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Drosophila IRBP bZIP heterodimer binds P-element DNA and affects hybrid dysgenesis

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In *Drosophila*, P-element transposition causes mutagenesis and genome instability during hybrid dysgenesis. The P-element 31-bp terminal inverted repeats (TIRs) contain sequences essential for transposase cleavage and have been implicated in DNA repair via protein–DNA interactions with cellular proteins. The identity and function of these cellular proteins were unknown. Biochemical characterization of proteins that bind the TIRs identified a heterodimeric basic leucine zipper (bZIP) complex between an uncharacterized protein that we termed "Inverted Repeat Binding Protein (IRBP) 18" and its partner Xrp1. The reconstituted IRBP18/Xrp1 heterodimer binds sequence-specifically to its dsDNA-binding site within the P-element TIRs. Genetic analyses implicate both proteins as critical for repair of DNA breaks following transposase cleavage in vivo. These results identify a cellular protein complex that binds an active mobile element and plays a more general role in maintaining genome stability.

P-transposable elements | DNA repair | IRBP18/CG6272 | Xrp1/CG17836 | IRBP complex

Transposable elements contribute significantly to the organization and evolution of all eukaryotic genomes. Recent estimates of transposon content within the *Drosophila melanogaster* genome are between 5% and 10%, and in humans over half the genome is composed of mobile elements (1, 2). Although many of these elements, including the *Drosophila* P-element transposon, are still active (3), the cellular mechanisms used to combat the genotoxic effects of DNA double-strand breaks (DSBs) generated by transpositional recombination are not fully understood. The *Drosophila* P-transposable element provides an excellent model for understanding the ancient mechanisms used by the cell to counteract newly invading parasitic mobile DNA elements (4).

The P-element transposon is a mobile DNA element that spread through wild populations of *D. melangaster* ∼100 y ago after most common laboratory strains were isolated (5, 6). P elements were identified by studying a genetic syndrome called "P-M hybrid dysgenesis." It was observed that males from wild populations (P strains) crossed to females from isolated laboratory stocks (M strains) yielded progeny that had germline mutations, temperature-sensitive sterility, and atypical male recombination (6). Reciprocal crosses yielded phenotypically normal progeny. The P element was shown to be the causative agent of these so-called P-M hybrid dysgenesis phenotypes by molecular analyses showing that P elements were present in variable locations in P strains yet totally absent from most M strains (7, 8).

The *Drosophila* P-element transposon encodes a GTP-dependent site-specific DNA transposase/integrase family enzyme (9, 10). At each end of the P-element transposon are perfect 31-bp terminal inverted repeats (TIRs), 11-bp internal inverted repeats that serve as enhancers of transposition, and internal 10-bp transposase binding sites (11–13) (Fig. 1*A*). The P-element transposase catalyzes DNA cleavage within the 31-bp TIRs to create 17-nt 3′ single-strand extensions at both the donor site and the transposon ends (14, 15). The cleaved P-element ends remain tightly bound by the transposase enzyme (10, 16). The flanking donor site DNA is released after P-element cleavage (16) and needs to be efficiently repaired by endogenous DNA repair mechanisms. Engineered somatic mobilization of as few as 15–20 small nonautonomous P elements by transposase leads to a temperature-dependent pupal lethality due to extensive DNA damage and apoptosis (17).

Repair of P-element–induced DNA breaks typically occurs through two distinct DSB repair pathways: nonhomologous end joining (NHEJ) or a variant of classical homologous recombinational repair, termed "Synthesis-Dependent Strand Annealing" (SDSA) (18–21) The choice between these two pathways is dictated by cell-cycle location, the availability of pathway substrates, and tissue type (22, 23).

In addition to containing sequences critical for transposase-mediated DNA cleavage, the 31-bp TIRs of the P element have been postulated to be important for DNA repair (24), perhaps through protein–DNA interactions. To date, the identity and functional role of endogenous *Drosophila* proteins bound to the 31-bp TIRs in the regulation of P-element transposition is undetermined. Previous genetic and biochemical data implicated *Drosophila* Ku70 as a protein that bound to the P-element TIRs (25). However, recombinant *Drosophila* Ku70 alone or as a heterodimer with Ku80 did not bind the 31-bp TIRs sequence-specifically.

