RecQ5 Interacts with Rad51 and Is Involved in Resistance of *Drosophila* to Cisplatin Treatment

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RecQ5 is a member of the RecQ family of DNA helicases. There are 5 RecQ members in humans. Defects in 3 of them, i.e., BLM, WRN, and RTS, cause Bloom, Werner, and Rothmund–Thomson syndromes, respectively. RECQL1 and RECQL5 have not been associated with any human disease, and their precise roles are unknown. Our previous study suggests that the lack of RecQ5, which is the *Drosophila* homolog of RECQL5, leads to the accumulation of DNA double-stranded breaks (DSBs). It is possible that RecQ5 is involved in DSB repair. However, little is known about this possible function of RecQ5 in DSB repair. Here, we report that Rad51 protein, which plays a critical role in DSB repair, interacted with RecQ5 *in vitro* and *in vivo* in *Drosophila*. The Rad51 protein interacted with the C-terminal region of RecQ5, as shown by the yeast two-hybrid method. Moreover, the C-terminal region of the RecQ5 protein and the central region of Rad51 interacted directly and specifically when examined by the glutathione-S-transferase pull-down method. Consistent with these results, when RecQ5 and Rad51 were co-expressed in *Drosophila* cells in culture, they became co-localized in nuclei and could be co-immunoprecipitated. Furthermore, RecQ5-deficient flies (*recq5*) were more sensitive to the chemotherapeutic agent cisplatin compared with wild-type ones. Also, Rad51 mutants (*rad51*) were more sensitive to cisplatin, with sensitivity similar to that of *recq5 rad51* double mutants. These data suggest that RecQ5 and Rad51 in *Drosophila* functioned for survival after the flies had been treated with cisplatin.

Key words RecQ; Rad51; cisplatin; repair; DNA damage; *Drosophila*

RecQ5 is a member of the RecQ family of DNA helicases, in which there are 5 members in humans. Defects in 3 of them, i.e., BLM, WRN, and RTS, cause Bloom, Werner, and Rothmund–Thomson syndromes, respectively, in humans. Genome instability and predisposition to cancer is a common feature of many RecQ helicase-deficient cells. Although RECQL1 and RECQL5 (human homolog of *Drosophila* RecQ5), the other 2 members of the RecQ family in humans, have not been associated with any human genetic diseases, *Recql5*-knock out mice show susceptibility to cancer,¹⁾ suggesting that RecQ5 may also play an important role in preventing cancer. However, the precise role of RecQ5 remains unknown.

In *Drosophila*, the frequencies of spontaneous and induced chromosomal aberrations are increased in RecQ5-mutant neuroblasts.²⁾ These data imply that double-stranded break (DSB) damage to DNA accumulates spontaneously in RecQ5 mutants.²⁾ Furthermore, the loss of maternally-derived RecQ5 leads to spontaneous mitotic defects in syncytial embryos. These mitotic defects are derived from anaphase DNA bridges, which link pairs of daughter nuclei. These nuclei concurrently exit from the cycle and are eliminated by *Drosophila* checkpoint kinase 2 tumor suppressor homolog (DmChk2)-dependent centrosome inactivation.³⁾ DmChk2 responds to DSB DNA lesions.⁴⁾ These findings suggest that the lack

of RecQ5 results in DSBs, which are recognized by the Chk2-checkpoint. However, little is known about the role of RecQ5 in DSB repair.

Cisplatin, which is *cis*-diamminedichloroplatinum(II), is a chemotherapeutic drug widely used for the treatment of several solid tumors, such as non-small-cell lung, ovarian, and testicular cancers. The mechanistic pathway by which this chemical exerts its function involves the induction of covalently linked DNA adducts.⁵⁾ Among these adducts, intrastrand G–G cross-links (65% of the adducts) are the major lesions induced by this drug,^{6,7)} and are considered to be responsible for the chemotherapeutic effects of cisplatin.^{7,8)} It is believed that the major pathway involves a DSB intermediate that is repaired by homologous recombination.⁹⁾ The recombination-repair system is necessary for DSB repair following cross-link removal.¹⁰⁾ Rad51 plays critical roles in DSB repair¹¹⁾ and DNA interstrand cross-link repair in eukaryotes.¹²⁾ Previously, we showed that cisplatin increased the level of RecQ5 protein in S2 cells.¹³⁾ It is thus possible that RecQ5 responds appropriately to DNA damage induced by cisplatin.

