様式３

論　文　内　容　の　要　旨

氏　名　（　李　珂　）	
論文題名	Budding yeast protein phosphatase 4 promotes meiotic chromosome axis formation through Hop1 assembly （出芽酵母における脱リン酸化酵素PP4はHop1を介して減数分裂期染色体軸構造の形成を促進する）

論文内容の要旨

Meiosis consists of one round of DNA replication followed by two rounds of chromosome segregation, which produces four haploid gametes from one diploid cell. Meiotic crossover formation between homologs generates a physical connection called chiasma which is essential for the first round of homologous chromosomes segregation (meiosis I), and simultaneously increases genetic diversity in gametes. Crossover formation requires a meiosis-specific chromosome axis-loop structure, providing a scaffold for Spo11-dependent meiotic DNA double-strand break (DSB) formation to initiate crossover. During the onset of meiotic prophase I, meiotic chromatins are folded into a dense array of loops emanating from proteinaceous linear architecture called the meiotic chromosome axis. Therefore, the establishment of chromosome axes by hierarchical assemblies of axial components is significant for axis formation. In budding yeast, Rec8, Red1 and Hop1 are essential axial components. Rec8 generates fundamental structure for the loading of the Hop1-Red1 complex to form the meiotic chromosome axis. The Hop1-Red1 complex, the main regulator of meiotic recombination, exhibits dynamical distribution on chromosome axes involved in the recruitment of Spo11-accessory proteins to promote meiotic DSB formation. $Mec1^{ATR}/Tel1^{ATM}$ kinases, functioning as sensors in the DNA damage response pathway, phosphorylate Hop1 proximity to DSB site to activate meiotic DSB dependent checkpoint. Protein phosphatase 4 (PP4) counteracts $Mec1^{ATR}/Tel1^{ATM}$ kinases and dephosphorylates Hop1 to inactivate the meiotic checkpoint and promote the transition to meiosis I. In addition, it is also reported that PP4 is possible to be a component of the meiotic chromosome structure. Therefore, I would like to reveal if PP4 functions independently of meiotic DSB formation in meiotic prophase I.

PP4 is a stable complex consisting of a Pph3 catalytic subunit and a Psy2 regulatory subunit. To avoid the structural effect of Pph3 protein absence, a catalytic-dead allele *pph3-H112N* was also analyzed besides the *pph3* null allele (*pph3*Δ) to elucidate the enzymatic necessity of PP4. In this study, I found the *pph3*Δ and *pph3-H112N* mutants showed delayed phosphorylation and dephosphorylation of Hop1 with the wild-type level of expression of Hop1 protein. Using immunostaining analysis, I revealed that the *pph3-H112N* and *pph3*Δ mutants show a significant delay in Hop1 and Red1 loading onto meiotic chromatin, but not in Rec8 loading. It suggested that the delayed phosphorylation of Hop1 was caused by delayed assembly of Hop1 protein onto chromatin. This delayed Hop1-Red1 loading caused by PP4 dysfunction was still observed in meiotic DSBs deficient mutation (*spo11-Y135F*) background, indicating the function of PP4 in Hop1 loading is meiotic-DSB independent. Moreover, the significant delay in Hop1 loading was not rescued in a $Mec1^{ATR}/Tel1^{ATM}$ meiosis-specific knockdown background (*pCLB2-MEC1 tel1*Δ), indicating this PP4 function is free from the $Mec1^{ATR}/Tel1^{ATM}$ activity. Co-immunoprecipitation analysis revealed that PP4 physically interacts with Hop1, but not with Red1 or Rec8. This suggests PP4 directly promotes recruitment onto chromatin or stabilization of Hop1 protein on chromosome axes. In addition, the deletion of the *PCH2* gene, which is required for Hop1 removal from the synapsed regions of chromosome axis, failed to restore Hop1

loading in the *pph3* mutants, indicating PP4 function is required for timely assembly of Hop1 protein onto chromatin.

Based on these results, I showed a novel role of PP4 activity which is completely distinguished from the known function of PP4 as a counteractor of $Mec1^{ATR}$/$Tel1^{ATM}$ kinases. That is during the onset of meiotic prophase I, PP4 promotes the loading of Hop1-Red1 onto chromatin which is required for the timely formation of meiotic DSBs and entire homolog synapsis. The comparable phenotype of *pph3-H112N* mutant and *pph3*Δ mutant indicates the requirement of PP4 catalysis, rather than structure, in this novel role. Therefore, I would like to propose a model that PP4 physically interacts and dephosphorylates Hop1 to promote efficient loading of Hop1-Red1 and form an axis-loop structure. In this model, Hop1 just after translation is phosphorylated possibly may explain the suppression of inappropriate binding Hop1 onto chromatin. I would like to discuss this novel role of PP4 activity in the regulation of meiotic chromosome morphogenesis in detail.

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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

Budding yeast protein phosphatase 4 promotes meiotic chromosome axis formation through Hop1 assembly

出芽酵母における脱リン酸化酵素 PP4 は Hop1 を介して、
減数分裂期染色体軸構造の形成を促進する

減数分裂期の組換えはゲノムの多様性の産生と、配偶子形成に必須の役割を果たしている。その仕組みの解明は配偶子形成時のゲノムの安定化の分子メカニズムの解明はこれまでに知られていない組換えの機能を明らかにすることが出来るばかりでなく、配偶子の機能不全などの医学的側面の理解に繋がることが期待できる。染色体はさまざまな遺伝子の機能発現の場として働くが、減数分裂期では染色体が軸―ループ構造を形成し、減数分裂期組換えなどを制御することが知られている。染色体の軸形成の仕組みやその制御の分子メカニズムについては体細胞期、減数分裂期ともほとんど分かっていない。

本申請研究は、減数分裂期の染色体の軸形成の仕組みを明らかにするために出芽酵母の脱リン酸化酵素の１つProtein phosphatase 4(PP4)に着目して、その機能解析を実施した。その結果、PP4は減数分裂期の軸構成に必須のタンパク質であるHop1と相互作用すること、その染色体への結合を促進することで、減数分裂期の染色体の軸形成を制御することを明らかにした。その成果をもとに、PP4による脱リン酸化反応を介した、新規の減数分裂期の軸構成モデルを提唱することが出来た。

本申請研究により、減数分裂期の軸構成における新しい制御の仕組みを明らかにした点において、学位に値する成果と言える。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。

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。

博士研究の一部は下記の国際誌の論文の共筆頭著者として発表している。

Li Ke and Shinohara Miki, Mec1/Tel1-independent role of protein phosphatase 4 in Hop1 assembly to promote meiotic chromosome axis formation in budding yeast. BioRix. doi: 10.1101/2021.05.10.443451