Abstract of Thesis

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Title

SUN domain protein, Mps3 localization and regulation on nuclear envelope (NE) of Yeast Meiosis

(出芽酵母の減数分裂期におけるSUNドメインタンパクMps3の核膜上局在と制御メカニズムの解明)

Abstract of Thesis::

Chromosome movements and Nuclear envelope (NE) remodeling are the important events in Meiotic Prophase-I (Early stage of meiosis-I). After entry into the meiotic prophase of yeast (*Saccharomyces cerevisiae*) cells, telomeres move on nuclear envelope and attaches to NE as well as forms transient clusters near the vicinity of Spindle pole body (SPB), equivalent to the mammalian centrosome. At early meiosis-I, a lot of key changes occurs on NE such nuclear envelope remodeling (Movements, deformations and enlargements). Telomere movements such as clustering and declustering were triggered by an INM (Inner Nuclear Membrane) protein, Mps3 (It has conserved SUN domain with Sad1-Unc84 homology). Nuclear envelope bound Mps3 tethers telomeres to the NE, which promotes chromosome motion inside the nucleus; this chromosome motion may promote the alignment and pairing of homologs, important for the homologous recombination as well as new offspring formation. Key feature of Mps3, it can change its cellular localization from mitosis to meiosis. During mitosis (vegetative growth of cell), Mps3 mostly localizes to SPB and when cells enter into meiosis, Mps3 forms multiple foci and clusters on NE. Important event during nuclear envelope remodeling is NE localization of Mps3. Biological significance and molecular mechanisms behind the NE remodeling, thus Mps3 localization on NE during meiosis is poorly understood. I have been looking to find both positive and negative factors, which can regulate NE localization of Mps3 during meiosis. Then, identified that Rec8, it is a meiosis-specific klesin component of cohesion complex involved in sister chromatid cohesin, is important for NE localization of Mps3. I identified that mitotic klesin, component of cohesin complex such as Scc1/Mcd1 over-expression during meiosis, can only complement the Mps3-NE localization function but not other meiotic events/functions. However, Scc1 cannot be sufficient to promote Mps3-NE localization in mitotic cells. Scc1contribution towards Mps3-NE localization is independent from mitosis

to meiosis. I design and construct an experimental system, which can express Rec8 even in mitotic yeast cells and found that the Rec8 is sufficient to promote Mps3-NE localization in mitotic cells. Through meiosis-specific cohesin component, Rec8 mediated Mps3-NE localization process in mitosis, I also identified Mps3 consensus sequence such 188T, 189S and 190S region also critical Mps3-NE localization. Which clearly shows that *mps3-AAA* (phospho defective) mutants with defective phosphorylation condition, Rec8 expression system, not able to induce Mps3-NE localization but it clearly increases similar to wild-type levels in *mps3-DDD* (phospho-mimetic) mutant. Mps3 N-terminus domain and Acidic domain, which are present in the nucleoplasmic region of Mps3, were contributing to Mps3-NE localization. Possibly, interaction with Rec8 might be critical for the regulation of nuclear envelope Mps3. *mps3Δ2-64 & mps3Δ65-145* domains of Mps3 are essential for the Mps3-NE localization during both meiosis as well as mitosis. Interaction of these regions of Mps3 with chromatin can contribute the chromosome movements (RPMs: Rapid Prophase Movements) inside the nucleus. Nuclear pore complexes (NPC) are large protein complexes present in the nuclear envelope and contribute a key role in nuclear envelope remodeling. Some of the nuclear pore proteins or nuclear porins promotes NE remodeling by differential regulation of Mps3 (Both positive or negative regulation of Mps3). In this study, identified Nup157, pom152 negative regulators for Mps3 localization on NE, these show complete coverage of Mps3 on NE in vegetative cells, which is not seen in wild-type cells. Nup42 and Nup53 have a minimal contribution in nuclear envelope remodeling. I identified Nup60 is positive regulator for Mps3-NE localization. During early meiosis, *nup157Δmps3Δ2-64 & nup157Δmps3Δ65-145* shows only SPB localization. Interestingly, after induction of meiosis, *nup157Δmps3Δ2-64 & nup157Δmps3Δ65-145* cells shows NE localization of Mps3 observed in meiotic time course analysis. Also identified two different regulations of Mps3 during meiosis. Mps3 N-terminus dependent as well as N-terminus independent meiosis-specific positive regulation of Mps3 under nucleoporin background cells. I have been addressing how Rec8 promotes Mps3-NE localization along with other factors such as Mps3 two domains of nucleoplasmic regions. I speculate that the interaction of Mps3 with Rec8, the meiosis-specific cohesin in nucleoplasm is a key factor for nuclear envelope localization of Mps3. These, suggest that chromosomes are the key regulator for NE functions such as NE remodeling.

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論文審査の結果の要旨及び担当者

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論文審査の結果の要旨

SUN domain protein, Mps3 localization and regulation on nuclear envelope (NE) of Yeast Meiosis .
酵母減数分裂期における SUN ドメインタンパク質, Mps3 の核膜上での局在と制御

減数分裂は生殖細胞を作り出す過程である。その過程で細胞は一度の複製を終えた後、二度の連続的な分裂により二倍体から一倍体の配偶子を形成する。減数分裂期組換えは減数第一分裂前期に起こり、その中でも交叉型組換えとシナプトネマ複合体形成は、第一分裂の正確な染色体分配に必須である。

減数分裂期の核内では染色体が劇的にその位置を変えて、運動する。特に染色体末端のテロメアが核膜に結合し、一過的に集合するブーケと言う配置を取ることが知られている。このテロマメアブーケの形成にはSUNドメインを持つ核膜タンパク質が必須の役割を果たすが、SUNドメインタンパク質の局在の制御については不明点が多い。

Jagadeeswara Rao Bommiによる本申請研究では、出芽酵母のSUNドメインタンパク質であるMps3の局在の仕組みを解析した。このタンパク質は体細胞分裂期では中心体様構造に局在するが、減数分裂期には加えて、核膜に局在する様になる。Mps3の核膜局在に関わる因子を探したところ、減数分裂期コヒーシンのサブユニットであるRec8がMps3の体細胞分裂期における核膜局在に十分であること、Rec8によるMps3の核膜局在にはMps3の核ラミナドメインが必要であることも見出した。染色体の構成要素が核膜のダイナミックスを制御する点で興味深い知見である。加えて、核膜孔の構成要素であるPom152, Nup157がMps3の核膜局在を抑制することも見出した。この結果は核膜孔構成要素と核膜タンパク質の連携を示していて、新規性が高い成果である。

本申請研究により、減数分裂期の核膜リモデリングについての新しい制御の仕組みを分子レベルで明らかにできた。今後の進展により、当該分野での研究の発展も大きく期待できる成果と言える。

よって、本論文は博士（理学）の学位論文として十分価値あるものと認める。