INTRODUCTION

RecQ helicases are evolutionally conserved enzymes required for the maintenance of genome stability.1) Mutations in 3 of the 5 known human RecQ helicase genes, BLM, WRN, and RTS, cause distinct clinical disorders, i.e., Bloom’s, Werner’s, and Rothmund-Thomson syndromes, respectively, which are characterized by genome instability and a predisposition toward cancer.2–4) As with RecQ1, no human genetic disease has been attributed thus far to a deficiency in the RecQ5 gene. RecQ5 has 3 alternative splicing products, the longest of which, RecQ5β, is localized only in nuclei and corresponds to RECV5/QE in Drosophila melanogaster.5,6) Previously, we showed that RECQ5/QE complements several phenotypes of sgs1, a mutant of the sole RecQ in yeast, and suggested that RECQ5/QE has common and unique RecQ functions in comparison with BLM and WRN and that the C-terminal domain has a specific function in the absence of Top3.7) All RecQ family members contain a catalytic helicase domain that comprises 7 highly conserved motifs found in many DNA and RNA helicases. RECQ5/QE protein (1058 aa) is composed of a short N-terminal region, a helicase domain, and a long unique C-terminal domain (~700 aa). Although no disease has yet been genetically linked to a mutation in RECQ5, the prominent roles of RecQ helicase in the maintenance of genome stability suggest that RECQ5 helicase is likely to be important in vivo. To acquire a better understanding of RECQ5 function, we investigated protein interaction with the C-terminal domain of Drosophila melanogaster RECQ5/QE. A portion of Drosophila melanogaster retrotransposon mdg3, which corresponds to a nucleocapsid protein (gag-NC), was identified by use of the yeast two-hybrid system as interacting specifically with it. Glutathione S-transferase (GST) pull down experiments indicated that the mdg3 gag-NC bound mainly to an acidic region in the C-terminal domain of RECQ5/ QE, which is adjacent to the RecQ helicase domain. The helicase activity of RECQ5/QE was stimulated by mdg3 gag-NC protein in vitro. These data suggest that RECQ5/QE helicase interacts physically and functionally with mdg3 gag-NC through the acidic region and that RecQ homologue might be involved in retrotransposition and genomic stability.

Key words —— RecQ, nucleocapsid, retrotransposon, recombination, repair, integration
dent upon the N-terminal domain of WRN,8) or by mediating interactions with other proteins, for example, binding of p53 by the C-terminal domain of WRN.9) Since no obvious motif was identified in the C-terminal domain of RECQ5/QE,5) we decided to investigate its interaction with other proteins.

Here we screened a *Drosophila* cDNA library with a RECQ5/QE unique C-terminal sequence as bait by using the yeast two hybrid method. Retrotransposon *mdg3* protein was identified to have a specific interaction with RECQ5/QE. *mdg3* is a long terminal repeat (LTR)-containing retrotransposon of *Drosophila melanogaster* that is related to human immunodeficiency virus (HIV). The implications of these findings for the retroviral life cycle and genome stability are discussed.

**MATERIALS AND METHODS**

**Yeast Two-Hybrid Screening** —— To generate pASRECQEΔN, we cloned a DNA fragment corresponding to amino acids 464–1058 of the RECQ5/QE into pAS-2 (Clontech, California, U.S.A.), as described earlier.7) Yeast strain CG-1945 expressing the Gal4 DNA binding domain (Gal4BD)-RECQ5/QEΔN fusion protein (pASRECQEΔN) was used as a host strain to screen a 3–21 hr *Drosophila* embryo cDNA library as recommended by the supplier of the plasmids and strain (Clontech). The two-hybrid cDNA library inserted in the plasmid pACT2 was used to transform the host strain containing integrated *lacZ* and *HIS3* reporter genes by using a modification of the lithium acetate method of Gietz et al.8) pACT2 utilizes the constitutive *ADH1* promoter for expression of a cDNA-encoded protein as a fusion protein with the activation domain of Gal4 (Gal4AD). An estimated ~10⁷ transformants were plated on medium lacking Trp, Leu, and His in the presence of 6 mM 3-aminotriazole. False positives were eliminated by generating Leu+ Trp– strains and assaying them for β-galactosidase activity, by co-transforming pACT2 clones and a control bait, pAS-lamin (pLAM5Δ-1), and by retransforming pACT2 clones into Y190 harboring pASRECQEΔN and reassaying for β-galactosidase activity and growth on Trp– Leu– His– medium with 25 mM 3-aminotriazole. Plasmids were recovered from HIS3+ and lacZ-positive clones by rescue in *Escherichia coli* and used to retransform Y190 expressing GAL4BD-RECQ5/QEΔN or other controls to verify the specificity of the two-hybrid interaction. Assays were performed in triplicate. Those cDNAs that induced the reporter genes in a bait-specific manner were identified by DNA sequencing.

