Polycomb Repressive Complex 2-Dependent and -Independent Functions of Jarid2 in Transcriptional Regulation in *Drosophila*

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Jarid2 was recently identified as an important component of the mammalian Polycomb repressive complex 2 (PRC2), where it has a major effect on PRC2 recruitment in mouse embryonic stem cells. Although Jarid2 is conserved in *Drosophila*, it has not previously been implicated in Polycomb (Pc) regulation. Therefore, we purified *Drosophila* Jarid2 and its associated proteins and found that Jarid2 associates with all of the known canonical PRC2 components, demonstrating a conserved physical interaction with PRC2 in flies and mammals. Furthermore, *in vivo* studies with Jarid2 mutants in flies demonstrate that among several histone modifications tested, only methylation of histone 3 at K27 (H3K27), the mark implemented by PRC2, was affected. Genome-wide profiling of Jarid2, Su(z)12 (Suppressor of zeste 12), and H3K27me3 occupancy by chromatin immunoprecipitation with sequencing (ChIP-seq) indicates that Jarid2 and Su(z)12 have very similar distribution patterns on chromatin. However, Jarid2 and Su(z)12 occupancy levels at some genes are significantly different, with Jarid2 being present at relatively low levels at many Pc response elements (PREs) of certain *Homeobox* (Hox) genes, providing a rationale for why Jarid2 was never identified in Pc screens. Gene expression analyses show that Jarid2 and E(z) (Enhancer of zeste, a canonical PRC2 component) are not only required for transcriptional repression but might also function in active transcription. Identification of Jarid2 as a conserved PRC2 interactor in flies provides an opportunity to begin to probe some of its novel functions in *Drosophila* development.

Different and distinct gene expression patterns are established during development, which need to be maintained and regulated. This is important to allow for the integrity of cell identity and thus the functional preservation of tissues and organs. However, at the same time, transcribed loci must be equipped with an intrinsic flexibility to regulate these expression patterns and initiate changes if necessary. The core components that are required for the maintenance of gene expression or gene repression have been characterized quite extensively to date (25, 40). *Trithorax* and *Polycomb* group genes play antagonistic roles in determining whether a gene is transcriptionally turned on or off, respectively (17, 34). In *Drosophila*, so far four distinct complexes, pleiohomeotic repressive complex (PhoRC), Polycomb repressive complex 2 (PRC2), Polycomb repressive complex 1 (PRC1), and recently Polycomb repressive deubiquitinase (PR-DUB) have been described to play a role in Polycomb group-mediated gene repression (1, 39–41, 44). However, little is known about the factors involved in controlling recruitment and activity of these complexes on chromatin or about the mechanisms that drive such changes (38). It should be expected that quite a significant number of proteins would convey Polycomb group-mediated transcriptional changes in order to allow an uncoupling of individual gene activity from that of a group of Polycomb group-controlled genes. Functional redundancy might account for part of the problem to discover such candidates. Furthermore, biochemical approaches might be hindered by the fact that such context-specific and more gene-specific recruiters are contained in only a minor fraction of Polycomb repressive complexes.

Recently, Jarid2 the founding member of the JmjC domain-containing protein family (16) which plays important developmental roles in mice and *Drosophila* (14, 18, 37), has been characterized as a component of PRC2 in embryonic stem (ES) cells (19, 22, 32, 33, 42, 53). The consensus indicates that in ES cells, PRC2 recruitment to many of its targets requires Jarid2. However, levels of bulk histone 3 trimethylated at K27 (H3K27me3) in ES cells depleted of Jarid2 were reported to be only slightly changed at best. This also holds true when individual PRC2 target genes are analyzed. Even though core components of the PRC2 complex were lost from chromatin in the absence of Jarid2, H3K27me3 was not reproducibly affected to a similar degree. Additionally, gene expression analyses in *jarid2*−/− ES cells did not confirm a genome-wide derepression of PRC2 target genes as would be expected for any core component of PRC2 (19).

