A core hSSB1–INTS complex participates in the DNA damage response

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Summary

Human single-stranded DNA-binding protein 1 (hSSB1) plays an important role in the DNA damage response and the maintenance of genomic stability. It has been shown that the core hSSB1 complex contains hSSB1, INTS3 and C9orf80. Using protein affinity purification, we have identified integrator complex subunit 6 (INTS6) as a major subunit of the core hSSB1 complex. INTS6 forms a stable complex with INTS3 and hSSB1 both *in vitro* and *in vivo*. In this complex, INTS6 directly interacts with INTS3. In response to the DNA damage response, along with INTS3 and hSSB1, INTS6 relocates to the DNA damage sites. Moreover, the hSSB1–INTS complex regulates the accumulation of RAD51 and BRCA1 at DNA damage sites and the correlated homologous recombination.

Key words: DNA damage response, hSSB1–INTS complex, Homologous recombination

Introduction

The integrity of our genome is constantly challenged by various types of DNA damage induced by replication errors or environmental hazards. Among those different types of DNA damage, DNA double-strand breaks (DSBs) are the most deleterious as they can cause chromosomal loss, fusion and translocation. Once DSBs occur, cells can sense and repair them by activating evolutionarily conserved pathways (Harper and Elledge, 2007; Rouse and Jackson, 2002; Sancar et al., 2004). Among these DSB repair pathways, homologous recombination (HR) repair is an error-free type of repair that ensures genomic stability (Aguilera and Gómez-González, 2008; Bartek et al., 2004; Ciccia and Elledge, 2010; Lukas and Bartek, 2004). It relies on the presence of an undamaged homologous DNA sequence as template to repair the broken genomic DNA. It is believed that the HR pathway is initiated by a 5′ to 3′ end resection at both sides of the broken site, generating a 3′ single-stranded DNA (ssDNA), which is motorized by RAD51 to invade the sister chromatid for the replication and recombination (Cejka et al., 2010; Gravel et al., 2008; Hopkins and Paull, 2008; Huertas, 2010; Mimitou and Symington, 2008; Nicolette et al., 2010; Niu et al., 2010; Takeda et al., 2007; West, 2003; Williams et al., 2008).

Previous evidence shows that human single-stranded DNA-binding protein 1 (hSSB1) plays an import role during HR repair (Richard et al., 2008). hSSB1 is a 211-residue polypeptide containing an oligonucleotide/oligosaccharide-binding (OB) fold domain at the N-terminus. Following DNA damage, hSSB1 quickly relocates to DNA damage sites and regulates focus formation of other DNA damage repair proteins, such as BRCA1 and RAD51 (Huang et al., 2009; Li et al., 2009; Richard et al., 2008; Skaar et al., 2009; Zhang et al., 2009b). Thus, depletion of hSSB1 impairs DSB repair. In response to DNA damage, hSSB1 could be phosphorylated by ATM and regulate ATM-dependent cell cycle checkpoint activation (Huang et al., 2009; Li et al., 2009; Richard et al., 2008; Skaar et al., 2009; Zhang et al., 2009b). From initial protein affinity purifications, hSSB1 was identified to associate with integrator complex subunit 3 (INTS3) and C9orf80 (also known as SOSS complex subunit C), to form a multi-subunit complex. C9orf80 is a 104-residue polypeptide and is considered as an adapter in the complex, whereas INTS3 is a much larger protein with 1043 residues and it directly interacts with the OB fold domain of hSSB1 and C9orf80 through its N-terminus (Huang et al., 2009; Li et al., 2009; Skaar et al., 2009; Zhang et al., 2009b). Similar to hSSB1, both INTS3 and C9orf80 are recruited to DNA damage sites and participate in HR repair (Huang et al., 2009; Li et al., 2009; Skaar et al., 2009; Zhang et al., 2009b). However, the function of INTS3 in the complex remains elusive. In this study, we identified INTS6 as another important subunit in the hSSB1–INTS complex. INTS6 participates in the DNA damage response through direct interaction with INTS3. In response to DNA damage induction, INTS6 relocates to the DNA damage sites and regulates homologous recombination (HR) by stabilizing RAD51 and RPA at DNA damage sites.