**Colonial 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.

**Preparation of Glutathione S-Transferase (GST)-Fusion Proteins** —— pET42b (Novagen, California, U.S.A.) plasmids were used to prepare GST fusion proteins. *ApaI*-Scal fragments of RECQ5/QE (8–1058 aa) derived from RECQ5/QE cDNA were blunt ended and ligated into the *Stul* site of pET42b. To construct the C-terminal domain fusion proteins (464–1058 aa), we removed the *BglII* fragments from pET42b-RECQ5/QE (8–1058 aa) plasmids. The acidic region of C-terminal domain (464–840 aa) plasmids was generated by ligating the *BglII-*XhoI fragments of RECQ5/QE into the *BglII*-XhoI sites of pET42b. The basic region of C-terminal domain (841–1058 aa) plasmids was generated by ligating the XhoI fragments of RECQ5/QE into the XhoI site of pET42b. GST fusion proteins were expressed and purified from BL21 Star (DE3) cells (Invitrogen, California, U.S.A.) transformed with the pET42b expression plasmids containing various portions of the RECQ5/QE fragments. Overnight cultures of these transformants were inoculated into LB at a 1 : 100 dilution and grown at 37°C to an *A₆₀₀* of 0.4–0.6. Isopropyl-β-D-thiogalactopyranoside was then added to a final concentration of 0.4 mM, and the cultures were allowed to grow for a further 3 hr. The cultures were harvested, resuspended in cold lysis buffer (20 mM potassium phosphate pH 7.5 containing 10% sucrose, 0.5 mM dithiothreitol, 1 mM PMSF, 1 mM EDTA, and 1 mg/ml lysozyme), and then chilled on ice for 30 min. Cells were then lysed by sonication, and the lysate was centrifuged at 9500 rpm for 60 min at 4°C. The supernatant was stored at –80°C and used for the GST pull down assay.

**In Vitro Transcription/Translation** —— The *Neol*-XhoI fragments of pACTmdg3gagNC were ligated into the *Neol*-SalI sites of pSPUTK (STRATAGENE, California, U.S.A.). The plasmids were used to generate [35S]methionine-labeled proteins in the TNT-coupled reticulocyte system (Promega, Wisconsin, U.S.A.). The reaction mixtures were incubated at 30°C for 90 min, and the
labeled proteins were immediately used for the GST pull down assay.

**GST Pull Down Assay** —– *In vitro* synthesized [35S]methionine labeled mdg3 gag-NC protein was incubated with GST fusion proteins (1 µg) bound to glutathione Sepharose 4B beads (10 µl, Amersham Biosciences, Buckinghamshire, U.K.) 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, Basel, Switzerland]) for 2 hr at 4°C. The beads were then washed 3 times in GST binding buffer, and the labeled proteins were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE). The gels were dried and analyzed by using a BAS2500 Imaging plate reader (Fuji, Tokyo, Japan).

**Helicase Assay** —– The SalI-HindIII fragment of pACTmdg3gagNC was cloned into XhoI-HindIII sites of pRSETA vector to produce His-tagged mdg3 gag-NC protein. The recombinant protein was purified by ProBond column chromatography according to the manufacturer’s manual (Invitrogen). Full-length RECQ5/QE protein was expressed in a baculovirus/insect cell system and was purified as described previously. Helicase assays were performed using 32P-labeled 20mer annealed with M13mp18 ssDNA, as described earlier. The reaction was initiated by the addition of RECQ5/QE protein and was incubated at 27°C for 10 min. The reaction products were separated on a 12% polyacrylamide gel. Dried gels were analyzed using the BAS 2500 Imaging plate reader (Fuji).

