A RECQ5–RNA polymerase II association identified by targeted proteomic analysis of human chromatin

Ozan Aygün*, Jesper Svejstrup*†, and Yilun Liu‡

*Mechanisms of Transcription Laboratory, Clare Hall Laboratories, Cancer Research UK London Research Institute, South Mimms, Hertfordshire EN6 3LD, United Kingdom; and ‡Department of Therapeutic Radiology, Yale University School of Medicine, New Haven, CT 06510

Communicated by Roger D. Kornberg, Stanford University School of Medicine, Stanford, CA, May 7, 2008 (received for review April 25, 2008)

Although the active forms of factors involved in DNA-related processes such as DNA replication, repair, and transcription are associated with chromatin, proteins are rarely purified from this source. Here, we describe a protocol for the isolation of chromatin-associated factors and use it to identify proteins interacting with human RNA polymerase II (RNAPII). Our data establish RECQ5 helicase as a bona fide RNAPII-associated protein. The RECQ5–RNAPII interaction is direct and is mediated by the RPB1 subunit of RNAPII, and RECQ5 appears to be the only member of the human RECQ family of helicases that associates with RNAPII. These data suggest an unexpected role for RECQ5 helicase at the interface of transcription and genomic stability.

genome integrity | transcription | BLM | WRN

Over the last decade, several analyses of the composition of human RNA polymerase II (RNAPII) complexes were reported. These studies typically used the soluble portion of a whole-cell extract or a high-salt extracted nuclear lysate as the starting point for purification (see refs. 1–5). Such studies identified various proteins that interact with soluble human RNAPII by either Western blotting or mass spectrometry. However, under native conditions, the majority of RNAPII in human nuclei is insoluble due to its strong, function-induced association with chromatin. Unfortunately, the conventional high-salt nuclear extraction methods (6) disrupt many physiologically relevant protein–protein interactions. Thus, the protein interactions of native human RNAPII associated with chromatin have not been exhaustively investigated.

To gain a more complete understanding of the mechanism of RNAPII transcription in human cells, we developed a strategy to isolate protein complexes from human chromatin under native conditions. This approach enables the separation and purification of chromatin-associated and nucleoplasmic (free) forms of nuclear proteins, respectively, under physiological salt conditions. We used it to perform an unbiased, targeted proteomic analysis of RNAPII complexes using mass spectrometry, which identified protein complexes that are specifically associated with the active form of human RNAPII and revealed proteins not previously known to interact with the polymerase.

We show that one of these proteins, the DNA helicase RECQ5, is a bona fide RNAPII-associated factor. RECQ5 is one of the five members of the human RECQ family of DNA helicases (7, 8). Members of this highly conserved family of DNA helicases play key roles in the maintenance of genome stability in all organisms examined. They are generally thought to act at the replication–recombination interface to suppress undesired recombination events that may occur due to stalled or damaged replication forks (7). Loss-of-function mutation in three members of the human RECQ family of helicases, namely, BLM, WRN, and RECQ4, have been directly associated with genetic diseases that are characterized by premature aging and predisposition to various types of tumors due to increased genomic instability (9–11). Moreover, mice lacking RECQ5 have genome instability and an increased incidence of cancer (12–14).

We show that among the five members of the human RECQ family, only RECQ5 is associated with human RNAPII. The RNAPII–RECQ5 interaction represents an unexpected connection between transcription and genomic stability.

Results

To perform a reliable and comprehensive proteomic study of the chromatin-associated form of human RNAPII, we established a generic strategy to purify chromatin-associated proteins under native conditions (Fig. 1*A*). HEK293 cells stably expressing a FLAG-tagged version of the RPB3 subunit of RNAPII at normal levels were established and used as a source for the purification. Highly purified and intact nuclei were prepared from 2.5×10^9 cells, the nuclei were lysed at a physiological salt concentration, and the soluble nucleoplasmic extract was recovered after centrifugation. The insoluble native chromatin was then resolubilized by using Benzonase, a pan-nuclease that efficiently digests both DNA and RNA. By applying this method, we were able to solubilize a significant proportion of even the strongest chromatin-binding proteins, such as histones, under physiological salt concentrations (see Fig. 7*A* in ref. 15) (data not shown). Next, RNAPII was purified from the nucleoplasmic and chromatin extracts by M2-agarose affinity chromatography. A mock, control purification was performed in parallel by using the same amount of untagged HEK293 cells to evaluate the degree of background in the RNAPII purification (Fig. 1*B Left*).