In this report, we purified a multisubunit Inverted Repeat Binding Protein (IRBP) complex that binds sequence-specifically to the outer 16 bp of the P-element 31-bp TIRs. The core DNA-binding subunits of this complex consist of a basic leucine zipper (bZIP) heterodimer between Xrp1 (CG17836) and a previously uncharacterized 18-kDa CAAT/Enhancer Binding Protein (C/EBP)

Significance

The P-element transposon is a mobile DNA that invaded the *Drosophila* genome approximately 100 y ago. P elements were identified by studying a genetic syndrome called "hybrid dysgenesis." The elements use their encoded transposase for mobility, but rely on host-cell factors for essential parts of their life cycle. Here we demonstrate biochemically that a *Drosophila*-encoded bZIP heterodimer binds to the P-element terminal 31-bp sequences. We used genetics to show that these proteins play a role in repairing DNA breaks caused by P-element transposase activity during hybrid dysgenesis and other types of DNA damage. These results provide an example of the mechanisms that the host genome uses to combat genome instability caused by foreign DNA invasion.

Author contributions: M.J.F., S.R., M.J.C., E.B., B.M., R.P.P., and D.C.R. designed research; M.J.F., S.R., M.J.C., E.B., B.M., R.P.P., and D.C.R. performed research; M.J.F., S.R., and E.B. contributed new reagents/analytic tools; M.J.F., S.R., M.J.C., E.B., B.M., R.P.P., and D.C.R. analyzed data; and M.J.F., S.R., E.B., and D.C.R. wrote the paper.

The authors declare no conflict of interest.

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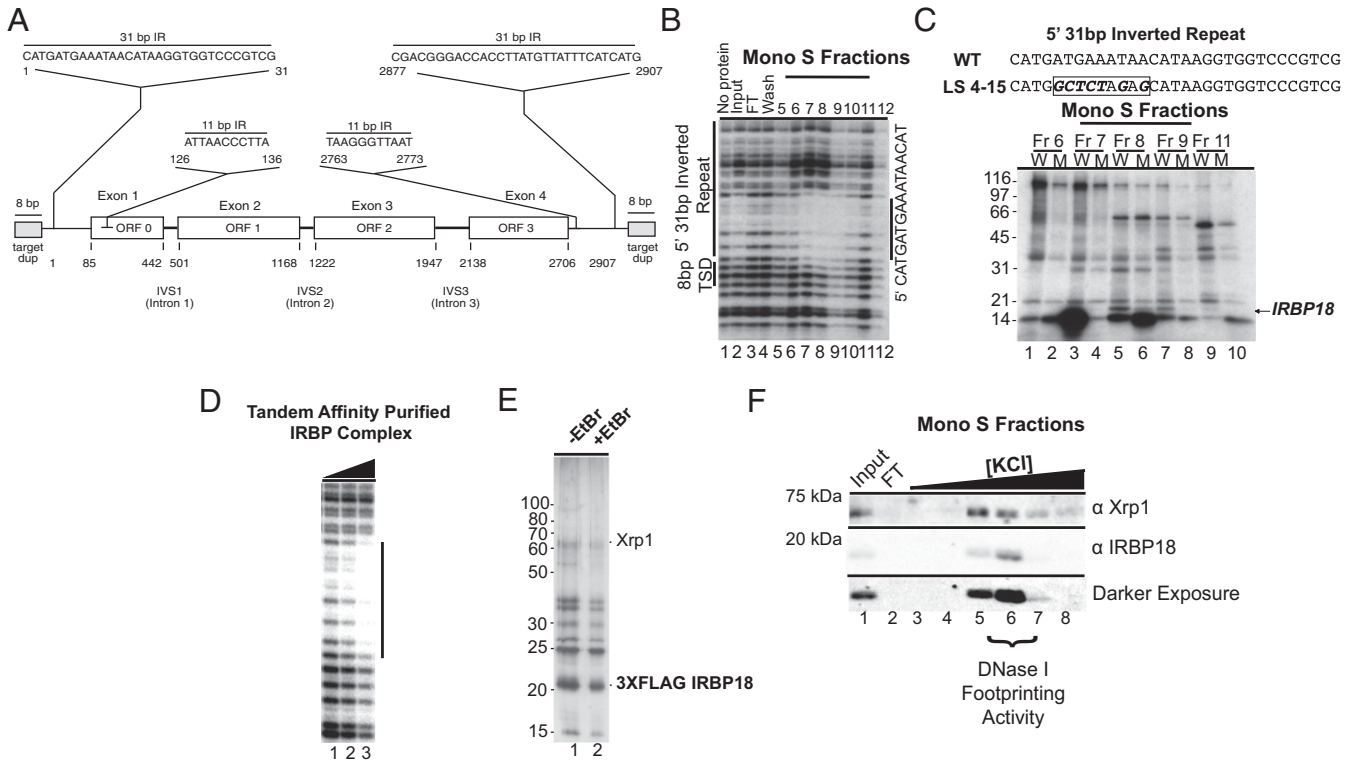