**RESULTS**

The yeast two-hybrid screening method was used to identify proteins that interacted with the C-terminal domain of RECQ5/QE. Since RECQ5/QE is expressed most preferentially in early embryos, Drosophila embryo cDNA Gal4AD fusion library DNA was introduced into CG1945 harboring Gal4BD fused to the C-terminal domain of RECQ5/QE and screened for being a His+ Leu+ Trp+ auxotroph as described in MATERIALS AND METHODS. Expression of the C-terminal domain of RECQ5/QE as a Gal4BD fusion protein was confirmed by immunoblot analysis of the yeast cell lysates with anti-RECQ5/QE and anti-Gal4BD antibodies (data not shown). Several colonies were obtained whose HIS3 and lacZ reporter genes had been activated. These colonies were considered to contain partners interacting with RECQ5/QE. To further verify this interaction, we isolated the Gal4AD-cDNA plasmids from these colonies and used them for simultaneous co-transformation with pASRECQ5/QEaN, empty vector, or pAS-lamin as a negative control in Y190 strain. Together with Gal4BD-RECQ5/QE C-terminal domain, a Gal4AD-cDNA clone, pACTmdg3gagNC, specifically activated the HIS3 reporter gene (Fig. 1A and 1B), and also the LacZ reporter gene (Fig. 1C), indicating that the activation depends on both the C-terminal domain of RECQ5/QE and the cDNA-encoded proteins. The cDNA sequence was 100% identical to the Gag nucleocapsid portion of mdg3 DNA (mdg3 gag-NC, 1091–1739 nt, Fig. 2). Thus, retrotransposon mdg3 gag-NC and the C-terminal domain of RECQ5/QE specifically activated HIS3 reporter gene and lacZ reporter gene in the yeast two hybrid system, suggesting specific interaction between them (Fig. 1).
mdg3 is an LTR-containing retrotransposon of Drosophila melanogaster that is related to HIV. mdg3 gag-NC was most related to HIV-2 nucleocapsid protein as well as HIV-1 nucleocapsid protein in having the characteristic 2 zinc fingers domains of the CCHC type (Fig. 2B).14)

To confirm physical interaction of mdg3 gag-NC with RECQ5/QE, we performed a GST pull down assay by using various fragments of RECQ5/QE. The mdg3 gag-NC bound the C-terminal domain of RECQ5/QE but not GST alone in the GST-pull down assay (Fig. 3), thus providing independent confirmation of the mdg3 gag-NC interaction with RECQ5/QE identified by the yeast two-hybrid system. The mdg3 gag-NC bound whole RECQ5/QE and the acidic region within the C-terminal domain, but not the basic region. These data suggest that the intact RECQ5/QE molecule interacts with mdg3 gag-NC through the acidic region.

Since mdg3 gag-NC binds to the RECQ5QE acidic region, which is adjacent to the helicase domain, this binding may have effects on helicase activity of RECQ5/QE. To investigate functional relationship between RECQ5QE and mdg3 gag-NC, we purified mdg3 gag-NC protein by tagging it with His, as described in MATERIALS AND METHODS, and examined its effect on the RECQ5QE helicase reaction (Fig. 4, lanes 3 and 5). The mdg3 gag-NC protein enhanced the helicase activity of RECQ5/QE (Fig. 4, lanes 4 and 6). In this condition, the mdg3 gag-NC itself did not cause any change in strand displacement (data not shown).

**DISCUSSION**

In this present study, we demonstrated that RECQ5/QE specifically bound to retrotransposon mdg3 gag-NC through its acidic region. In addition, RECQ5/QE helicase activity in vitro was enhanced by mdg3 gag-NC protein.

The mdg3 element is localized at 15–17 sites on different chromosomes of Drosophila melanogaster, with its location varying from one animal to another; and it move around mostly to the sites of intercalary heterochromatin.15) The ORF of the mdg3 sequence encodes a putative retrotransposon polyprotein of 1449 aa and a portion of gag-NC was most related to HIV-2 nucleocapsid protein having 2 zinc fingers domains of the CCHC type (Fig. 2B).14)
strongly activate magnesium-dependent LTR-DNA strand transfer by integrase (IN). This activation relies on the zinc fingers of NCp7. It was proposed that these zinc fingers function to stabilize IN at the LTR ends and to promote the formation of a nucleoprotein complex competent for integration. These properties of NCp7 are believed to be conserved among nucleocapsid proteins of retrotransposon and retrovirus origins. RECQ5/QE is mainly localized in the nucleus, and the integration process of retrotransposons, like retrovirus, occurs there. Although no in vitro system is yet available to study mdg3 integration, RECQ5/QE helicase activity would provide new insight into retrotransposition.

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REFERENCES