Results and Discussion

INTS6 is a major subunit in the hSSB1–INTS complex

The hSSB1–INTS3–C9orf80 complex has been shown to participate in Ataxia-telangiectasia mutated kinase (ATM) activation and HR repair following DSBs (Huang et al., 2009; Li et al., 2009; Richard et al., 2008; Skaar et al., 2009; Zhang et al., 2009b). To examine whether other subunit(s) may exist in this complex, we performed unbiased tandem affinity purification using INTS3 as the bait. Mass spectrometry analysis revealed that besides hSSB1, INTS6 (also known as DDX26a) is another major partner of INTS3 (Fig. 1A; supplementary material Fig. S1A). Previous studies have shown that INTS6 is a subunit in the

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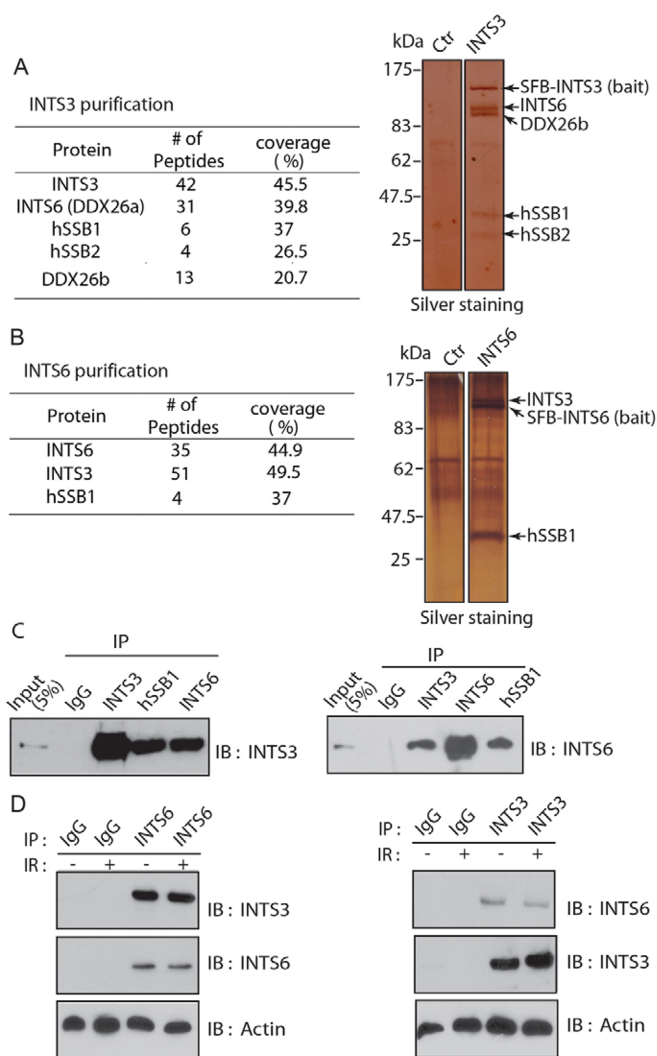

Fig. 1. INTS6 associates with INTS3 and hSSB1. (A,B) Cell lysates of 293T cell stably expressing SFB-tagged (S tag, FLAG tag, and streptavidin-binding peptide tag) INTS3 (A) or INTS6 (B) or control (Ctr) were subjected for tandem affinity purifications. Final elutes were separated by 12% SDS-PAGE and visualized by silver staining and mass spectrometry. The results of peptide coverage are shown in the table. (C) INTS6 interacts with INTS3 and hSSB1. 293T cells were lysed with NETN buffer and then analyzed by immunoprecipitation (IP) and western blotting with anti-INTS3, anti-hSSB1 or anti-INTS6 antibodies. Whole cell lysates were also blotted (input). An irrelevant IgG was used as the IP control. (D) The interaction between INTS3 and INTS6 is independent of DSBs. 293T cells were treated with 0 or 10 Gy of IR. One hour after IR, cells were lysed and analyzed with the indicated antibodies. A blot with anti-β-actin antibody was used as a protein loading control.

integrator complex that associates with hSSB1 (Baillat et al., 2005; Skaar et al., 2009). Using the stringent purification, we did not identify other subunits of the integrator complex that associate with INTS3. Meanwhile, we also identified hSSB2 and DDX26b, paralogs of hSSB1 and INTS6, respectively. Because the transcription of the hSSB2 and DDX26b genes is much lower than that of hSSB1 and INTS6 (supplementary material Fig. S1B) (Richard et al., 2008; Zhang et al., 2009b), our study mainly focused on hSSB1 and INTS6. To validate our initial purification results, we performed reverse purification using INTS6 as the bait, and confirmed by mass spectrometry analysis, that INTS3 and hSSB1 were associated with INTS6 (Fig. 1B; supplementary material Fig. S1C), indicating that INTS6 is indeed a major subunit in the hSSB1–INTS3 complex.