Fig. 1. IRBP18 and Xrp1 bind the P-element inverted repeats. (*A*) Diagram of the organization of the P-element transposon. (*B*) DNase I protection assay of the 5′-end of the P element with Mono S column fractions. The sequence indicates the portion of P-element TIR bound by the IRBP complex. (*C*) Autoradiograph of an 8–20% gradient SDS/PAGE containing UV photochemical cross-linking reactions performed with the Mono S fractions with a wild-type (lanes 1, 3, 5, 7, and 9) or LS 4–15 mutant (lanes 2, 4, 6, 8, and 10) ^{32}P-labeled, BrdUrd-substituted 5′ inverted repeat DNA probe. (*D*) DNase I protection assay of the purified IRBP complex eluted from FLAG antibody resin. (*E*) Silver stain SDS/PAGE analysis of the 3XFLAG-eluted complex separated by 12% SDS/PAGE. FLAG immunoaffinity chromatography was performed in the presence (+) and absence (–) of ethidium bromide (EtBr) (150 μg/mL). (*F*) Immunoblot analysis of MonoS column fractions for the presence of IRBP18 and Xrp1 with affinity-purified antibodies (1:2,000).

family member, we termed "Inverted Repeat Binding Protein 18 kDa" (IRBP18/CG6272). Purified recombinant IRBP18/Xrp1 heterodimer reconstitutes high-affinity and sequence-specific dsDNA binding to TIRs. In vivo analyses indicate that the IRBP complex protects cleaved donor DNA ends and facilitates efficient repair of DSBs created after transposase cleavage. Moreover, a null mutation in the IRBP18 gene enhances somatic hybrid dysgenesis, and other genetic experiments indicate that the IRBP complex is critical for general DNA break repair in the absence of P elements. Taken together, our data shed light on endogenous cellular mechanisms used to recognize and repair newly invading transposable elements in their host *D. melanogaster* genome.

Results

The P-Element Inverted Repeat Binding Protein Complex Is a bZIP Heterodimer. Because the P element only recently invaded *Drosophila* genomes, we postulated that any proteins that could bind to the 31-bp TIR predate the P element and would thus have to be present in the absence of P elements. To identify proteins that recognize and bind to the P-element 31-bp TIRs, we performed ultraviolent (UV)-photochemical protein–DNA cross-linking experiments with partially purified Kc cell nuclear extracts that were fractionated using five sequential chromatographic steps (Fig. S1*A*). The presence of the IRBP DNA binding was determined after each chromatographic step by DNase I protection assay. Fractions 6–11 from the Mono S column containing the peak IRBP DNase I protection activity (Fig. 1*B*, lanes 6–11) were incubated with either wild-type or mutant BrdUrd-substituted ^{32}P-labeled 31-bp inverted repeat DNA UV cross-linking probes (Fig. 1*C*). The mutant inverted repeat DNA probe contained several nucleotide substitutions within the outer half of the 31-bp TIR (Fig. 1*C*) (12). Following UV cross-linking and nuclease treatment, samples were fractionated by SDS/PAGE. In protein fractions with peak IRBP DNase I protection, an ∼18-kDa protein was strongly cross-linked to the wild-type inverted repeat probe (fractions 8 and 9; Fig. 1*C*, lanes 5 and 7), but only weakly cross-linked to the mutant inverted repeat probe (Fig. 1*C*, lanes 6 and 8). Although other proteins in the Mono S fractions 7–9 were bound to the wild-type DNA probe (Fig. 1*C*, lanes 3–8), none of these proteins were comparable to the 18-kDa protein in terms of signal enrichment on the wild-type versus mutant probes.

The 18-kDa protein was digested with Lys-C, and peptides were subsequently purified by HPLC. Two isolated peptides were sequenced and used to search the protein database. The two peptides mapped to the protein product of an uncharacterized *Drosophila* gene, *CG6272* (Fig. S1*B*). This protein product is IRBP18. IRBP18 is a bZIP DNA-binding protein within the CAAT/enhancer binding protein (C/EBP) superfamily of transcription factors (26) and is most closely related to human C/EBPγ (Fig. S2) (27).

We next asked if IRBP18 was necessary for sequence-specific binding to the P-element TIRs. Addition of affinity-purified rabbit polyclonal anti-IRBP18 antibodies blocked protection of partially purified native IRBP binding (Fig. S3*A*, lanes 7–10), whereas control reactions with nonspecific rabbit IgG had no effect on IRBP binding (Fig. S3*A*, lanes 3–6). Recombinant IRBP18 protein alone expressed in either prokaryotic or eukaryotic protein expression systems did not exhibit the characteristic IRBP DNase I protection pattern at the concentrations used (Fig. S3*B*). These

observations suggested that IRBP18 is necessary, but not sufficient, to reconstitute sequence-specific IRBP DNA binding to the P-element TIR and led us to examine whether other proteins in a potential IRBP complex might be required for IRBP DNA binding.