As expected, the core subunits of human RNAPII were observed in both purifications (Fig. 1*B Right* and *C*). Interestingly, however, the overall patterns of eluted proteins differed dramatically between the free and chromatin-associated polymerases. Among the two, the chromatin-associated RNAPII exhibited a much more complex proteomic composition, suggesting a great diversity of protein complexes associated with the enzyme that is actively engaged in transcription.

RECQ5 Is a Bona Fide RNAPII-Associated Protein in Human Chromatin. The low background in our purification prompted us to identify the protein bands observed in the elution profiles of human RNAPII. To this end, we excised the individual protein bands and performed mass spectrometric peptide identification analysis. Proteins identified by this analysis are labeled in Fig. 1*B* and 1*C*.

Relatively few RNAPII-interacting proteins were identified in the free form of the polymerase isolated from the nucleoplasmic extract. Significantly, the main such protein was FCP1, the CTD phosphatase involved in recycling RNAPII after transcriptional termination (Fig. 1B *Right*). A few other proteins, such as the general transcription factor TFIIF, also were identified.

Author contributions: O.A., J.S., and Y.L. designed research; O.A. and Y.L. performed research; O.A. and Y.L. contributed new reagents/analytic tools; O.A., J.S., and Y.L. analyzed data; and O.A., J.S., and Y.L. wrote the paper.

The authors declare no conflict of interest.

Freely available online through the PNAS open access option.

†To whom correspondence should be addressed. E-mail: j.svejstrup@cancer.org.uk.

© 2008 by The National Academy of Sciences of the USA

 www.pnas.org/cgi/doi/10.1073/pnas.0804424105

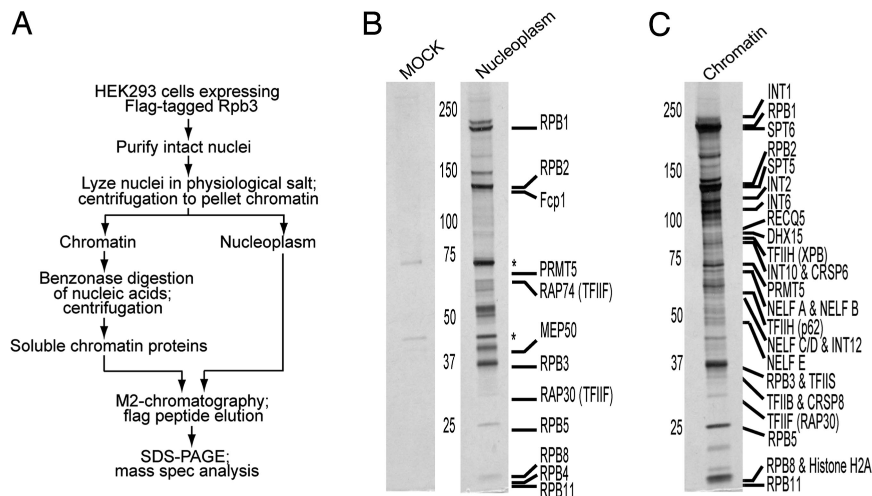

Fig. 1. Mass spectrometric analysis of purified human RNAPII complexes. (*A*) Outline of the purification procedure. (*B* and *C*) Equal amounts of the M2 chromatography eluates from control (Mock), nucleoplasm (*B*), and chromatin fractions (*C*) were fractionated on a 4–12% bis-Tris gradient protein gel and analyzed by silver staining. Asterisks indicate background protein bands that also are present in the control purification. The mock purification looked similar whether originating from nucleoplasm or chromatin. For MS analysis, a duplicate gel was analyzed by SYPRO Ruby staining, and protein bands were excised for mass spectrometric analysis. Identified proteins are labeled to the right of the respective protein bands.