In this report, to address the role of RecQ5 in DNA repair, we analyzed the relationship between RecQ5 and Rad51, both *in vitro* and *in vivo*. Furthermore, we measured the sensitivity of *recq5*, *rad51*, and *recq5 rad51* *Drosophila* mutants to cisplatin. The results suggest that, in *Drosophila*, RecQ5 cooperated with Rad51 and was involved in the resistance to DNA damage induced by cisplatin.

The authors declare no conflict of interest.

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MATERIALS AND METHODS

Yeast Two-Hybrid Method To generate pASRECQ5ΔN, we cloned a DNA fragment corresponding to amino acids 464–1058 aa of RecQ5 (RecQ5 C, Fig. 1A) into pAS-2 (Clontech), as described earlier.¹⁴ For pACT2-Rad51, *Drosophila* Rad51 cDNA was inserted into *Bam*HI–*Sac*I sites of pACT2 (Clontech). Yeast strain Y190 was transformed with pASRECQ5ΔN to express the Gal4 DNA binding domain (Gal4BD)-RECQ5ΔN fusion protein as described earlier.¹⁴ The transformed clones were checked for growth on Trp[−] Leu[−] His[−] medium containing 25 mM 3-aminotriazole and assayed for β-galactosidase activity by performing the colony-lift assay.

Colony-Lift Filter Assay Fresh colonies were lifted onto Whatman #5 filters. After the filters had been frozen completely in liquid nitrogen, they were incubated at 30°C in 100 mM NaPO₄ (pH 7.0) containing 10 mM KCl, 1 mM MgSO₄, 0.3% β-mercaptoethanol, and 0.3 mg/mL 5-bromo-4-chloro-3-indolyl-D-galactopyranoside, as described earlier.¹⁴

Construction of Rad51 Truncated Series Plasmid LD35801 (Berkeley *Drosophila* Genome Project), which contains the Sp6 promoter and *Drosophila* Rad51 cDNA cloned into the 5' *Eco*RI site and the 3' *Xho*I site of pOT2 (for Rad51-W, Fig. 2A), was digested with *Ban*II and ligated to form Rad51-A (Fig. 2A). Plasmid LD35801 was digested with *Bst*BI and *Xcm*I and ligated to a linker (5'-CGGGCC ACAACCAAAATGGGC-3', 5'-CCCATTGTTGTG GCC-3') to produce Rad51-B (Fig. 2A). The Rad51-B was cut with *Sph*I and *Fsp*I, blunted, and ligated to a linker to make Rad51-C (Fig. 2A). For Rad51-D, Rad51-B was cut with *Bgl*II, and ligated to produce Rad51-D (Fig. 2A).

In Vitro Transcription/Translation The plasmids of the *Drosophila* Rad51 truncated series were used to generate [³⁵S]methionine-labeled proteins in the TNT-coupled reticulocyte system (Promega), as described earlier.¹⁵ The reaction mixtures were incubated at 30°C for 90 min, and the labeled proteins were immediately used for the glutathione-S-transferase (GST) pull-down assay.