To identify putative IRBP18-interacting proteins that might be required for IRBP DNA binding, we used a combination of conventional chromatography and tandem affinity purification, as outlined in Fig. S4 *A* and *B* and detailed in *SI Text*. Briefly, nuclear extracts were prepared from 8 to 10 L of *Drosophila* S2 cells expressing a stably integrated ZZ-TEV-3XFLAG (two protein A modules; a tobacco etch virus (TEV) protease site; three tandem copies of the short FLAG monoclonal antibody epitope)-tagged version of IRBP18 (Fig. S4*A*). Protein expression of the ZZ-TEV-3XFLAG IRBP18 fusion protein was ∼1.5-fold higher than the endogenous IRBP18 protein level, as assessed by immunoblot analysis (Fig. S4*A*). Complexes eluted under native conditions with a synthetic 3XFLAG peptide were assayed for sequence-specific IRBP DNA binding by DNase I protection on the P-element 5′ TIR (Fig. 1*D*). The IRBP DNase I protection pattern observed with this affinity-purified material was indistinguishable from that of the native complex (Fig. 1*D*, lanes 2 and 3; Fig. S3*A*, lane 2). Silver-stained SDS/PAGE analysis of the complex revealed the presence of several protein species that were resistant to ethidium bromide treatment, suggesting that protein–protein, not DNA–protein interactions, were involved (Fig. 1*E*).

Mass spectrometric analysis of these tandem-affinity purified complexes identified Xrp1/CG17836 as a potential bZIP protein partner for IRBP18 (Fig. S4*D*). Xrp1 is a drosophilid-specific bZIP protein with an additional AT hook DNA-binding motif, similar to those found in the high-mobility group class of DNA-binding proteins (28). The bZIP interaction between IRBP18 and Xrp1 was determined independently from previous predictions and experiments (29, 30).

We next examined if both IRBP18 and Xrp1 copurified in the native IRBP fractionation. We performed immunoblot analysis on MonoS column fractions used in the UV photo cross-linking experiments (Fig. 1*F*). We observed a near-perfect correlation between the presence of IRBP18, Xrp1, and sequence-specific IRBP DNA binding (Fig. 1*F*, lanes 5–7).

Several other proteins were identified by mass spectrometry (Fig. S4*D*). At present we have not characterized these proteins further and have instead focused on the function of the IRBP18/Xrp1 heterodimer and its relationship to P-element transposition.

Recombinant IRBP18/Xrp1 Reconstitutes Sequence-Specific IRBP DNA Binding. To determine if the IRBP18/Xrp1 heterodimer alone is sufficient to reconstitute sequence-specific IRBP DNA binding in vitro, we used DNase I protection with purified recombinant proteins. Briefly, recombinant versions of IRBP18 (3XFLAG-IRBP18) and Xrp1 (His_6-Xrp1) were expressed in bacteria either as monomers or as a heterodimer (*SI Text*). The proteins were then purified to apparent homogeneity as determined by SDS/PAGE analysis (Fig. 2*A*). DNA binding was measured by titrating IRBP18, Xrp1, and the heterodimer in DNase I protection assays (Fig. 2*B*). The IRBP18/Xrp1 heterodimer displayed the highest affinity for the IRBP site and displayed the characteristic IRBP protection pattern. At low concentrations of the heterodimer, protection was observed within the target-site duplication (Fig. 2*B*, lanes 10 and 11). Additionally, there were hypersensitive DNase I cleavage sites that extended into the P-element sequence (Fig. 2*B*, lanes 8–11 and 14–17; indicated by asterisks). Xrp1 alone was able to bind to P-element DNA with an ∼20-fold lower affinity than the IRBP18/Xrp1 heterodimer (Fig. 2*B*, lanes 14–17). IRBP18 was also able to bind weakly and only at a very high concentration (Fig. 2*B*, lane 6). These experiments clearly demonstrate that the IRBP18/Xrp1 heterodimer is the bona fide IRBP DNA-binding protein.

Next we asked if the IRBP18/Xrp1 heterodimer and the 87-kDa P-element transposase enzyme could simultaneously bind the same DNA molecule. When increasing amounts of purified P-element transposase were added to the DNase I protection reactions, binding of the IBRP18/Xrp1 heterodimer to the P-element inverted repeats was unaltered (Fig. 2*C*, lanes 7–15; see TNP and IRBP solid lines). This experiment indicates that these proteins can co-occupy the same DNA molecule by interacting independently with their respective binding sites.