In contrast to the limited number of proteins in the nucleoplasm, numerous proteins with known links to RNAPII were identified in the chromatin-derived polymerase fraction, whereas virtually no proteins were identified that could immediately be disregarded as contaminants (beyond the few also identified in the control purification) (Fig. 1*C Left*) (data not shown). Strikingly, and in agreement with the idea that it was the actively engaged versions of the polymerase that had been isolated, chromatin-associated human RNAPII was highly enriched with regulators of transcription elongation such as SPT5, SPT6, NELF complex, and TFIIS, the recently described Integrator complex, and the RNA helicase DHX15 (involved in pre-mRNA splicing), suggesting that the chromatin fraction, as expected, is a highly relevant starting point for discovering and characterizing transcription elongation factors. Moreover, several proteins involved in transcriptional initiation and promoter clearance (mediator, TFIIH, TFIIF, and TFIIB) also were found. Intriguingly, we also identified proteins that were not previously linked to RNAPII. Among these proteins was the helicase RECQ5. Because of the high confidence in the relevance of the RNAPII proteomic data, the numerous other identified proteins inspired, we decided to characterize this connection in further detail.

To verify the mass spectrometry data, we established HEK293 cells stably expressing FLAG-tagged human RECQ5 and we examined the RECQ5–RNAPII interaction in these cells by coimmunoprecipitation from the solubilized chromatin fraction. As shown in Fig. 2*A*, human RNAPII indeed coimmunoprecipitated with tagged RECQ5. We then performed a large-scale immunopurification of RECQ5 from native chromatin as described above for RNAPII (see Fig. 1*A*). Strikingly, a nice RECQ5–RNAPII complex was uncovered in this experiment, suggesting that a substantial fraction of RECQ5 is associated with RNAPII in human chromatin (Fig. 2*B*). Interestingly, RECQ5 also copurified with the DNA replication processivity factor PCNA and recombination protein RAD51, although these proteins were perhaps somewhat less stoichiometric than RNAPII in the fraction. Interactions between RECQ5 and that of PCNA and RAD51 have been reported by others previously (13, 16). Taken together, these results indicate that RECQ5 is a bona fide RNAPII-associated factor in human chromatin.

RNAPII Associates Directly with RECQ5 via the RPB1 Subunit. The next obvious question is whether RECQ5 interacts directly with human RNAPII or whether other proteins mediate the interaction. To address this question, we performed *in vitro* interaction

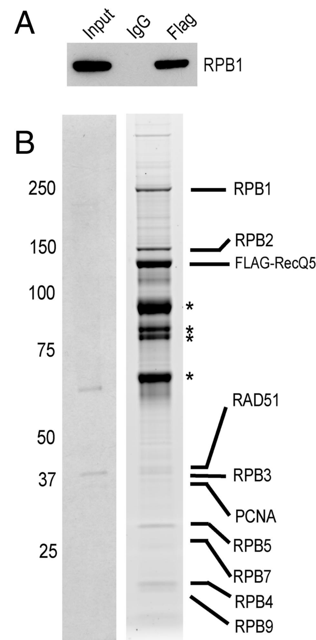

Fig. 2. RECQ5 is a bona fide RNAPII-associated protein in human chromatin. (*A*) Western blotting showing the coimmunoprecipitation of RNAPII with RECQ5. Native chromatin prepared from HEK293 cells stably expressing FLAG-RECQ5 was solubilized and used for immunoprecipitation either with anti-FLAG antibody or nonspecific mouse IgGs. RNAPII was detected with anti-Rpb1 (4H8) antibody. (*B*) Immunopurification of FLAG-RECQ5 from stable HEK293 cells. Native chromatin was prepared from FLAG-RECQ5 expressing stable cells as described in Fig. 1*A*. The M2 eluate was resolved by 4–12% bis-Tris gradient PAGE, stained by SYPRO Ruby, and analyzed with a PhosphorImager. Proteins identified by mass spectrometry are labeled to the right of the respective protein bands. Asterisks indicate four C-terminal degradation products of the FLAG-RECQ5 protein. The lane on the left represents the mock purification.