GST Pull-Down Assay The C-terminal region (464–1058 aa) of RecQ5 fused with GST protein (GST-RecQ5 C) was prepared as described earlier.¹⁴ *In vitro* synthesized [³⁵S]-methionine-labeled Rad51 protein was incubated with GST fusion protein (1 μg) bound to glutathione Sepharose 4B beads (10 μL, Amersham Biosciences) in 150 μL of GST binding buffer (50 mM potassium phosphate pH 7.5, containing 150 mM KCl, 1 mM MgCl₂, 10% glycerol, 1% Triton X-100, and protease inhibitors [Roche]) for 2 h at 4°C. The beads were then washed 3 times in GST binding buffer, and the labeled proteins were separated by sodium dodecyl sulfate (SDS) 12% polyacrylamide gel electrophoresis.¹⁵ The gels were dried and analyzed by using a BAS2500 Imaging plate reader (Fuji), as described earlier.¹⁴

Immunocytology and Co-immunoprecipitation For construction of pCaSpeR-hs-3HA-RecQ5 (RecQ5 8–1058 aa), the *Apa*LI–*Ase*I fragment of RecQ5 cDNA from LD21474 (Berkeley *Drosophila* Genome Project) was ligated into the *Stu*I site of pCaSpeR-hs (a gift from Masayuki Miura). The resulting plasmid DNA was cut with *Hap*I and *Sac*II, and ligated to synthetic DNA (5'-CAC AACCAAATGTACCCCFACG ATG TGC CCG ATT ACG CCA TGT ACC GCTAC GAT GTG

CCC GAT TAC GCC ATG TAC CCC TAC GAC GTG CCC GAT TAC GCC GGC-3', 5'-CGGCGT AAT CGG GCA CGT CGT AGG GGT ACATG GCG TAA TCG GGC ACA TCG TAG CGG TACAT GGC GTA ATC GGG CAC ATC GTA GGG GTA CAT TTTGGTTGTG-3').

For construction of pCaSpeR-hs-FLAG-Rad51 (Rad51 11–336 aa), the *Ear*I–*Ase*I fragment of Rad51 cDNA¹⁶ and synthetic DNA (5'-CAC AACCAAATGGATTACAAGGA TGACGACGATAAG-3', 5'-CTAGCTTATCGTCGTCTGTCATCCTTAAATCCATTGTTGTG-3') were ligated to *Sac*II–*Xba*I sites in pCaSpeR-hs.

S2 cells were transfected by use of a SuperFect transfection kit (QIAGEN) and incubated at 27°C for 1 d. The heat-shock promoter was activated by 37°C treatment twice, for 30 min each time, with a 1-h interval between treatments. Cells were harvested at 18 h after heat induction. These cells were stained with 1:1000-diluted anti-FLAG antibody M2 (Sigma), anti-HA antibody HA.11 (Santa Cruz), and 0.3 μg/mL Hoechst 33258. S2 cells (in 100-mm dishes) were collected and lysed in 600 μL of lysis buffer (50 mM Tris-HCl, 150 mM NaCl, 0.5% NP-40, 1 mM PMSF). After centrifugation, the pellet was extracted with 600 μL of IP wash buffer (20 mM Hepes [pH 7.4], 150 mM NaCl, 1 mM EDTA, 1% polyoxyethylene (9) octylphenyl ether (NP-40), 1 mM phenylmethylsulfonyl fluoride (PMSF), protease inhibitor cocktail (complete mini Roche)) for 30 min, and sheared by extrusion through a 25G needle. The supernatant (180 μL) was precleaned with protein G Sepharose beads for 30 min, mixed with 2 μL of anti-HA antibody overnight, and then added to 20 μL of protein G Sepharose. The precipitates were resuspended and washed 3 times with 400 μL of IP wash buffer each time. The resulting pellet was resuspended in SDS sample buffer. Immunoblot analysis was carried out as described earlier.¹³