We next examined the interactions between INTS6, INTS3 and hSSB1. We first generated an anti-INTS6 antibody against endogenous INTS6, which recognized a polypeptide with a molecular mass of 100 kDa. Moreover, knockdown of INTS6 by siRNA suppressed the expression of this protein, suggesting that our antibody recognizes endogenous INTS6 specifically (supplementary material Fig. S1D). Using this antibody, we performed co-immunoprecipitation (co-IP) and reverse co-IP. The results showed that INTS6 indeed associates with INTS3 and hSSB1 endogenously (Fig. 1C).

Because both hSSB1 and INTS3 participate in the DNA damage response, we investigated whether DNA damage regulates the interaction between INTS3 and INTS6. Cells were treated with 10 Gy of ionizing radiation (IR). Lysates were collected and subjected to co-IP and reverse co-IP. We found that the interaction between INTS3 and INTS6 occurs constitutively regardless of the IR treatment, suggesting that assembly of the complex is independent of DNA damage (Fig. 1D).

The C-terminus of INTS3 directly binds to the C-terminus of INTS6

To study how the hSSB1–INTS complex is assembled, we use siRNAs to knock down INTS3 and examine the association between hSSB1 and INTS6 *in vivo*. As shown in Fig. 2A, downregulation of INTS3 by specific siRNA treatment abolished the association between hSSB1 and INTS6. However, depletion of hSSB1 or INTS6 did not affect the interaction between INTS3 and INTS6 or INTS3 and hSSB1 (Fig. 2A), indicating that INTS3 mediates the interaction between INTS6 and hSSB1.

We and others have demonstrated that the N-terminus of INTS3 interacts with hSSB1 (Huang et al., 2009; Skaar et al., 2009; Zhang et al., 2009b). From the protein folding analysis we determined that INTS3 contains two well-folded areas that are linked by a small hinge region (supplementary material Fig. S2A). Therefore, we generated a series of internal deletion mutants of INTS3 according to its folded structure to examine which region directly interacts with INTS6. As shown in Fig. 2B, deletion of the C-terminal 881–1042 amino acids of INTS3 abolished the interaction between INTS3 and INTS6. Similar to the N-terminal region, the C-terminal region of INTS3 is also evolutionarily conserved (supplementary material Fig. S2B), suggesting that this region could serve as a protein–protein binding motif. To examine which region of INTS6 interacts with INTS3, we also generated internal deletion mutants of INTS6 based on the predicted protein folding (supplementary material Fig. S2A). We found that D8, the C-terminal deletion mutant of INTS6, abolished the interaction with INTS3 (Fig. 2C). Similar to the D8 mutant of INTS6, the C-terminal deletion mutant of DDX26b also abolished its interaction with INTS3 (supplementary material Fig. S2C). Interestingly, both INTS6 and DDX26b contain a highly conserved region that is also present in other DDX26 orthologous (supplementary material Fig. S2D). Taken together, these results suggest that the C-terminus of INTS6 and INTS3 interact with each other to form a stable complex.

We then examined which region of hSSB1 was required for its interaction with INTS6. As shown in Fig. 2D, the N-terminal

