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(Summary)

Protecting the integrity of genomic sequence information is integral to the existence of any living organism. Double-stranded DNA (dsDNA) is the molecule of inheritance that encodes our genomic information. The DNA double helix is invariably prone to modification and damage through exposure to endogenous and exogenous agents. Endogenous DNA damage can arise from the DNA molecule's interaction with its immediate surroundings. Exogenous factors are genotoxic chemicals can also cause DNA damage. The most severe form of DNA damage is a double-strand break (DSB). A single DSB divides a normal chromosome into two pathological chromosomes and can lead to genome instability, which is a potent driver of cell death and tumorigenesis. Homologous recombination (HR) is the mechanism responsible for accurately repairing DSBs. The RecA-family recombinase Rad51 forms a right-handed helical filament on single-stranded DNA that possesses the enzymatic activities central to HR. Although Rad51 is the key protein in HR, multiple auxiliary factors — including Rad52, Rad54, Rad55-Rad57, and Swi5-Sfr1 — interact with Rad51 to promote its recruitment to DNA damage sites and potentiate its activity. The recruitment of Rad51 to DSBs is strongly dependent on Rad52 (Miyazaki et al., 2004). Consistently, *rad52*Δ yeast strains show a marked reduction in the formation of DNA damage induced Rad51 foci (Gasior et al., 2001; Lorenz et al., 2009), which are cytological entities that represent Rad51 nucleoprotein filaments at sites of ongoing DNA repair. Rad54 belongs to the Swi2/Snf2-family of proteins, which utilize the energy derived from ATP hydrolysis to translocate on dsDNA (Pazin & Kadonaga, 1997; Amitani et al., 2006). In yeast, *rad54*Δ strains are as sensitive to DNA damage as *rad51*Δ, indicating that, much like Rad52, Rad54 is essential for Rad51 activity in vivo (Jiang et al., 1996; Muris et al., 1997). The most established role of Rad54 in HR is in promoting Rad51-driven D-loop formation and strand exchange (Ceballos & Heyer, 2011). How this is achieved at the molecular level is not exactly known, but it has been proposed that strand invasion by the Rad51 presynaptic filament is facilitated by the fact that Rad54 translocation on dsDNA leads to some local DNA unwinding (Ristic et al., 2001). Rad55 and Rad57 are both paralogs of Rad51 that share significant sequence similarity in their ATPase core domain (Bonilla et al., 2020). Yeast two-hybrid analysis suggested that Rad57, not Rad55 interacts with Rad51 in *S. pombe* (Tsutsui et al., 2001). Biochemical reconstitutions demonstrated that Rad55-Rad57 can stabilize the Rad51 presynpatic filament against disruption by the Srs2 anti recombinase (Liu et al., 2011d). These in vitro results are supported by cytological analysis in yeast demonstrating that Rad51 foci are reduced in the absence of Rad55/Rad57 (Akamatsu et al., 2007). Sfr1 was first identified in *S. pombe* as an interacting partner of Rad51 that forms a complex with Swi5 to promote Rad51-dependent DNA repair (Akamatsu et

al., 2003). Biochemical reconstitutions demonstrated that Swi5-Sfr1 stimulates Rad51-driven DNA strand exchange by potentiating Rad51's ATPase activity and stabilising the Rad51 presynaptic filament (Haruta et al., 2006; Kurokawa et al., 2008). In *S. pombe*, the *rad57*Δ and *sfr1*Δ mutants show relatively mild sensitivity to DNA damage, while the *rad57*Δ *sfr1*Δ double mutant is as sensitive as the *rad51*Δ single mutant. Based on this additivity, it was proposed that Rad55-Rad57 and Swi5- Sfr1 function independently of each other to promote Rad51-dependent DNA repair (Akamatsu et al., 2003; Akamatsu et al., 2007). However, it was recently shown that Rad55-Rad57 can suppress defects in the physical interaction between Swi5-Sfr1 and Rad51 (Argunhan et al., 2020). Moreover, partially purified Rad55-Rad57 was shown to interact with purified Swi5-Sfr1, suggesting that, while capable of functioning independently of each other, the two auxiliary factors collaboratively promote HR in *S. pombe* (Argunhan et al., 2020). Despite the many studies on auxiliary factors, it is still unclear how they interact with Rad51. In this study, I characterised an acidic patch of *Schizosaccharomyces pombe* Rad51 that is comprised of three residues (E205, E206, and D209) and protrudes out of the exterior of the Rad51 nucleoprotein filament on the opposite face to the catalytic domain. I refer to this acidic patch as the PAP (Protruding Acidic Patch). An *S. pombe* strain harbouring mutations in all three residues of the PAP (*rad51-EED*) phenocopies *rad51*Δ, indicating that the PAP is essential for Rad51-dependent DNA repair. The DNA repair defect of *rad51-EED* is due to impairments in the mechanisms promoting recruitment of Rad51 to DNA damage sites. Pertinently, mutation of the PAP abolished the physical interaction with Rad52 and impaired complex formation with Rad55- Rad57. Furthermore, biochemical reconstitutions demonstrated that mutation of the PAP also impaired the interaction with Rad54, indicating that a single motif is important for the interactions with multiple auxiliary factors. I propose that the PAP is a fundamental, evolutionarily conserved motif of Rad51 that facilitates interactions with auxiliary factors and is therefore essential for recombinational DNA repair.