Cisplatin Sensitivity Sensitivity of developing larvae to cisplatin (*cis*-diaminedichloroplatinum(II), Sigma-Aldrich) was performed as described by Boyd *et al.*¹⁷ Adults were crossed in plastic vials such that mutant and control classes were generated among the progeny. Adults were removed after 2 d of egg laying. After 1 additional day, 600 μL of 0.05–0.50 mM cisplatin in water was added to the medium. To calculate sensitivity, we counted the adult progeny and then determined the ratio of mutant progeny to control progeny. Controls were balanced, heterozygous siblings in the same vial. The *recq5*^{D1} (*recq5*²) and *Df(3L)fz-D21* (*Drosophila* Genetic Resource Center) are deficient in RecQ5. *Dp(1; Y)BS*; *ru*¹ *st*¹ *e*¹ *spn-A*¹ *ca*¹/TM3, *Sb*¹ (*rad51*, Bloomington *Drosophila* Stock Center) contains a mutation for spindle-A, the Rad51 homolog, in *Drosophila*.¹⁸ For the double-mutant experiments, *Df(3L)fz-D21-sp-A*¹/TM6B were crossed to *recq5*^{D1}-*spn-A*¹/TM3. Progenies with the genotypes *recq5*^{D1}-*spn-A*¹/TM6B (control) and *recq5*^{D1}-*spn-A*¹/*Df(3L)fz-D21-sp-A*¹(*recq5 rad51* double mutant) were compared. Heterozygosity for *recq5* did not affect the survival of *rad51* mutants and *vice versa* (data not shown). At least 500 individuals were counted for each point. *Df(3L)fz-D21-sp-A*¹/TM6B and *recq5*^{D1}-*spn-A*¹/TM3 flies were generated by recombination between *spn-A*¹ and *Df(3L)fz-D21* or *recq5*^{D1}.²

RESULTS

Physical Interaction between Rad51 and RecQ5

Fig. 1. Rad51 Interacts Specifically with RecQ5 in the Yeast Two-Hybrid System

(A) Schematic representation of *Drosophila* RecQ5 molecule and RecQ5 C-terminal region (RecQ5 C). The closed and open boxes indicate the helicase domain and the extended region homologous in the RecQ family, respectively. (B, C) Y190 yeast cells containing lacZ and HIS3 reporter genes were co-transformed with plasmids pAS2-1 and pACT2 (–/–), pASRECQ5ΔN and pACT2 (RecQ5 C/–), pAS2-1 and pACT2-Rad51 (–/Rad51), pASRECQ5ΔN and pACT2-Rad51 (RecQ5 C/Rad51, duplicate) or pASRECQ5ΔN and pLAM5'-1 (RecQ5 C/lamin). The transformed clones were incubated on SD/– Trp/– Leu (B) or SD/– HIS (C) plates. The β-galactosidase activities were measured by performing the colony-lift filter assay (D) as described in Materials and Methods.

Previously we reported that *Drosophila* RecQ5 can complement several phenotypes of *sgs1* in yeast, including the synthetic growth defect with *srs2*, the suppression of slow growth in *top3*, the hypersensitivity to hydroxyurea and methyl methanesulfonate, and the elevated frequency of homologous recombination and sister chromatid exchange.¹⁹ These past findings suggest that RecQ5 exhibits an evolutionally conserved RecQ function *in vivo*. The above phenotypes of the *sgs1* mutation is related to Rad51-dependent homologous recombination.^{20,21} In addition, Sgs1, the only RecQ of *Saccharomyces cerevisiae*, interacts with yeast Rad51.²² It has thus been speculated that RecQ5 interacts with yeast Rad51, which is 77% similar to *Drosophila* Rad51.¹⁶ Therefore, we examined whether *Drosophila* Rad51 protein could interact with the *Drosophila* RecQ5 by using the yeast two-hybrid method. *Drosophila* RecQ5 consists of a short N-terminal region, a helicase domain, and a unique C-terminal region¹⁵ (Fig. 1A). The helicase domain in RecQ5 is in common with