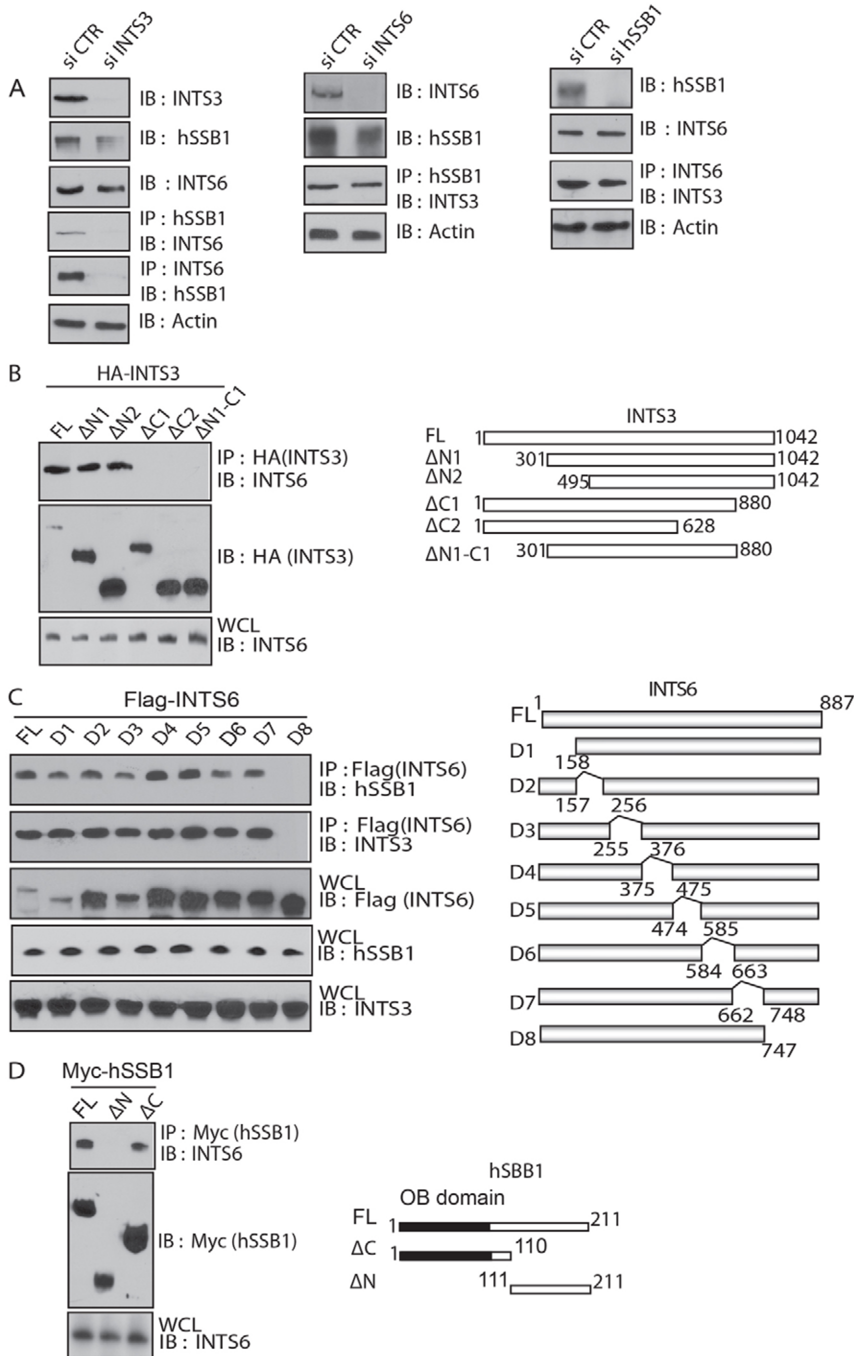

Fig. 2. Mapping the interaction regions of INTS6 and INTS3. (**A**) INTS3 mediates the interaction between INTS6 and hSSB1. U2OS cells were treated with control, INTS3, INTS6 or hSSB1 siRNA. Cell lysates were analyzed by IP and western blotting with the indicated antibodies. (B) The C-terminus of INTS3 interacts with INTS6. HA-tagged wild-type INTS3 and deletion mutants (as shown on the right) were expressed in 293T cells. Cell lysates were analyzed using the indicated antibodies. The expression levels of exogenous INTS3 proteins were examined by western blotting with anti-HA antibody. (C) The C-terminus of INTS6 interacts with INTS3. FLAG-tagged wild-type INTS6 or DDX26b and their deletion mutants were expressed in 293T cells. The interactions between INTS6 and INTS3 were examined by IP and western blotting with the indicated antibodies. (D) The OB fold domain of hSSB1 interacts with INTS3. Myc-tagged wild-type and deletion mutants of hSSB1 were expressed in 293T cells. The interactions between hSSB1and INTS6 were examined by IP and western blotting with the indicated antibodies.

deletion mutant, but not the C-terminal deletion mutant of hSSB1, disrupted the interaction with INTS6. Because the N-terminal OB fold domain of hSSB1 directly binds INTS3, it is probable that INTS3 mediates the association between hSSB1 and INTS6.

INTS6 forms a stable complex with INTS3, hSSB1 *in vitro* and *in vivo*

To investigate whether endogenous hSSB1, INTS3 and INTS6 form a stable complex *in vivo*, we analyzed these proteins in nuclear extracts of 293T cells using size-exclusion chromatography. As shown in Fig. 3A, hSSB1, INTS3 and INTS6 co-existed in cell lysate fractions corresponding to a molecular mass of ~300 to ~500 kDa.