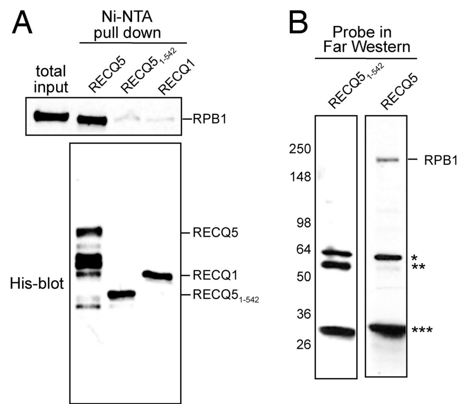

Fig. 3. Direct interaction between RNAPII and RECQ5 via the C-terminal region of RECQ5 and the RPB1 subunit of RNAPII. (*A*) Ni-NTA pull-down of RNAPII using purified, His-tagged RECQ5, RECQ5$_{1-542}$, and RECQ1. His-tagged proteins attached to Ni-NTA beads were incubated with purified RNAPII. After washing, the beads were boiled, and bound proteins were detected by SDS/PAGE, followed by Western blotting using 4H8 (RPB1, *Upper*) and anti-His antibodies (*Lower*). (*B*) Far Western blot analysis of RECQ5 interaction with individual RPB subunits of RNAPII. Purified RNAPII (from a RECQ5-FLAG purification) was separated by denaturing SDS/PAGE and transferred onto nitrocellulose membrane. Membranes were either incubated with recombinant His-RECQ5 (*Right*) or His-RECQ5$_{1-542}$ (*Left*), and membrane-bound, His-tagged proteins were then detected by anti-His antibody. Asterisks indicate contaminating mouse IgG heavy and light chains derived from the polymerase purification procedure that were recognized directly by secondary anti-mouse IgG antibody. The origin of the band detected better with the truncated RECQ5 probe (labeled by a double asterisk) is unknown.

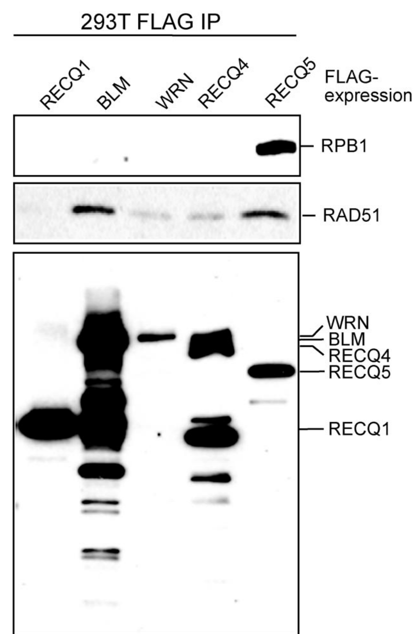

Fig. 4. RNAPII interacts with RECQ5, but not with other helicases in the RECQ5 family. FLAG-RECQ complexes were immunoprecipitated from 293T cells by using FLAG M2-agarose beads. The presence of RNAPII, RAD51, and FLAG-RECQ in each of the RECQ complexes was determined by SDS/PAGE, followed by Western blotting with mouse 4H8 mAb (RPB1, *Top*), rabbit anti-RAD51 (*Middle*), and rabbit anti-FLAG antibody (*Bottom*).

experiments using highly purified human RNAPII (15) and either His-tagged, full-length RECQ5, a truncated version (RECQ5$_{1-542}$), or the RECQ1 protein, from *Escherichia coli*. Ni-NTA pull-down experiments showed that RECQ5 indeed binds directly to RNAPII (Fig. 3*A*). In contrast, only very small amounts of RNAPII were pulled down with RECQ1 and the C-terminally truncated version of RECQ5, suggesting that the C-terminal region of RECQ5 is critical for polymerase interaction. We next investigated which subunit of human RNAPII is responsible for the interaction. To this end, we fractionated RNAPII by denaturing SDS/PAGE and analyzed its association with RECQ5 by Far Western blot analysis using the different His-tagged RECQ5 proteins as probes (Fig. 3*B*). This analysis indicated that binding of full-length RECQ5 is via the largest subunit of human RNAPII, RPB1 (Fig. 3*B Right*). The truncated RECQ5$_{1-542}$ protein, which could not bind to RNAPII, also failed to bind the RPB1 subunit in the Far Western blot analysis (Fig. 3*B Left*). Together, these results indicate that the RECQ5–RNAPII interaction is direct and mediated via the C-terminal region of RECQ5 and the RPB1 subunit of human RNAPII.