Fig. 2. GST-Rad51 C Pull-Down of Rad51

(A) Schematic representation of truncated *Drosophila* Rad51 molecules. Closed and open boxes indicate Walker motifs A and B, respectively.³⁷ (B) Physical interaction between Rad51 protein and RecQ5. A series of [³⁵S]-methionine-labeled Rad51 proteins were pulled down by GST-fused RecQ5 protein. Lanes 1, 6, and 7, whole Rad51-W (1–336 aa); lanes 2 and 8, Rad51-A (1–103 aa); lanes 3 and 9, Rad51-B (62–336 aa); lanes 4 and 10, Rad51-C (62–206 aa); lanes 5 and 11, Rad51-D (62–123, 312–336 aa); lanes 1–5, 20% of input; lane 6, pull-down by GST alone; lanes 7–11, pull-down by GST-Rad51 C. Bars at the right side of the panel indicate molecular weight markers (kDa). The asterisks at the left side of the panel correspond to the expected molecular weights of the truncated Rad51 proteins (W*, A*, B*, C*, D*).

that in the RecQL1, BLM, WRN, and RTS proteins,¹⁵ and the relatively large C-terminal region is likely important in functionally distinguishing these members of the RecQ helicase family. To analyze the role of the C-terminal region in RecQ5, we previously performed a yeast two-hybrid method in a *Drosophila* embryo cDNA library by using the C-terminal region (residues 464–1058 aa) of RecQ5 as bait.¹⁴ Expression of the C-terminal region of RecQ5 as a Gal4BD fusion protein (Gal4BD-Rad51 C) was confirmed by immunoblot analysis of yeast cell lysates with anti-Rad51 and anti-Gal4BD antibodies (data not shown). We used Gal4BD-Rad51 C for simultaneous co-transformation with Gal4AD-Rad51 plasmid, empty vector or pAS-lamin as a negative control in the Y190 strain (Fig. 1B). Together with Gal4BD-Rad51 C, the Gal4AD-Rad51 plasmid activated the HIS3 reporter gene (Fig. 1C, RecQ5 C/Rad51), and also the LacZ reporter gene (Fig. 1D, RecQ5 C/Rad51). Neither empty vector nor pAS-lamin activated them with Gal4BD-Rad51 C (Figs. 1C, D, RecQ5 C/– & RecQ5 C/lamin). Gal4AD-Rad51 did not activate the reporter genes without RecQ5 C (Figs. 1C, D, –/Rad51). Thus, Gal4AD-Rad51 and Gal4BD-Rad51 C specifically activated both the HIS3 reporter gene and lacZ reporter gene in the yeast two-hybrid system, suggesting specific interaction

Fig. 3. Immuno Co-localization of RecQ5 and Rad51 to the Nuclei

Anti-HA staining of a field of cells transfected with HA-tagged RecQ5 (A), FLAG-tagged Rad51 (E) and both (I). The same field stained with anti-FLAG antibody (B,F,J) and with Hoechst 33258 for DNA (C,G,K). The merged image is also shown (D,H,L). Two cells can be seen expressing FLAG-Rad51 and HA-RecQ5 (I,J,L). In both cells the proteins had been localized to the nucleus.

between Rad51 and the C-terminal region of RecQ5 (Fig. 1).

RecQ5 Binding Domain in Rad51 To confirm the physical interaction of Rad51 with RecQ5, we performed a GST pull-down assay by using various fragments of Rad51 (Fig. 2A). The whole region of the Rad51 protein bound to GST-fused to the C-terminal region of the RecQ5 protein (Fig. 2B, lane 7), whereas GST alone did not pull down the whole Rad51 (Fig. 2B, lane 6), suggesting specific interaction between Rad 51 and the C-terminal region of RecQ5. The C-terminal region of RecQ5 also bound to the central domain, 62–336, 62–206 aa of Rad51 (Fig. 2B, lanes 9, 10, respectively), but not to the N-terminal (1–103, 62–123 aa) and C-terminal (312–336 aa) regions of Rad51 (Fig. 2B, lanes 8, 11), *in vitro*. This pattern indicates that amino acid residues 124–206 of Rad51 were important for the interaction with RecQ5.