To dissect the protein–protein interaction within the complex, we next investigated the association between hSSB1, INTS3 and INTS6 *in vitro*. Sf9 insect cells were co-infected with baculoviruses encoding SBP-tagged (streptavidin-binding peptide tag) hSSB1, SFB-INTS6 and glutatione *S*-transferase (GST)–INTS3. When INTS3 was pulled down using glutathione beads, both hSSB1 and INTS6 were found to associate with INTS3 (Fig. 3B). When SFB–INTS6 was co-expressed with GST–INTS3 N-terminus or C-terminus in Sf9 cells, we found that only the C-terminus, but not the N-terminus of INTS3,

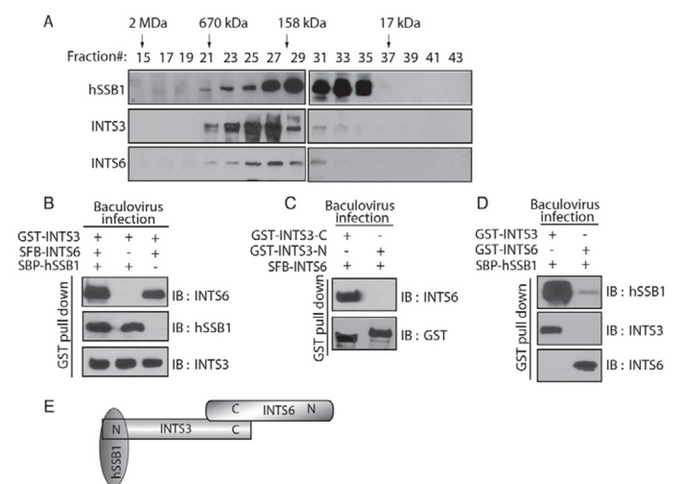

Fig. 3. INTS6 forms a stable complex with INTS3, hSSB1 *in vitro* and *in vivo*. (A) 293T cell extracts were analyzed by size-exclusion chromatography on a Superose 6 gel filtration column. Proteins eluted from the indicated fractions were separated by SDS-PAGE and analyzed by western blotting using antibodies against INTS3, INTS6 and hSSB1. (B–D) Each recombinant protein was harvested from Sf9 cells with individual or combinations of virus infection and examined by western blotting with the indicated antibodies. (E) Schematic of direct binding of the INTS3 with INTS6 and hSSB1. N, N-terminus; C, C terminus.

directly bound to INTS6 (Fig. 3C). Moreover, little direct interaction was detected between hSSB1 and INTS6 (Fig. 3D). These results further confirm that the N-terminus of INTS3 interacts with hSSB1 and the C-terminus of INTS3 directly binds to INTS6. We also performed the GST pull-down assay to confirm the direct interaction between the C-terminus of INTS3 and the C-terminus of INTS6 (supplementary material Fig. S3). These results further confirm that the N-terminus of INTS3 interacts with hSSB1 and the C-terminus of INTS3 directly binds to INTS6 (Fig. 3E). Besides hSSB1, INTS3 and INTS6, C9orf80 has been identified as another subunit in the complex (Huang et al., 2009; Li et al., 2009; Skaar et al., 2009). However, owing to its small size, we did not identify it in our mass spectrometry analyses. Nevertheless, we named the complex containing hSSB1, C9orf80, INTS3 and INTS6 as the hSSB1–INTS complex.

INTS6 relocates to DNA damage sites in response to DSBs and regulates homologous recombination

In response to DSBs, both hSSB1 and INTS3 relocate to DNA lesions and colocalize with γH2AX (Huang et al., 2009; Li et al., 2009; Richard et al., 2008; Skaar et al., 2009; Zhang et al., 2009b). Because INTS6 is a subunit in the hSSB1–INTS complex, we examined the formation of IR-induced foci (IRIF) of INTS6 in U2OS cells treated with 10 Gy of IR. As shown in Fig. 4A, INTS6 relocated to DNA damage sites and colocalized with INTS3 following IR treatment, suggesting that INST6, like INTS3, participates in the DNA damage response. To investigate which region targets INTS6 to DNA damage sites, we examined the formation of IRIF of the deletion mutants of INTS6. Interestingly, the D8 mutant, but not other mutants, disrupted the IRIF for INTS6 (supplementary material Fig. S4A). Moreover, depletion of INTS3 by siRNA significantly