IRBP18 and Xrp1 Promotes Donor-Site DNA Repair After P-Element DNA Cleavage. Because the IRBP-binding sites and the P-element DNA cleavage sites overlap, the IRBP complex has long been suggested to play a role in the P-element transposition reaction, perhaps by stabilizing DNA ends following transposase-mediated DNA cleavage or to facilitate DNA repair (24, 31). We therefore asked if the IRBP complex might function to promote DNA repair after P-element excision.

Transposase cleavage and cellular DNA repair efficiency can be quantitated using a cell-culture–based assay for P-element excision (21). We first asked by ligation-mediated PCR (LM-PCR)

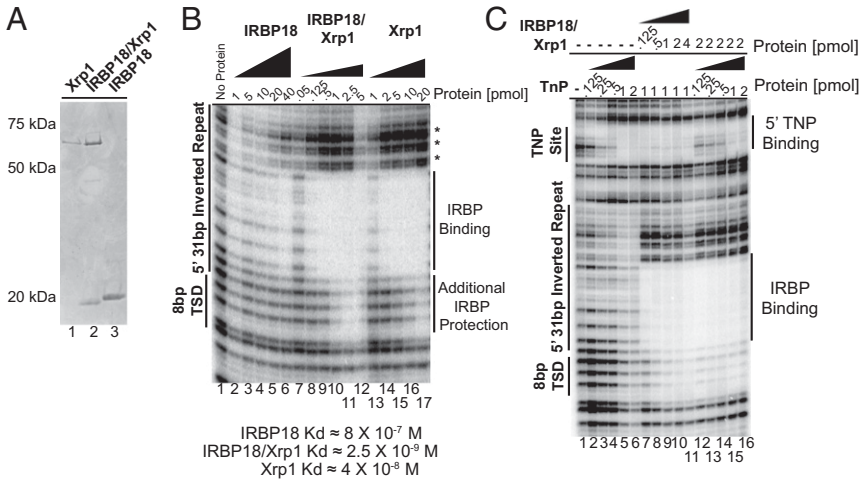

Fig. 2. Recombinant IRBP18/Xrp1 heterodimer reconstitutes sequence-specific IRBP DNA binding. (*A*) Coomassie-stained SDS/PAGE of purified recombinant proteins. (*B*) DNase I protection assay with IRBP18, IRBP18/Xrp1 heterodimer, and Xrp1. The asterisks indicate sites of hyper-DNase I sensitivity. Solid lines indicate regions of protection. Apparent K_d values for binding based on half-maximal protection are indicated below the panel. (*C*) DNase I protection assay IRBP18/Xrp1 proteins and P-element transposase. Solid lines indicate regions of protection.

on P-element reporter plasmids recovered from *Drosophila* S2 cells if the IRBP complex plays a role in transposition cycle (Fig. S5*A*). This assay directly detects the presence of both flanking donor and the P-element transposon ends following transposase-mediated excision in vivo in cells where IRBP18 was knocked down by RNA interference (RNAi) (Fig. S5*B*). The LM-PCR signal from the flanking donor DNA end was decreased in the IRBP18 RNAi-treated samples compared with control samples (Fig. S5*C*, lanes 3 and 4). By contrast, the P-element transposon ends were unaffected (Fig. S5*C*, lanes 1 and 2). This suggests that the IRBP complex does not affect transposase cleavage activity in vivo, but rather plays a role in the repair of the donor site after DNA cleavage.

Additionally, RNAi knockdown of either IRBP18 or Xrp1 resulted in a reduction of the normalized DNA repair efficiency to 45–55% of the control level. The combination treatment with dsRNA against both IRBP18 and Xrp1 resulted in no further reduction in DNA repair efficiency (Fig. S5*D*). The P-element excision–DNA repair assay was also performed with cells that had been transfected with either anti-IRBP18 or rabbit IgG antibodies not specific to IRBP18. In vivo antibody blocking of IRBP18 yielded a similar reduction in DNA repair efficiency to that observed with the RNAi knockdowns, (Fig. S5*E*), again suggesting that the IRBP complex promotes efficient repair of P-element–induced DSBs in vivo.

We next examined the qualitative nature of deletions surrounding the donor-site DNA repair events on the recovered plasmids under each RNAi condition (21). In both the IRBP18 and Xrp1 RNAi-treated samples, we observed an increase in the number of deletions compared with the control RNAi-treated group. The double IRBP18 and Xrp1 RNAi-treated samples were similar to the Xrp1-alone, RNAi-treated group. The increase in the frequency of deletions at the donor DNA site after IRBP18 or Xrp1 RNAi knockdown, along with the LM-PCR data (Fig. S5*F*), suggests that the IRBP complex acts in the repair of flanking donor-site DNA ends after transposase cleavage and P-element excision.