RecQ5 Is Localized in Nuclei Next, we checked the localization of RecQ5 and Rad51 in *Drosophila* cells in culture. S2 cells were transfected with HA-tagged RecQ5 (HA-RacQ5 [8–1058 aa], Figs. 3A–D), FLAG-tagged Rad51 (FLAG-Rad51 [11–336 aa]), Figs. 3E–H) or both (Figs. 3I–L), and then stained with anti-HA antibody, anti-FLAG antibody, and Hoechst33258. RecQ5 was detected in the nucleoplasm (Fig. 3A).^{23–25} Rad51 itself has no detectable nuclear localization signal (NLS),²⁶ and so was not found in the nucleus; however, significant levels of it were found in the cytoplasm (Fig. 3F), as similarly reported previously.^{26,27} HA-RecQ5 and FLAG-Rad51 were co-localized in the nuclei (Figs. 3I–L). These data suggest that Rad51 became localized together with RecQ5 in the S2 nuclei.

HA-RecQ5 Co-immunoprecipitates with FLAG-Rad51

To test whether Rad51 would bind to RecQ5 *in vivo*, we prepared extracts of S2 cells that had been transfected with FLAG-Rad51, HA-RecQ5 or both. The proteins were immunoprecipitated with anti-HA antibody and then subjected to immunoblotting using anti-HA and anti-FLAG antibodies. The anti-HA antibody immunoprecipitated HA-RecQ5 (Fig. 4, lane 8), whereas it did not pull down FLAG-Rad51 when no

Fig. 4. HA-RecQ5 Co-immunoprecipitates FLAG-Rad51

S2 cells were transfected with FLAG-tagged Rad51 (lanes 2, 6), HA-tagged RecQ5 (lanes 4, 8) or both (lanes 3, 7). Cell extracts were prepared, immunoprecipitated with anti-HA antibody and analyzed by Western blotting using anti-HA antibody (HA) or anti-FLAG (FLAG), as described in Materials and Methods.

HA-RecQ5 was used for co-transfection (Fig. 4, lane 6). When cells were co-transfected with FLAG-Rad51 and HA-RecQ5, anti-HA antibody immunoprecipitated HA-RecQ5 along with FLAG-Rad51 (Fig. 4, lane 7). These data suggest that RecQ5 interacted physically with Rad51 in S2 cells.

Cisplatin Sensitivity of *recq5*, *rad51*, and *recq5 rad51* Mutants

Cisplatin is a chemotherapeutic agent that forms many different adducts with DNA, removal of some of which would require formation of a DNA DSB.²⁸ Previously, we showed that cisplatin increases the level of RecQ5 protein in S2 cells.¹³ To check the functional interaction between RecQ5 and Rad51, we measured the sensitivity of flies to cisplatin. Crosses were designed to produce heterozygotes and homozygotes. Cisplatin was added to the food of 2nd larvae. After eclosion, the number of adult flies was counted and the observed number of homozygotes was compared with the expected number of homozygotes from the observed number of heterozygotes. As a result, the *recq5* mutant was shown to be sensitive to cisplatin (Fig. 5), suggesting that RecQ5 was involved in resistance to DNA damage induced by cisplatin. The *rad51* flies (mutation in Rad51) were even more sensitive to the drug, as were the double mutant, *recq5 rad51*, ones (Fig. 5). The addition of the RecQ5 mutation to the Rad51 mutation

Fig. 5. Cisplatin Sensitivity of *recq5*, *rad51*, and *recq5 rad51* Mutants

Survival of *recq5* (open circle), *rad51* (closed box), and *recq5 rad51* (open triangle) mutants after treatment with cisplatin. Percent survival is given relative to the survival of wild-type controls (heterozygous siblings; see Experimental Procedures for details). Bars indicate standard deviation of at least 2 independent experiments. Cisplatin was added to the medium as described in Materials and Methods.

did not appear to have any additive or synergistic effect on cisplatin sensitivity. These data suggest that RecQ5 and Rad51 in *Drosophila* functioned for survival after the flies had been treated with cisplatin.