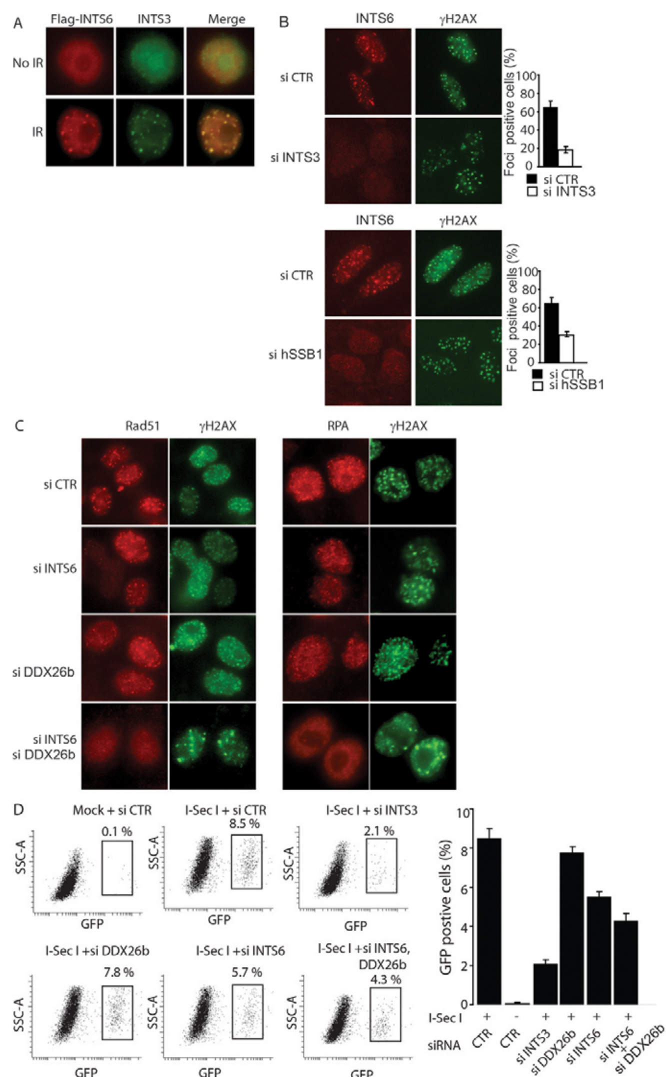

Fig. 4. INTS6 relocates to the DNA damage sites following DNA DSBs. (A) INTS6 colocalizes with INTS3 follow IR treatment. Wild-type INTS6 or its deletion mutants were expressed in U2OS cells. Cells were treated with 10 Gy of IR and immunostained with the indicated antibodies. (B) Knockdown of INTS3 or hSSB1 abolished the IRIF of INTS6. U2OS cells were treated with the indicated siRNA for 48 hours, irradiated with 10 Gy and immunostained with the indicated antibodies. (C) Knockdown of INTS6 and DDX26b impairs IRIF of RAD51 and RPA. U2OS cells were treated with control or INTS6 and DDX26b siRNA for 48 hours and then irradiated with 10 Gy of IR. Cells were fixed 6 hours after irradiation and co-stained with the indicated antibodies. (D) Knockdown of INTS6 and DDX26b impairs HR repair. U2OS-DrGFP cells were treated with the indicated siRNAs. The siRNA-treated cells were then infected by adeno-I-SecI. The percentage of GFP-positive cells was examined by flow cytometry. Data are averages (±s.e.m.) from three independent experiments.

decreased the IRIF of INTS6 (Fig. 4B and supplementary material Fig. S4B). These results suggest that the interaction with INTS3 is important for formation of INTS6 IRIF. Depletion of hSSB1 also impaired formation of INTS6 IRIF (Fig. 4B), whereas depletion of INTS6 and DDX26b did not affect the IRIF of either INTS3 or hSSB1 (supplementary material Fig. S4C). Taken together, these results suggest that INTS6 is recruited to DNA lesions through its interaction with INTS3 and hSSB1.