RECQ5 Is the only Member of the Human RECQ Family of Helicases That Interacts Strongly with RNAPII. We next wanted to investigate whether the interaction with RNAPII was unique for RECQ5 or whether other RECQ family helicases also associate with human polymerase. To this end, we established 293T cells stably expressing FLAG-tagged versions of all five members of the human RECQ helicase family. Each RECQ protein was then immunoprecipitated from the respective cell lines on M2-agarose beads, and the immunoprecipitates were analyzed by Western blotting. Previous studies suggested that RECQ family helicases play a role in recombinational repair through their interactions with RAD51 (see references in ref. 13). Consistent with these observations, we detected RAD51 in the RECQ5–FLAG complex by mass spectrometric analysis (Fig. 2*B*) and Western blot analysis by using α-RAD51 antibody (Fig. 4). RAD51 also was coimmunoprecipitated with BLM and, to a lesser extent, WRN and RECQ4 (Fig. 4). However, only RECQ5, but none of the other four RECQ proteins, coimmunoprecipitated significant amounts of RNAPII (Fig. 4). Mass spectrometric analysis of purifications of all five human RecQ helicases confirmed this result (Y.L., unpublished data). We conclude that RECQ5 is the only RECQ family helicase that associates with RNAPII.

Discussion

In this article, we report an approach to specifically purify and dissect the proteomic composition of chromatin-associated proteins. Using this strategy to identify proteins associated with RNAPII, we provide evidence for an interaction between RECQ5 DNA helicase and the active polymerase in human chromatin. Moreover, we show that RECQ5 associates directly with the RPB1 subunit of RNAPII and that RECQ5 is the only member of the human RECQ family that is associated with RNAPII.

Purification from Human Chromatin. The unexpected discovery of RECQ5 as an RNAPII-associated factor suggests that our understanding of the human RNAPII transcription machinery remains incomplete and also highlights the potential benefits of generally studying native protein complexes in the context of chromatin. It should be mentioned that, although we initially only observed RECQ5 in the chromatin-associated RNAPII fraction, RNAPII was found in both the chromatin-associated and the nucleoplasmic (or free) form of RECQ5 (Fig. 2) (data not shown), and the interaction of FLAG-RNAPII with RECQ5 also can be detected in the nucleoplasmic extract by Western blotting. We believe that starting purification from the chromatin fraction simply makes it possible to enrich/detect more interactions because of the functional setting; the same protein–protein interactions also can typically be detected in the nucle-

oplasm, but to a much smaller extent, and often only by Western blot analysis.

RECQ5 Is an RNAPII-Associated Factor. The results presented in this article provide strong evidence for a potential direct role of RECQ5 in some aspect of RNAPII transcription. Indeed, the interaction may link transcription and genomic stability in an unexpected manner. The RECQ family of helicases is known to play an essential role in the maintenance of genomic stability in all organisms studied so far (7, 8). Thus, they have been proposed to act at the interface of stalled replication forks and recombination, where they suppress undesired recombination events that may occur in response to replication fork collapse. Interestingly, recent genetic studies have shown that recq5$(^{-/-})$ mice are viable, but exhibit elevated levels of intrinsic recombination in the form of sister chromatid exchange (13, 14). Moreover, *Recq5*$(^{-/-})$ mice develop various types of tumors, further supporting an important role for RECQ5 in the maintenance of genomic stability in mammalian cells (13). In a recent study, RECQ5 was proposed to be the mammalian homologue of the budding yeast protein Srs2 (13), which is involved in restoring stalled replication forks and suppressing recombination events (17). However, although we cannot rule it out, the data presented here open a number of alternative scenarios to this possibility. First, RECQ5 might play completely separate roles in transcription and genome integrity. Second, RECQ5 may only be involved in transcription. It could then affect genome integrity indirectly through the regulation of, for example, repair genes. Finally, RECQ5 may be involved in suppressing transcription-associated recombination. Pioneering studies in budding yeast have demonstrated that a head-on collision of the transcription and replication machineries in S phase is a major source of recombination (18, 19), and evidence for a similar mechanism in mammalian cells has recently been obtained (20). Interestingly, we found that human RECQ5 copurifies with RAD51 and PCNA in addition to RNAPII, which could support the idea that RECQ5 plays a role at the interface of transcription, recombination, and replication. We also found that RECQ5 is the only member of the human RECQ family that is associated with RNAPII, supporting the idea that, among the five RECQ helicases, RECQ5 helicase has a specialized role. Further studies are required to understand the mechanism underlying elevated genomic instability in cells lacking RECQ5 and how it is related to RNAPII transcription through chromatin.