DISCUSSION

Here, we have shown both *in vitro* and *in vivo* the interaction between Rad51 and RecQ5. The physical interaction between RecQ5 and Rad51 was confirmed by using 3 independent experimental methods, *i.e.*, the yeast two-hybrid method (Fig. 1), GST pull-down method (Fig. 2), and co-immunoprecipitation (Fig. 4).

In *Drosophila* S2 cells in culture, Rad51 co-localized with RecQ5 in the nucleus (Fig. 3). Rad51 itself has no detectable NLS.²⁶⁾ A potential NLS with the sequence KRPRSQQENPESQPQKRPR exists in the C-terminus of human RecQ5β.²⁴⁾ Nuclear entry of the Rad51 protein likely requires its interaction with another protein that contains a functional NLS, such as Brca2,²⁷⁾ Rad51C,²⁶⁾ or RecQ5.

RecQ5-deficient flies (*recq5*) were more sensitive to the chemotherapeutic agent cisplatin than the wild-type ones; and the Rad51 mutants (*rad51*) were more sensitive to cisplatin than the RecQ5 mutants. It is possible that the RecQ5 function is partially substituted by other proteins in affording resistance to cisplatin. Alternatively, Rad51 and RecQ5 may function in separate pathways for resistance to cisplatin. To distinguish between these possibilities, we examined cisplatin sensitivity in the *recq5 rad51* double mutants. If these genes were required in separate pathways, the double mutants, which would be defective in both pathways, should show an additive or synergistic effect on cisplatin sensitivity. However, we found that these *recq5 rad51* double mutants were almost identical in sensitivity to the *rad51* single mutants (Fig. 5). These data imply a model in which RecQ5 would be required for a subset of Rad51-dependent repair of DNA damage caused by cisplatin, although other models could also account for it. Since cisplatin increases the level of RecQ5 protein in S2 cells,¹³⁾ Rad51 would cooperate with RecQ5 in response to

DNA damages in *Drosophila*.

Members of the RecQ family share homology in their RecQ helicase domain. Although the C-terminal regions of RecQ5, Sgs1, and BLM share little sequence homology, the C-terminal region of yeast Sgs1 (1299–1447 aa) interacts with yeast Rad51 in the yeast two-hybrid system.²²⁾ The C-terminal region of human BLM (1267–1417 aa) also interacts with human Rad51 in the yeast two-hybrid system.²²⁾ The C-terminal region of RecQ would be important for the interaction of Rad51 in the same species. The C-terminal region of HsRecQ5 binds with several proteins, such as HsRad51,^{29–32)} PCNA,^{29,31–33)} and RNP11.^{32,34–36)} The C-terminal region of RecQ5 would thus be expected to coordinate RecQ5 function with that of other proteins *in vivo*.

Rad51-W (1–336 aa), Rad51-B (62–336 aa), and Rad51-C (62–206 aa) bound to GST-RecQ5 (464–1058 aa), whereas Rad51-A (1–103 aa) and Rad51-D (62–123, 312–336 aa) did not (Fig. 2B). These data suggest that the central region of Rad51 (124–206 aa) was important for the interaction of Rad51 with RecQ5. This region contains the walker A motif (Fig. 2A),³⁷⁾ which corresponds to the ATP-binding site, and is conserved well in Rad51. Recently, a BRC repeat variant, named BRCv, was identified in HsRecQ5, and was shown to be essential for RecQ5 to interact with Rad51.³⁸⁾ BRC repeats interact with Rad51 and promote homologous recombination by regulating the Rad51 recombinase activity in the tumor suppressor protein Brca2 (breast cancer type 2 susceptibility protein).³⁹⁾ Exceptionally, only *Drosophila* RecQ5 contains 2 putative BRCv repeats; whereas BRCv is present as a single copy in RecQ5 from most species. It is possible that tandem BRCv repeats in *Drosophila* RecQ5 would work synergistically to activate homologous recombination in nuclei as seen in Brca2.⁴⁰⁾

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