We and others have shown that both hSSB1 and INTS3 are important for IRIF formation of RAD51 and the RAD51-dependent HR repair (Huang et al., 2009; Richard et al., 2008; Skaar et al., 2009; Zhang et al., 2009b). Because INTS6 is a major subunit in the hSSB1–INTS complex, we examined the role of INTS6 in the IRIF formation of RAD51, as well as in HR repair. We found that only depletion of INTS6 or DDX26b mildly reduce IRIF of RAD51, suggesting that INTS6 and DDX26b may have redundant role in the regulation of IRIF formation of RAD51. However, depletion of INTS6 and DDX26b together resulted in no IRIF formation of RAD51 (Fig. 4C; supplementary material Fig. S4D), but did not affect the expression level of RAD51 (supplementary material Fig. S4E). Similar to the depletion of INTS3, lack of INTS6 and DDX26b also impaired I-SceI-induced HR repair using the direct repeat green fluorescent protein (DR-GFP) report system (Fig. 4D). We also used another set of siRNA to target INTS6 and DDX26b and examined the IRIF formation of RAD51 and HR repair. The same results were obtained, which excluded the siRNA off-target effect (supplementary material Fig. S4F). Taken together, these results suggest that INTS6 is the functional partner of INTS3 and hSSB1 in regulating HR repair by stabilizing RAD51 at DNA damage sites. Moreover, lacking both INTS6 and DDX26b, cells were hypersensitive to IR treatment (supplementary material Fig. S4G). This confirms the important role of INTS6 and DDX26b in DNA damage repair. During HR repair, RAD51 replaces replication protein A (RPA) to motorize the ssDNA invasion into sister chromatids. To study whether the hSSB1–INTS complex regulates the earlier step during HR, we investigated single-stranded DNA formation by examining the formation of RPA IRIF. Interestingly, knockdown of INTS6 and DDX26b reduced the accumulation of IRIF of RPA (Fig. 4C; supplementary material Fig. S4D), indicating that the hSSB1–INTS complex might regulate DNA end resection, the initial step of HR.

We and others found that some subunits in the integrator complex participate in the DNA damage response, whereas others may not be involved (Cotta-Ramusino et al., 2011; Huang et al., 2009; Li et al., 2009; Skaar et al., 2009; Zhang et al., 2009b). Using stringent affinity purification and reverse affinity purification, we only found that INTS3 and INTS6 strongly interacted with each other. It is possible that other integrator complex subunits also associate with the hSSB1–INTS complex in a similar way to the Mre11–Rad50–Nbs1 (MRN) complex, and function in the similar or different pathway in response to DNA damage. However, based on our purification results, the core complex only contains INTS3 and INTS6 but not other integrator subunits. The organization of this complex might resemble the Fanconi anemia (FA) core complex, which contains at least nine core subunits, and the core subunits could associate with other proteins during the DNA damage response (Deans and West, 2011; Wang, 2007). In addition, DDX26b is likely to be a paralog of INTS6 and have a redundant role with INTS6 to activate HR repair. It has been reported that the level of hSSB2 is much lower than that of hSSB1. Similarly, the level of DDX26b is also lower than that of INTS6. Moreover, INTS6 interacts with both hSSB1and hSSB2, and so does DDX26b (supplementary material Fig. S4H). Thus, the redundant function of these gene products may ensure that the HR repair, a key biological event for cell viability, will not be easily prevented by spontaneous genetic mutations.

Both INTS6 and DDX26b contain the DEAD (Asp-Glu-Ala-Asp) motif that is often found in DNA/RNA helicases. However, the DEAD motifs is located in the unfolded region of INTS6 and DDX26b. There is no conserved helicase domain in these two proteins; and we could detect neither the helicase activity nor the DNA translocase activity *in vitro*. However, we could not rule out the possibility that INTS6 and DDX26b are atypical DNA helicases that facilitate the unwinding of DNA at damage ends in the presence of other functional partners.

Materials and Methods

Plasmids, siRNA and antibodies

For protein affinity purification and other analyses, full-length cDNA of INTS3 or INTS6 was cloned into pS-FLAG-SBP vector. Truncation mutants of INTS3 or SSB1 were described previously (Zhang et al., 2009b). Internal deletion mutants of INTS6 and an siRNA-resistant INTS3 mutant were generated using the QuikChange site-directed mutagenesis kit (Stratagene). For GST- or His-tagged protein, INTS6, hSSB1 or INTS3 full length or fragments were cloned into the pGEX-4T or PET-28a vector accordingly. hSSB1 was cloned into pEGFP-C1 vector for GFP fusion. The siRNA sequences targeting hSSB1, INTS3, INTS6 and DDX26b are: 5′-GCAGAAGAUUGGAGAAUUCdTdT-3′ (hSSB1); 5′-GGACA-AAGUACUCCAGCUAdTdT-3′ (INTS3); 5′-GGAAAGAAAUUGAUGCAUUd-TdT-3′ (INTS6); 5′-GGAAACAAGUUAACAAGUAdTdT-3′ (DDX26b). siRNAs were transfected into the cells using Oligofectamine (Invitrogen) according to the manufacturer's instructions.

