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論　文　内　容　の　要　旨

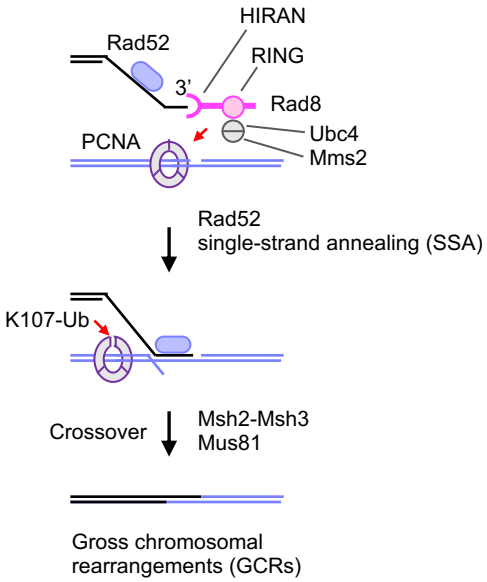
氏　名　（　Su Jie　）	
論文題名	Fission yeast Rad8-dependent PCNA ubiquitination at lysine 107 promotes gross chromosomal rearrangements （分裂酵母Rad8によるPCNAのリシン107のユビキチン化が染色体異常を促進する）

論文内容の要旨

Faithful repair of DNA damage is crucial for maintaining genome integrity. Spontaneous double strand breaks (DSBs) and stalled replication forks can induce homologous recombination (HR). HR is considered as an error-free DNA repair mechanism, as it uses intact homologous DNA as the template. However, homologous recombination can occur between ectopic sites from repetitive sequences and when accompanied with crossover or through break-induced replication (BIR), can lead to gross chromosomal rearrangements (GCRs). GCRs, such as deletion, translocation, and inversion, can cause cell death and diseases including cancer. However, how GCRs occur remains unclear. Rad51 recombinase, a key HR factor, catalyzes DNA strand exchange with homologous duplex DNA molecules. Rad51 promotes gene conversion, a non-crossover type of recombination. Loss of Rad51 reduces gene conversion causing the DNA damage be repaired by GCRs. In fission yeast, loss of Rad51 greatly increases the formation of isochromosomes and truncation. Isochromosomes that contain mirror-imaged arms formed between centromere inverted repeats. Truncations formed by addition of *de novo* telomeres to broken ends. Yeast Rad52 promotes Rad51 loading onto DNA, but this function is not conserved in mammals. Both yeast and mammalian Rad52 catalyzes single-strand annealing (SSA) of complementary single-stranded DNA (ssDNA) molecules. SSA is independent of Rad51. Rad52-dependent SSA can cause GCRs, as the *rad52-R45K* mutation, which impairs SSA *in vitro*, largely reduces isochromosome formation in *rad51*Δ cells. Mus81, a crossover-specific endonuclease is required for Rad52-dependent GCRs probably by cleaving the joint molecule formed by Rad52 to crossover products. Rad52-dependent SSA can also lead to gene conversion, but Mus81 is not required for gene conversion. Does the recruitment of Mus81 determines the fate of the joint molecule leading to GCRs, but not gene conversion? The detailed mechanism of Rad52-dependent GCRs remains unclear.

Here, I provide genetic evidence that fission yeast Rad8 and Mms2-Ubc4 E2 conjugating enzymes catalyze PCNA lysine 107 (K107) ubiquitination to promote Rad52-dependnet GCRs. Rad8 E3 ligase is the homolog of budding yeast Rad5 and human HLTF. Using an extra chromosome, ChL^C to monitor GCRs in fission yeast, I found that loss of Rad8 reduced GCRs in *rad51Δ* cells. Two types of GCRs were detected using ChL^C in *rad51*Δ cells: isochromosome and truncation. *rad8Δ* reduced 75% of the isochromosomes formed in *rad51Δ* cells, but did not affect chromosomal truncations, showing that Rad8 is specifically required for homology-mediated GCRs. Rad8 contains three conserved domains: HIRAN, SNF2/SWI2 translocase, and RING finger domains. The HIRAN domain mediates binding to 3' ends of ssDNA. The translocase domain contains an ATP binding motif and catalyzes translocation on double-stranded DNA (dsDNA). The RING domain binds ubiquitin E2 enzyme and transfers ubiquitin from E2 to the substrate. Mutations in HIRAN and RING but not translocase domain of Rad8 reduced GCRs, suggesting that the abilities of Rad8 to bind the 3'-end of ssDNA and to catalyze ubiquitination are required for isochromosome formation. With the aid of Mms2-Ubc13 and Mms2-Ubc4 E2 ubiquitin-conjugating complexes, Rad8 catalyzes ubiquitination of the same substrate, PCNA, but at different sites, K164 and K107, respectively. PCNA ubiquitination at K164 promotes a template switching

