macromolecular crowding in the NPC, the author utilized a fluorescent protein-based probe, GimRET, and successfully quantified subtle changes in the local crowding states of the individual subunits upon the oxidative stress. Furthermore, the author developed a single-molecule imaging technique of the crowding probe, as well as utilized fluorescence recovery after photobleaching method to measure the kinetics of nuclear transport. Finally, based on such highly unique and quantitative approaches, the author demonstrated that oxidative stress increased disulfide bonds formed among several nucleoporins, and reduced the transport activity through the pore. This finding is providing a new mechanism of how oxidative stress regulates nuclear transport, and, therefore, has a big impact on the cell biological research field.

This thesis substantiates the candidate's extensive and wide knowledge of life sciences, demonstrates expert research capability in the field of cell biology, and presents new discoveries that contribute to the profound understanding and further development of the candidate's research field. Moreover, the thesis is written logically and coherently, which satisfies the degree requirement that the thesis shall serve a as a valuable document for future reference. On January 28th, 2021, the PhD thesis oral examination was held. Pursuant to this oral examination, the thesis examination committee hereby concludes that the candidate has passed all of the requirements for the degree of Doctor of Philosophy in Life Sciences.

The thesis, thesis summary (Form 1), and thesis evaluation summary (Form 2) will be published through the Kyoto University Research Information Repository. If the thesis cannot be published on the website immediately after the degree is awarded, due to patent application, journal publication constraints, or other reasons, please indicate the earliest date below that the thesis can be published.

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Publication date of the thesis summary (Form 1) and thesis evaluation summary

(Form 2) : mm dd , yyyy