Materials and Methods

Plasmids. ORFs encoding human Rpb3 and RECQ5 were cloned into pIRES-puro (Clontech) and pCDNA4/TO (Invitrogen), respectively, both with a FLAG tag. cDNA encoding full-length human RECQ1, BLM, and RECQ4 were PCR-amplified from a HeLa cDNA library. WRN cDNA was amplified from pEGFP-WRN (21). Sequences were confirmed by DNA sequencing. To generate His-tagged RECQ1 and RECQ5, RECQ1, and RECQ5, full-length cDNA was cloned into pET16b between NdeI–BamHI and NdeI–XhoI sites, respectively. pET16b-RECQ5$_{1-542}$ was generated by BamHI digestion of pET16b-RECQ5 and recircularization of the plasmid. The Stop codon of RECQ5$_{1-542}$ was provided by vector sequence downstream of the BamHI-cloning site. To generate mammalian FLAG expression vectors for comparison of RECQ proteins, the AgeI–XhoI fragment of pEGFP-C1 (Clontech) was replaced by AgeI–XhoI fragment made with annealed oligonucleotides (5′-ACCGGTGTCGCCACCATGGGAGATTATAAAGATGATGATAAAGGAGATTAT-3′ and 5′-AAAGATGATGATAAACATATGCTCGAG-3′) containing a Kozak consensus sequence and 2× FLAG, generating pCMV-FLAG. RECQ1 was then cloned between NdeI–BamI sites of pCMV-FLAG. RECQ4 and RECQ5 were cloned between NdeI–XhoI sites, whereas BLM and WRN were cloned into the XhoI site of pCMV-FLAG.

Cell Culture and Establishment of Stable Cells. HEK293 and 293T cells were grown in monolayer in DMEM containing 10% FBS (Gibco) in a 5% CO_2/95% air atmosphere at 37°C. For large-scale protein purification, stable cells were adapted to growth in suspension culture containing RPMI and 5% FBS.

To generate HEK293 cells stably expressing FLAG-tagged Rpb3, cells were transfected with pIRESpuroFLAG-RECQ5 and selected in 0.5 μg/ml puromycin (Sigma). To obtain FLAG-RECQ5 stable cells, 293-TREX cells (Invitrogen) were transfected with pCDNA4/TO-FLAG-RECQ5 and selected with 600 μg/ml zeocin (Invitrogen) and 5 μg/ml blasticidin (Invitrogen). Expression of FLAG-RECQ5 is controlled by 1 μg/ml tetracycline (Sigma). To generate 293T cells stably expressing FLAG-RECQ constructs, either ApaLI linearized or circular pCMV-FLAG plasmids were cotransfected with pGK vector carrying the puromycin resistance gene into 293T cells by using FuGENE HD transfection reagent (Roche). Then, 24 h after transfection, cells were trypsinized and plated in 96-well plates with medium containing 2 μg/ml puromycin. Single clones were obtained and screened for FLAG-tagged protein expression by Western blot analysis. Positive clones were further amplified to establish stable cell lines.

Immunopurification of Protein Complexes from Native Chromatin. Stable HEK293 clones were grown in large-scale suspension cultures up to 6–12 liters. Cells were lysed with cytoplasmic lysis buffer [10 mM Tris·HCl (pH 7.9), 0.34 M sucrose, 3 mM $CaCl_2$, 2 mM magnesium acetate, 0.1 mM EDTA, 1 mM DTT, 0.5% Nonidet P-40 and protease inhibitors] (Roche), and intact nuclei were pelleted by centrifugation at 3,500 × *g* for 15 min. Nuclei were washed with cytoplasmic lysis buffer without Nonidet P-40 and then lysed with nuclear lysis buffer [20 mM HEPES (pH 7.9), 3 mM EDTA, 10% glycerol, 150 mM potassium acetate, 1.5 mM $MgCl_2$, 1 mM DTT, 0.1% Nonidet P-40, and protease inhibitors] by homogenization. The nucleoplasmic fraction was cleared by centrifugation at 15,000 × *g* for 30 min. The chromatin-enriched pellet was then resuspended in nuclease incubation buffer [150 mM Hepes (pH 7.9), 1.5 mM $MgCl_2$, 150 mM KOAc, 10% glycerol, and protease inhibitors], and DNA and RNA in the suspension were digested with 0.15 unit/μl benzonase (Novagen). The sample was cleared by centrifugation at 20,000 × *g* for 30 min, and the supernatant containing the solubilized native chromatin proteins was collected. For negative control purification, the same extracts were prepared from the same amount of untagged HEK293 cells.

