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研究課題名（和文）Functional analysis of Rad51 activation by Rad55-Rad57 and Swi5-Sfr1

研究課題名（英文）Functional analysis of Rad51 activation by Rad55-Rad57 and Swi5-Sfr1

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研究成果の概要（和文）：DNA二本鎖切断は最も危機的なDNA損傷であり、この修復には相同組換えが重要な働きをする。Rad51リコンビナーゼは相同組換え反応における中心タンパク質であるが、正常に機能するためには多くの補助因子が必要である。本研究では、Rad51とSwi5-Sfr1補助因子の物理的および機能的相互作用を詳細に解析した。その結果、Sfr1のN末端にRad51と結合する領域を明らかにした。このSfr1変異株の解析から、別の補助因子複合体Rad55-Rad57が、Swi5-Sfr1とRad51間の相互作用の欠陥を抑制することを発見した。以上の結果をもとに、相同組換えの分子機構の新しいモデルを提案した。

研究成果の学術的意義や社会的意義
相同組換えは、哺乳類の腫瘍形成を防ぐために重要であるゲノムの安定性を維持するための不可欠な生体機構であり、また、減数分裂時の配偶子の生成に重要な役割を果たす。 したがって、相同組換えは、細胞増殖と生殖という2つの生命の中心的な特徴で重要な役割を果たしている。 重要なことは、相同組換の分子メカニズムが酵母からヒトまで高度に保存されていることである。したがって、酵母細胞に関する我々の発見は、ヒト細胞におけるDNA修復プロセスの理解に繋がり、そのため、がんや老化、不妊といった人類にとって極めて重要な現象の詳細な分子メカニズムの解明の基盤知識として貢献する。

研究成果の概要（英文）：DNA damage is an unavoidable consequence of life. DNA double stranded breaks are the most severe form of DNA damage, and their repair by homologous recombination (HR) is critical for maintaining genome stability. The central protein in HR is Rad51, but Rad51 requires several auxiliary factors to promote HR, including Swi5-Sfr1. We characterized the physical and functional interaction of Swi5-Sfr1 with Rad51 by employing an interdisciplinary approach. Our experiments demonstrated that the N-terminal half of Sfr1 contains two regions that bind Rad51. Biochemical analysis demonstrated that the stimulation of Rad51 activity by Swi5-Sfr1 is substantially diminished when both sites are mutated. However, cells expressing the mutated form of Sfr1 were not sensitive to DNA damage. We discovered that Rad55-Rad57, an independent auxiliary factor complex, was suppressing the defect in the Swi5-Sfr1 to Rad51 interaction. These results revealed novel insights into the molecular mechanisms of HR.

研究分野：分子生物学

キーワード：DNA repair　Genome stability　Double-strand break　Homologous recombination　Rad51　Swi5-Sfr1　fiision yeast

様　式　Ｃ－１９、Ｆ－１９－１、Ｚ－１９（共通）

１．研究開始当初の背景

DNA damage can occur when cells are exposed to external genotoxins such as ionizing radiation. However, even natural cellular processes such as DNA replication can result in DNA damage. The vast majority of these damage events are ultimately inconsequential due to the highly efficient DNA repair processes that safeguard genome integrity. The maintenance of genome stability is crucial, as genome destabilization is a hallmark of diseases such as cancer. The most severe type of DNA damage is a DNA double-strand break (DSB), in which both strands of the double helix are broken. Homologous recombination (HR) is a mechanism that identifies a segment of DNA that shares sequence identify (i.e., homology) with the DSB site then utilizes this intact DNA as a template to repair the DSB. Importantly, any genetic information that is lost at the DSB site is recovered during this process. At the molecular level, the Rad51 protein binds the single-stranded DNA (ssDNA) that is exposed at the DSB site to form a nucleoprotein filament; this filament is the entity capable of conducting HR. However, although Rad51 is the central protein in this process, many proteins assist Rad51 during HR. There are several distinct groups of such proteins, and they are collectively referred to as recombination auxiliary factors. The exact mechanistic differences between these factors remain to be elucidated.

２．研究の目的

The importance of HR can be most easily appreciated when considering human health. Mutations in HR proteins are commonly found to be the underlying cause of many diseases that are associated with genome instability. While such diseases may vary in severity, a unifying trait is cancer predisposition. Furthermore, an increasingly common finding is that mutations in recombination auxiliary factors such as BRCA2 and RAD51C results in cancer susceptibility. Thus, HR is critically important for maintaining genome stability and protecting against diseases such as cancer.

The ability of HR to identify homologous sequences is also exploited during meiosis, a specialized cell division that gives rise to gametes. During meiosis, a single DNA replication event is linked to two tandem nuclear divisions, resulting in the halving of ploidy. Furthermore, during this process, parental chromosomes are shuffled such that each gamete receives a unique combination of alleles. Defects in HR can result in miscarriages, disorders associated with aneuploidy-such as Down's syndrome-and in extreme cases, infertility. Thus, HR is essential for fertility in sexually reproducing organisms, where it is also responsible for driving evolution by generating genetic diversity upon which natural selection can act.