pathway of DNA damage tolerance, while the role of ubiquitination at PCNA K107 is unclear. *mms2* and *ubc4*, but not *ubc13* reduced GCRs. PCNA K107R but not K164R reduced GCRs in *rad51*Δ cells. *rad8-RING*, *ubc4,* and PCNA K107R mutations reduced GCRs in epistatic manner, suggesting that, with the aid of Ubc4-Mms2, Rad8 ubiquitinates PCNA at K107 to promote GCRs. I also found that the *rad52-R45K*, *mus81*Δ and PCNA K107R mutations epistatically reduced the GCR rate, showing that PCNA K107 works with Mus81 in Rad52-dependent GCRs. PCNA is a homotrimer forming ring structure. K107 is located at the PCNA-PCNA subunits interface, raising the possibility that K107 ubiquitination weakens the subunits interaction. Remarkably, an interface mutation D150E greatly increased the GCR rate of *pcn1-K107R* cells, showing that the interface mutation bypasses the requirement of PCNA K107 in GCRs. D150E mutation also increased the GCR rate of *rad8-RING* cells, showing that the mutation bypasses Rad8 ubiquitination activity. Taken together, these results suggest that Rad8-Ubc4-Mms2-dependent PCNA K107 ubiquitination weakens PCNA-PCNA subunits interaction to cause GCRs. I hypothesize that PCNA K107 ubiquitination destabilizes the PCNA trimer, which cause the sliding of PCNA to expose ssDNA that can used by Rad52-dependent SSA and/or make space for Mus81 recruitment. This study has uncovered a role of the non-canonical PCNA ubiquitination at K107 in Rad52-dependent GCRs.



様式 7

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

自然発生的なDNA損傷が起きると、ゲノム内の反復配列を介して、転座などの染色体異常が発生することがある。染色体異常は、細胞死や病気の要因となる。その一方、進化にも貢献し得る重要な生命現象である。しかし、染色体異常が起きる仕組みについては明らかとなっていない。SU Jie（蘇傑）氏は、分裂酵母を用いて、ユビキチン・リガーゼ Rad8/HLTF が 3 量体リング状蛋白複合体を形成する PCNA の 107 番目リシン残基（K107）をユビキチン化し、それにより反復配列を介した染色体異常を促進することを示した。ゲノム維持に働く相同組換え因子 Rad51 を破壊すると、セントロメア領域の反復配列を介して形成する同腕染色体と染色体末端欠失が増加する。しかし、Rad8 を破壊すると同腕染色体が特異的に減少することを発見した。*rad8* 点変異株の解析から、ユビキチン化活性に必須な RING フィンガーと 3'末端 DNA 結合ドメイン HIRAN が染色体異常の発生に重要であることを明らかにした。二重変異株の解析から、Rad8 と PCNA K107 が染色体異常に同一経路で働くことを明らかにした。これらの結果から、DNA 末端に結合した Rad8 が PCNA K107 をユビキチン化することで染色体異常を促進することが考えられる。興味深いことに、PCNA K107 は PCNA-PCNA 結合領域に存在することから、ユビキチン化により PCNA リング構造が変化すると予想される。実際、PCNA-PCNA 結合に影響する PCNA D150E 変異を PCNA K107R 変異株に導入すると、染色体異常の顕著な増加が見られた。これらの結果から、Rad8 による PCNA K107 のユビキチン化は、PCNA 複合体のリング構造を変化させることで染色体異常を促進すると考えられる。

SU Jie 氏の博士論文は、主に遺伝学的な実験データに限られるが、PCNA K107 ユビキチン化の報告は殆ど例がなく、染色体異常に於ける PCNA K107 ユビキチン化の役割を明らかにした初めての研究論文である。そのため、SU Jie 氏の研究成果は、今後、染色体異常の分子メカニズムを解明するための重要な礎となる。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を認める。