Nucleoplasmic and chromatin extracts were separately applied to M2-agarose beads (Sigma) (≈400 μl of packed beads per 6 liters of starting culture) and incubated for 4 h at 4°C. After binding of the protein complexes, beads were washed extensively with the washing buffer [20 mM HEPES (pH 7.9), 150 mM KCl, 0.5 mM EDTA, 0.1% Triton X-100, 10% glycerol, and protease inhibitors]. Finally, purified protein complexes were eluted by using FLAG elution buffer [20 mM HEPES (pH 7.9), 150 mM KCl, 0.5 mM EDTA, 400 μg/ml 3× FLAG peptide, 10% glycerol, and protease inhibitors]. Eluates were resolved in 4–12% bis-Tris gradient PAGE and analyzed by silver staining. When the protein bands were excised for mass spectrometry, the gels were stained with SYPRO Ruby (Invitrogen).

Mass Spectrometric Analysis. The sample peptides were generated by the *in situ* tryptic digestion of the gel bands. LC/MS/MS analysis of the peptides was performed by a Thermo LTQ-XL ion trap mass spectrometer. The resulting mass spectrometry data were then searched against the SwissProt protein database by using the SEQUEST protein-searching algorithm.

Antibodies. The antibodies used were rabbit anti-FLAG pAb (Sigma), mouse anti-His mAb (Clontech), and mouse anti-pCTD mAb 4H8 (Upstate Biotechnology). Rabbit anti-RAD51 antibody was from Stephen C. West (Cancer Research UK).

Protein Expression and Purification. His-tagged RECQ1, RECQ5, and RECQ5$_{1-542}$ were overexpressed in *E. coli* BL21(DE3)-RIPL (Stratagene). Cells were grown at 16°C for 6 h after the addition of 250 μM IPTG to induce protein expression. Cells were collected by centrifugation and resuspended in lysis buffer A [50 mM KPO_4 (pH 7.5), 0.5 M KCl, 10% glycerol, and 0.2% Triton-X-100] containing 5 mM imidazole, 1 mg/ml lysozyme, and protease inhibitors. After incubating on ice for 30 min, the lysates were sonicated, and insoluble material was removed by centrifugation. The supernatant was loaded onto Ni-NTA (Qiagen), and the beads were washed with 50 volumes of lysis buffer A containing 100 mM immidazole. His-tagged proteins were either eluted from Ni-NTA with Ni elution buffer [50 mM KPO_4 (pH 6.0), 0.05% Triton X-100, 0.3 M KCl, 0.5M immidazole, and 10% glycerol] or kept as Ni-NTA bound for nickel pull-down analyses.

Nickel Pull-Down and Far Western Blot Analyses. For nickel pull-down analyses, His-tagged proteins bound to Ni-NTA were incubated with purified

BIOCHEMISTRY

human RNAPII (15) in binding buffer [40 mM Tris (pH 7.5), 0.02% Triton X-100, 0.25 M KCl, and 10% glycerol] containing 0.5 mg/ml BSA. The reaction was incubated for 2 h at 4°C, and the beads were washed five times with 50 volumes of binding buffer. The bound proteins were eluted by SDS/PAGE loading buffer and fractionated on SDS/PAGE, followed by Western blot analyses. To carry out Far Western blot analyses, RNAPII from a FLAG-RECQ5 purification was separated on SDS/PAGE, followed by Western transfer to nitrocellulose membrane. The membranes were blocked with 5% milk in PBS and incubated overnight with PBS containing 5% BSA, 0.01% Tween-20, and either 0.5 ng/μl His-RECQ5 or His-RECQ5$_{1-542}$ at 4°C with rotation. The membranes were washed three times with PBS containing 0.01% Tween-20, and the His-tagged proteins were detected by further incubation with mouse anti-His antibody, followed by anti-mouse horseradish peroxidase.