IRBP18 Mutant Flies Display a Severe Somatic Dysgenesis Phenotype. We next assayed the role of IRBP18 in repair of DNA breaks generated at chromosomal P elements following transposase-mediated DNA cleavage. Somatic mobilization of 15–20 P elements at 25 °C results in complete larval and pupal lethality, so called "somatic dysgenesis," whereas at lower permissive temperatures (18 °C and 21 °C) viable survivors can be obtained (17). We asked whether at permissive temperatures if IRBP18 homozygous mutants increase the severity of the somatic dysgenesis phenotype. Flies homozygous for the Birmingham 2 chromosome (Birm2; a second chromosome containing 17 nonautonomous P elements) and heterozygous on the third chromosome for the *CG6272/IRBP18* null allele (Fig. S6) were crossed with flies carrying a wild-type second chromosome and a recombinant third chromosome with $CG6272^{WHf05006}$ and a somatically expressed P-element transposase source, P Δ2–3 (99B) (32) (Fig. 3*A*). If IRBP18 had no effect on somatic dysgenesis, then the expected phenotypic ratio should be 1:1:1:1. However, adult $CG6272^{WHf05006}$ homozygous mutant flies had a significant decrease in viability compared with heterozygous siblings carrying the transposase source. This killing phenotype was exacerbated at elevated temperatures (16% viability at 18 °C compared with 6% at 21 °C) (Fig. 3*B*). Because flies carrying the Birm2 chromosome have no detectable somatic transposase activity (17), the defects in repair of P-element–induced chromosomal DNA breaks are presumably attributable to loss of IRBP18 and the IRBP complex. These observations indicate a role for IRBP18 in both genome stability and DNA repair in vivo.

IRBP18 Is Essential for General DNA DSB Repair. To examine a potential role of IRBP18 in general DNA DSB repair, we tested the sensitivity of the IRBP18 mutant strain to the DNA-damaging agents methylmethane sulfonate (MMS) and ionizing radiation (IR). We performed self-crosses with heterozygous $CG6272^{WHf05006}$/TM3, Sb flies (Fig. S7*A*). In the absence of DNA-damaging agents we observed the expected phenotypic ratio of 2:1 heterozygous-to-homozygous adult flies. Sensitivity to DNA-damaging agents was detected by changes in the phenotypic ratio due to death of the *CG6272/IRBP18* homozygous mutant class. Homozygous mutant *CG6272/IRBP18* flies displayed a dose-dependent sensitivity/lethality to both MMS and IR, compared with observed ratios from the control groups (Fig. S7 *B* and *C*).

To ensure that the defective DNA repair phenotypes are attributable only to the IRBP18 mutant allele, we generated a 1.5-kb genomic DNA-rescuing transgenic *Drosophila* strain that encodes only the *CG6272/IRBP18* gene locus (Fig. S8*A*). We crossed flies heterozygous for a recombinant third chromosome