In summary, by maintaining genome stability during the mitotic cell cycle and ensuring the fidelity of ploidy reduction during the meiotic cell cycle, HR is critical for two central aspects of life-growth and reproduction. Additionally, HR is involved in several other aspects of chromosome biology, including rDNA and telomere maintenance. Elucidating the functional differences among recombination auxiliary factors will lead to a better understanding of HR, which can have an impact on a variety of topics related to human health.

３．研究の方法

This study involved an interdisciplinary approach to investigate the Swi5-Sfr1 auxiliary factor complex. Primarily, the power of yeast genetics was combined with classical and modern biochemical approaches. Additionally, in collaboration with the lab of Prof. Hideo Takahashi at Yokohama City University, NMR spectroscopy was employed to study protein structure and protein-protein interactions. A chemical biology technique known as site-specific proximity crosslinking was also utilized to validate the protein-protein interaction analysis. By bringing together several different disciplines, we were able to probe the function of the Swi5-Sfr1 complex with more freedom.

４．研究成果

A previous study had demonstrated that the Swi5-Sfr1 complex can be separated into two functional modules: the N-terminal half of Sfr1 (Sfr1N) and the C-terminal half of Sfr1 in complex with Swi5 (Swi5-Sfr1C). Although Sfr1N was shown to physically interact with Rad51, it did not exert any stimulatory effect on Rad51 activity. By contrast, Swi5-Sfr1C was shown to activate Rad51, despite not forming a detectable complex with it. To gain structural insights into Swi5-Sfr1C, the authors crystalized the complex and demonstrated that it forms an elongated, kinked

molecule. Because Sfr1N could not be crystalized and did not show any activator function, it was mostly neglected and assumed to be less important for the function of Swi5-Sfr1. To test this notion, I created fission yeast (*Schizosaccharomyces pombe*) strains lacking either the entire *sfr1* gene (*sfr1*$^{-}$), just the C-terminal half (*sfr1N*), or just the N-terminal half (*sfr1C*). Interestingly, both *sfr1N* and *sfr1C* strains showed the same DNA damage sensitivity as the *sfr1*$^{-}$ strain, indicating that, not only is Sfr1N as important as Sfr1C for DNA repair, but both modules are essential for the in vivo function of Sfr1.

Since Sfr1N could not be studied by X-ray crystallography, NMR structural biology was employed instead. This analysis indicated that Sfr1N is intrinsically disordered and constitutes a flexible module within the Swi5-Sfr1 ensemble. Moreover, NMR interaction analysis identified two sites with Sfr1N that interact with Rad51, referred to as Site 1 and Site 2. Consistently, several residues within these two sites could be crosslinked to Rad51 in a site-specific proximity crosslinking assay (Figure. 1).