ACKNOWLEDGMENTS. We thank Mark Skehel, the Protein Analysis and Proteomics Laboratory at Cancer Research UK Clare Hall Laboratories, and the Gygi Taplin Mass Spectrometry Facility at Harvard University for mass spectrometry analysis of proteins; Anindya Roy for help and advice throughout this project; Rebecca L. Barnes for her contribution to constructing the pCMV-FLAG vector and establishing a 293T:FLAG-RECQ4 stable cell line; and Stephen C. West (Cancer Research UK, South Mimms, U.K.) for his generous gift of anti-RAD51 antibody. This work was supported by a Cancer Research U.K. grant (to J.S.) and a Bloom's Syndrome Foundation and American Cancer Society grant (to Y.L.).

1. Cho H, *et al*. (1998) A human RNA polymerase II complex containing factors that modify chromatin structure. *Mol Cell Biol* 18:5355–5363.
2. Maldonado E, *et al*. (1996) A human RNA polymerase II complex associated with SRB and DNA-repair proteins. *Nature* 381:86–89.
3. Jeronimo C, *et al*. (2007) Systematic analysis of the protein interaction network for the human transcription machinery reveals the identity of the 7SK capping enzyme. *Mol Cell* 27:262–274.
4. Jeronimo C, *et al*. (2004) RPAP1, a novel human RNA polymerase II-associated protein affinity purified with recombinant wild-type and mutated polymerase subunits. *Mol Cell Biol* 24:7043–7058.
5. Robert F, *et al*. (2002) A human RNA polymerase II-containing complex associated with factors necessary for spliceosome assembly. *J Biol Chem* 277:9302–9306.
6. Dignam JD, Lebovitz RM, Roeder RG (1983) Accurate transcription initiation by RNA polymerase II in a soluble extract from isolated mammalian nuclei. *Nucleic Acids Res* 11:1475–1489.
7. Bachrati CZ, Hickson ID (2008) RecQ helicases: Guardian angels of the DNA replication fork. *Chromosoma*, 117:219–303.
8. Hickson ID (2003) RecQ helicases: Caretakers of the genome. *Nat Rev Cancer* 3:169–178.
9. Ellis NA, *et al*. (1995) The Bloom's syndrome gene product is homologous to RecQ helicases. *Cell* 83:655–666.
10. Yu CE, *et al*. (1996) Positional cloning of the Werner's syndrome gene. *Science* 272:258–262.
11. Kitao S, *et al*. (1999) Rothmund–Thomson syndrome responsible gene, RECQL4: Genomic structure and products. *Genomics* 61:268–276.
12. Branzei D, Foiani M (2007) RecQ helicases queuing with Srs2 to disrupt Rad51 filaments and suppress recombination. *Genes Dev* 21:3019–3026.
13. Hu Y, *et al*. (2007) RECQL5/Recql5 helicase regulates homologous recombination and suppresses tumor formation via disruption of Rad51 presynaptic filaments. *Genes Dev* 21:3073–3084.
14. Hu Y, *et al*. (2005) Recql5 and Blm RecQ DNA helicases have nonredundant roles in suppressing crossovers. *Mol Cell Biol* 25:3431–3442.
15. Anindya R, Aygun O, Svejstrup JQ (2007) Damage-induced ubiquitylation of human RNA polymerase II by the ubiquitin ligase Nedd4, but not cockayne syndrome proteins or BRCA1. *Mol Cell* 28:386–397.
16. Kanagaraj R, *et al*. (2006) Human RECQ5beta helicase promotes strand exchange on synthetic DNA structures resembling a stalled replication fork. *Nucleic Acids Res* 34:5217–5231.
17. Macris MA, Sung P (2005) Multifaceted role of the *Saccharomyces cerevisiae* Srs2 helicase in homologous recombination regulation. *Biochem Soc Trans* 33:1447–1450.
18. Prado F, Aguilera A (2005) Impairment of replication fork progression mediates RNA polII transcription-associated recombination. *EMBO J* 24:1267–1276.
19. Aguilera A (2002) The connection between transcription and genomic instability. *EMBO J* 21:195–201.
20. Gottipati P, Cassel TN, Savolainen L, Helleday T (2008) Transcription-associated recombination is dependent on replication in mammalian cells. *Mol Cell Biol* 28:154–164.
21. von Kobbe C, Bohr VA (2002) A nucleolar targeting sequence in the Werner syndrome protein resides within residues 949–1092. *J Cell Sci* 115:3901–3907.