Rabbit anti-INTS6 antibody was raised against GST–INTS6 [amino acids (aa) 477–887]. Anti-Myc antibody was purchased from Covance. Anti-FLAG and anti-β-actin antibodies were purchased from Sigma. Anti-DDX26b and anti-RPA2 antibodies were purchased from Abcam (ab95083) and Cell Signaling, respectively. Rabbit anti-INTS3, anti-hSSB1, anti-phospho-H2AX and RAD51 antibodies were obtained as previously described (Zhang et al., 2009b).

Protein purification and size-exclusion chromatography

Purification of SFB triple tagged protein (S, FLAG and SBP tags) was described previously (Zhang et al., 2009a). To search for binding partners of INTS3 and INTS6, 1 liter cultures of 293T cells stably expressing SFB–INTS3 and SFB–INTS6 were harvested and washed with PBS. Cells were lysed with 30 ml ice-cold NETN buffer (0.5% NP-40, 50 mM Tris-HCl pH 8.0, 2 mM EDTA and 300 mM NaCl). The soluble fraction was incubated with 0.5 ml streptavidin-conjugated agarose beads. The beads were washed with NETN buffer three times. Associated proteins were eluted with 2 mM biotin in PBS and further incubated with 50 µl S-beads (Novagen). The bound proteins were then eluted with SDS sample buffer, and analyzed by 10% SDS-PAGE and mass spectrometry.

Baculoviruses infection and GST pull down assays

For baculovirus, DNA fragments containing INTS3, INTS6, hSSB1, INTS3N (aa 1–628) and INTS3C (aa 593–1042) were subcloned into pFastBac vector with or without an epitope tag, and baculoviruses were generated according to manufacturer's instructions. After cells were infected with individual or combinations of baculoviruses for 48 hours, the Sf9 cells were harvested, washed with PBS and lysed with 10 ml ice-cold NETN buffer. The soluble fraction was incubated with 30 µl glutathione–agarose beads for 1 hour at 4℃, the beads were washed three times with NETN buffer and bound proteins were analyzed by SDS-PAGE followed by western blotting with the indicated antibodies.

Cell culture, cell lysis, immunoprecipitation and western blotting

Human cancer cell lines were maintained in RPMI 1640 medium with 10% fetal calf serum and cultured at 37℃ in 5% CO_2 (v/v). Cells were irradiated using a JL Shepherd ^{137}Cs radiation source at the indicated doses. Cells were lysed with NETN buffer containing 10 mM NaF and 50 mM glycerophosphate. Immunoprecipitation and western blotting were performed following standard protocol as described previously (Zhang et al., 2009a).

Homologous recombination assay

The assay was established and modified by Dr Jasin's group (Weinstock et al., 2006). Briefly, U2OS cells stably expressing a single copy of DR–GFP were transfected with siRNA as indicated. SiRNA-treated cells were infected with adenovirus-encoded I-SecI (adeno-I-SecI). Cells were harvested 2 days after infection and subjected to flow cytometry analysis, and the GFP-positive cell population was measured.

Immunofluorescence staining

Cells grown on coverslips were fixed with 3% paraformaldehyde for 20 minutes and permeabilized with 0.5% Triton X-100 in PBS for 5 minutes at room

temperature. Samples were blocked with 5% goat serum and then incubated with primary antibody for 60 minutes. Samples were washed three times and incubated with secondary antibody for 30 minutes. The coverslips were mounted onto glass slides and the cells visualized using a fluorescence microscope. To examine IRIF, cells were cultured on coverslips and irradiated with 10 Gy, followed by recovery for 4 hours, unless otherwise specified. To quantify DNA damage-induced foci, we usually choose nine fields from each slide and count 500 cells. To avoid non-specific staining, cells with at least three clear foci were considered as foci-positive cells.

Primers and siRNA

For quantitative PCR (QPCR) assays total RNA was prepared using Trizol (Gibco-Invitrogen) and used for synthesis of first strand cDNAs with Superscript II reverse transcriptase (Invitrogen). Real-time PCR was performed in 25 μl reactions in 96-well plates using 7300 Real Time PCR system, Power SYBR Green PCR master mix (Applied Biosystems). Primers for QPCR reactions are summarized in supplementary material Table S1.

siRNA sequences targeting INTS6 and DDX26b were: 5′-GCCAAAAUA-AAGAACAAdTdT-3′ (siINTS6#2), and 5′-GUACAAUGAACUUGGAUAUd-TdT-3′ (siDDX26b#2).

Author contributions

F.Z. and X.Y. conceived and designed the project. F.Z. and T.M. performed the experiments. F.Z. and X.Y. prepared the manuscript. All authors discussed and commented on the manuscript.

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