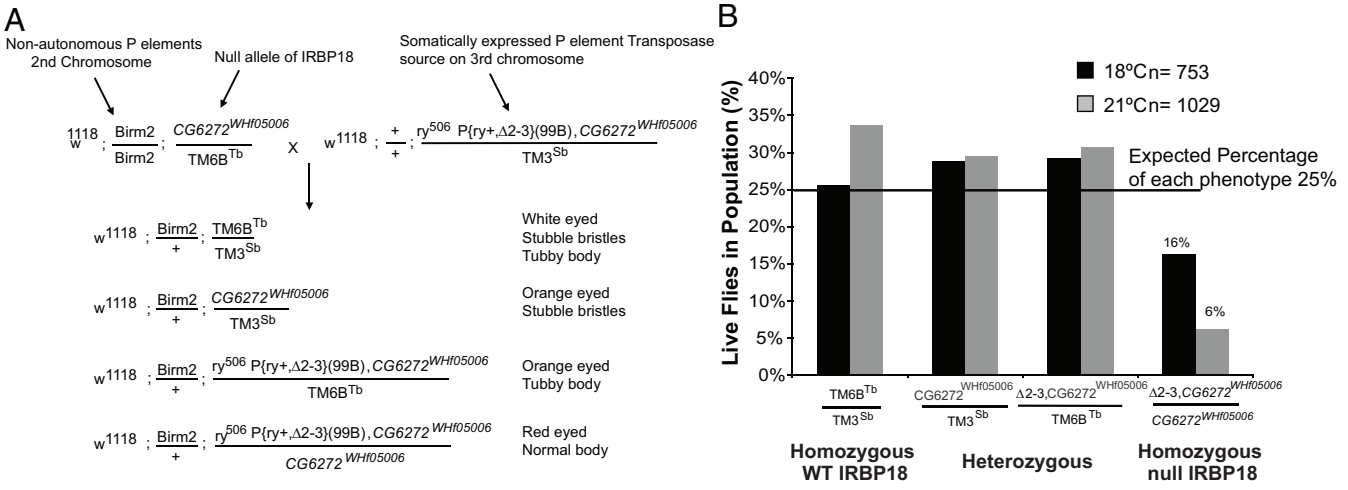

Fig. 3. IRBP18 null mutant flies exhibit increased somatic dysgenesis lethality. (*A*) Somatic dysgenic crosses were performed at permissive and semipermissive temperatures (18 °C and 21 °C), flies of each genotype/phenotype were counted, and the data are summarized in *B*. See *SI Text* for fly strains. (*B*) On the *x* axis is each genotype (third chromosome only), and on the *y* axis is the percentage of live adult flies of each genotype within the entire adult population. The data for the crosses at 18 °C are in black bars and at 21 °C in gray bars.

(CG6272^{WHf05006} and the P{w$^+$; 1.5kb CG6272}) (Fig. S8*B*) with heterozygous CG6272^{WHf05006}/TM3, Sb flies and treated the resulting third-instar larvae from this cross with MMS (0.03%) or IR (10 Gy) (Fig. S8*C*). Expression of the transgene resulted in complete rescue of both the MMS and IR sensitivity of the initial IRBP18 mutant (Fig. 4). Thus, using independent and distinct genetic approaches, we have demonstrated that IRBP18 is critical for efficient DNA DSB repair in *Drosophila*.

Discussion

A role for the IRBP complex in the P-element transposition reaction has been postulated since initial identification of its sequence-specific DNA-binding properties (24, 25, 31, 33). The organizational overlap between IRBP DNA binding and transposase cleavage sites makes the IRBP complex a prime cellular candidate to influence some aspect of the P-element transposition cycle. Several putative IRBP proteins copurified with observed IRBP DNA-binding activity or unambiguously promoted DNA repair posttransposase P-element cleavage (Ku 70 and mus309/DmBLM) (31, 34). None, however, could reconstitute site-specific DNA binding to the P-element 31-bp TIRs.

In this report, we identified a bZIP heterodimer between a C/EBP family member IRBP18 and a drosophilid-specific protein Xrp1 as the sequence-specific DNA-binding subunits of a larger multiprotein IRBP complex that binds to the P-element TIRs. We demonstrated that these proteins work in concert to facilitate efficient DNA repair following P-element transposase-mediated DNA cleavage. Finally, these proteins are required more generally in the cellular DNA damage response and DSB repair in the absence of P elements.

Repair of P-element–induced DNA breaks occurs predominantly through two distinct DSB repair pathways: NHEJ or a variant of classical homologous recombinational repair, SDSA (18–21) The choice between these two pathways is dictated by cell-cycle location, the availability of pathway substrates, and tissue type (22, 23). Because the IRBP homozygous mutant males are sterile, we cannot at present use any of the established DNA repair reporter strains to determine in which DNA repair pathway IRBP18 and Xrp1 participates. We also look forward to determining if the IRBP18/Xrp1 heterodimer can bind directly to different DNA repair intermediates and thus provide a direct link between the bZIP heterodimer and DNA repair.

A role for bZIP proteins and specifically mammalian C/EBP proteins in DNA repair is well established (35, 36). In human and mouse keratinocytes, UV-B UV DNA damage induces p53-dependent transcriptional activation of both the C/EBPα and β genes (37). C/EBPα expressed in prostate cancer cells where it interacts with the DNA repair proteins Ku p70/p80 heterodimer and the poly (ADP ribose) polymerase 1 (PARP-1) (38). Notably, *Drosophila* p53 up-regulates three bZIP proteins (*CG6272*/*IRBP18*, *CG17836*/*Xrp1*, and *CG15479*/*Mabiki*) upon DNA damage (26, 28, 39). *CG15479*/*Mabiki* is a novel regulator of caspase-independent cell death of excess cells in the expanded head region of 6×-bcd embryos and is thought to work in concert with other caspase-independent cell death mechanisms to ensure proper development (40). Genetic deletion of the *CG17836*/*Xrp1* gene resulted in a DNA repair phenotype when challenged with ionizing radiation (28). Additionally, the Dmp53 DNA damage-induced apoptotic response was unaffected in the Xrp1 mutants, suggesting that Xrp1 functions to preserve genome stability through a pathway independent of apoptosis (28). Although we have demonstrated that IRBP18 and Xrp1 share a similar function in DNA repair, more experiments are needed to understand how these proteins work downstream of p53 transactivation.