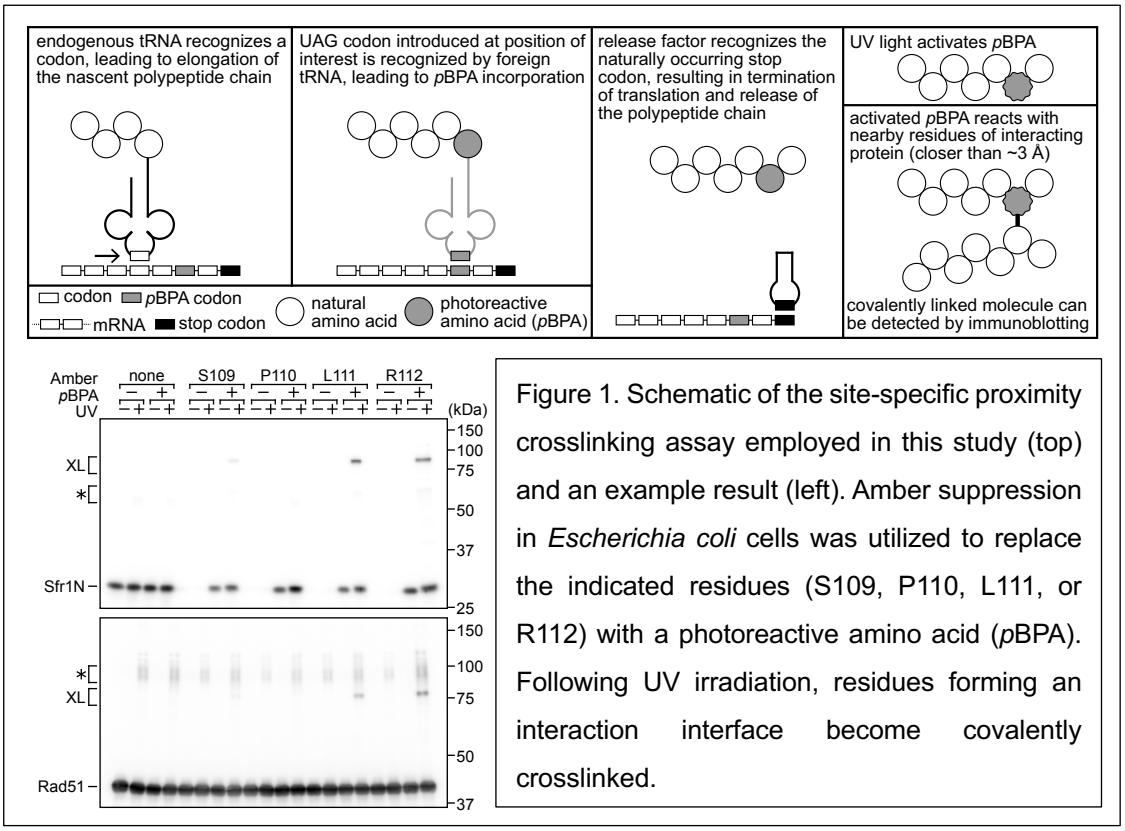

Figure 1. Schematic of the site-specific proximity crosslinking assay employed in this study (top) and an example result (left). Amber suppression in *Escherichia coli* cells was utilized to replace the indicated residues (S109, P110, L111, or R112) with a photoreactive amino acid (*p*BPA). Following UV irradiation, residues forming an interaction interface become covalently crosslinked.

Next, different versions of Swi5-Sfr1 were purified for biochemical analysis, including the wild-type complex, the 3A mutant (Site 1 residues mutated), the 4A mutant (Site 2 residues mutated), and the 7A mutant (Sites 1 and 2 residues mutated). While the 3A and 4A mutants only had a reduced interaction with Rad51, the 7A mutant had completely lost the interaction, indicating that the two sites interact cooperatively with Rad51. Since this demonstrated that the physical binding of Swi5-Sfr1 to Rad51 is mediated by Sites 1 and 2, I next examined whether the ability of Swi5-Sfr1 to simulate Rad51 was affected by these mutations. Consistent with the physical binding data, the 3A and 4A mutants only showed a small reduction in Rad51 stimulation, whereas the 7A mutant showed a substantial reduction. Taken together, these biochemical results demonstrated that the stimulation of Rad51 activity by Swi5-Sfr1 was mediated by Sites 1 and 2 in the intrinsically disordered N-terminal domain of Sfr1.

Finally, I sought to understand what happens to DNA repair mediated by Rad51 when the physical interaction between Rad51 and Swi5-Sfr1 is disrupted. For this purpose, the different mutations at Site 1 and/or 2 were introduced into fission yeast cells. The efficiency of DNA repair was not affected by any of the

mutations, which was particularly surprising for the 7A mutant. These results suggested that, although the stimulation of Rad51 by Swi5-Sfr1 was abrogated in vitro, something in the cell was rescuing this defect. I hypothesized that perhaps Rad55-Rad57 was responsible for this rescue. To test this, I constructed a strain in which the *rad55* gene was deleted (*rad55*$^{-}$) and the *sfr1* locus contained the 3A, 4A or 7A mutations. Importantly, the 3A or 4A mutations further sensitized *rad55*$^{-}$ cells to DNA damage, although not to the level of the *sfr1*$^{-}$ mutation. Strikingly, the *rad55*$^{-}$ *sfr1-7A* strain was as sensitive to DNA damage as the *rad55*$^{-}$ *sfr1*$^{-}$ strain. These results indicated that defects in the interaction between Swi5-Sfr1 and Rad51 could be suppressed by a Rad55-Rad57-dependent mechanism—contrary to the previous proposal that Swi5-Sfr1 and Rad5-Rad57 promote Rad51 activity independently of each other. One possible explanation for this was that Rad55-Rad57, which can interact with Rad51, could act as a molecular bridge to recruit Swi5-Sfr1-7A to Rad51. In support of this model, subsequent experiments suggested that Rad55-Rad57 could form a complex with Swi5-Sfr1.

In conclusion, this study provided molecular insights in the stimulation of Rad51 by Swi5-Sfr1, and in doing so, identified a novel interplay between two distinct groups of auxiliary factors that were previously thought to function independently of each other. Future work will focus on attempting to reconstitute this interplay in vitro using biochemical approaches in order to better understand the relationship between Rad51, Swi5-Sfr1 and Rad55-Rad57.

５．主な発表論文等

〔雑誌論文〕　計5件（うち査読付論文　5件／うち国際共著　0件／うちオープンアクセス　4件）

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３．雑誌名 Nature communications	６．最初と最後の頁 -
掲載論文のDOI（デジタルオブジェクト識別子） なし	査読の有無 有
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オープンアクセス オープンアクセスではない、又はオープンアクセスが困難	国際共著 －

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〔図書〕　計0件

〔産業財産権〕

〔その他〕

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6．研究組織

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