D. melanogaster uses multiple endogenous mechanisms to limit P-element transposition. Expression of catalytically active transposase is restricted to the germline by tissue-specific pre-mRNA splicing regulation (41, 42). The germline piwi-interacting RNA pathway has been demonstrated to repress transposition in trans and plays a critical role in host adaptation to newly invaded P elements (4, 43–45). We propose that the IRBP18/Xrp1 heterodimer recognizes new P elements and that its native function is to facilitate repair of breaks to maintain genomic stability during a genotoxic event such as ionizing radiation or the massive P-element mobilization that occurs following a hybrid dysgenic cross.

bZIP proteins are well suited to recognize newly invaded foreign DNA due to their inherit ability to form multiple heterodimers. IRBP18 and Xrp1, for example, form heterodimers with other several other bZIP proteins; the net result is expansion of the repertoire of DNA sequences that can be bound (26, 29, 30). This library of bZIP dimers can be deployed to recognize foreign DNA as part of a survival mechanism against the genome instability created by foreign DNA invasion. In humans, mice and *Drosophila* p53 transactivate steady-state levels of several bZIP proteins in response to DNA damage (28, 37, 39, 46, 47). It is unclear how changes in steady-state levels of these DNA repair proteins determine dimer formation or function. What is clear is that bZIP proteins are important players in DNA repair and maintenance of genome stability. In this respect, the IRBP18/Xrp1 heterodimer is a newly identified component of the interconnected pathways to combat the genotoxic effects of mass invasion/mobilization of transposons.

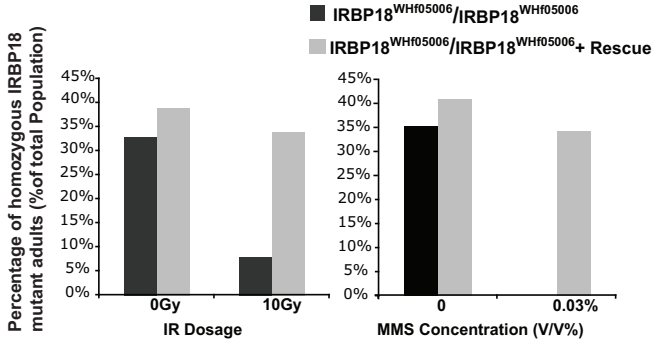

Fig. 4. IRBP18 is essential for general DNA DSB repair. On the *x* axis, doses of IR and concentrations of MMS are indicated, and on the *y* axis, the percentages of homozygous IRBP mutant (± rescue transgene) adult flies of each genotype within the entire adult population tested are indicated.

Materials and Methods

IRBP UV Photochemical Cross-Linking. UV photochemical cross-linking assays were performed using ^{32}P-labeled, bromodeoxyuridine-substituted DNA probes prepared as described (25). The pN/P175 (wild-type) and pHSX-LS4-15 (mutant) plasmids were used for generating TIR cross-linking probes (12).

DNaseI Protection Assay. DNase I protection experiments using the pN/P175 plasmid were performed as described (12, 33)

Microsequencing. MonoS fractions were concentrated and separated by SDS/PAGE and electroblotted onto a 0.2-μm PVDF membrane (48). The excised 18-kDa protein was digested with Lys-C and purified by HPLC. Peptide peaks were sequenced on a model 477A sequenator (Applied Biosystems). Database searches were conducted with BLAST. The Berkeley *Drosophila* Genome Project (BDGP) and GenBank accession numbers for the IRBP18 cDNA clones are GH10915 and AI114193, respectively.

***Drosophila* Strains.** The *Drosophila* strains used were ry^{506} P{ry$^+$,Δ2–3}(99B)/TM6B^{Tb}, Birm2; Sb/TM6^{Tb}, CyO/Sco, and w^{1118}; CG6272^{WHf05006}/TM6^{Tb} (Exelixis Collection at the Harvard Medical School, Boston) (49). All flies were raised on standard cornmeal, molasses, and yeast medium at 25 °C.

Somatic Dysgenesis Experiment. Flies for the somatic dysgenesis cross were allowed to mate at 25 °C for 2 d. The flies were then placed at 18 °C and 21 °C for 4 d (*SI Text*).

BIOCHEMISTRY

DNA Damage Agents and Treatments. w^{1118}; $CG6272^{WHf05006}$/TM3,Sb flies were self-crossed or crossed to w^{1118}; $CG6272^{WHf05006}$, P{w^+; 1.5-kb CG6272}/TM3,Sb. Third-instar larvae were treated with MMS (Sigma) and IR (γ-rays from a ^{137}Cs source: T. Cline, University of California, Berkeley, CA) at the indicated doses.

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