To further address whether Jarid2 constitutes a core PRC2 component, it is involved in recruitment of PRC2 to chromatin, and regulates H3K27 methylation in *Drosophila*, we have purified a Jarid2 complex from flies and performed a global *in vivo* analysis of Suppressor of zeste 12 (Su(z)12) and H3K27me3 occupancy in *Jarid2* mutant animals. Our data confirm that *Drosophila* Jarid2 purifies with the core members of the PRC2 complex. In imaginal discs, global H3K27me3 levels are only weakly but reproducibly affected under *Jarid2* mutant and *Jarid2*-overexpressing conditions. Our genome-wide studies suggest that in *Drosophila*, under physiological conditions, Jarid2 does not appear to be a canonical component of the PRC2 complex as PRC2 recruitment is not altered on most target genes in *Jarid2* mutant animals. Interestingly,
overexpression of Jarid2 results in reduced Su(z)12 binding and changed chromatin compaction on polytene chromosomes, highlighting a possible role for Jarid2 in altering chromatin architecture. Genome-wide, Jarid2 and Su(z)12 binding correlate very well. However, certain loci, such as Homeobox (Hox) genes, differ significantly from this pattern. Here, Jarid2 occupancy on Polycomb response elements (PREs) is often very low where usually the highest enrichment for Su(z)12 can be observed. Gene expression analyses suggest a PRC2-dependent and -independent role for Jarid2 in transcriptional regulation. Jarid2 appears to be involved in the regulation of a certain number of PRC2 target genes and also transcriptionally controls a subset of genes independently of PRC2. Our data not only imply a function for Jarid2 and PRC2 in transcriptional repression but also support a possible role for both Jarid2 and PRC2 in active transcription on genes that are occupied by these factors.

MATERIALS AND METHODS

Construction of a FLAG-HA-Jarid2 line. Genomic DNA encompassing the jarid2 locus encompassing in an attB-P[acman]-CR-BW plasmid (clone number CH322-118D12) served as a platform for N-terminally FLAG tagging Jarid2. First, the galk-positive and counterselection scheme was used to N-terminally insert a galk cassette in frame of the Jarid2 open reading frame (ORF) according to a previously published protocol (48).

The FLAG-HA sequence with galk homology arms was amplified with the primer pair Jarid2 FLAG A forward and Jarid2 FLAG A reverse and the pair Jarid2 FLAG B forward and Jarid2 FLAG B reverse (see sequences below in “Primers”) using clone CH322-118D12 as a template. The galk cassette was exchanged with the FLAG-HA PCR product with galk homology arms as described previously (48).

FLAG affinity purification. Six grams of 6- to 18-h-old embryos were homogenized with a tissue tearer (Tissue-Tearor, model 985370-395; Bio-momology arms as described previously (48). The FLAG-HA sequence with galk homology arms was amplified with the primer pair Jarid2 FLAG A forward and Jarid2 FLAG A reverse and the pair Jarid2 FLAG B forward and Jarid2 FLAG B reverse (see sequences below in “Primers”) using clone CH322-118D12 as a template. The galk cassette was exchanged with the FLAG-HA PCR product with galk homology arms as described previously (48).

MudPIT analysis. Flag affinity-purified samples were precipitated with trichloroacetic acid and subjected to MudPIT analysis.
performed on a Nutator at room temperature for 20 min with 300 incubated for 5 min on a Nutator at room temperature, and centrifuged 4°C. After centrifugation for 2 min at 1,000 rpm at 4°C, the protein A-agarose was washed in 5 ml of RIPA buffer and centrifuged at 4°C. Sixty microliters of protein A-agarose and centrifuged for 5 min at 2,000 g at 4°C. The supernatant was kept, and the elution step was repeated. Elution fractions were pooled as aspirated, resuspended in 5 ml of buffer A1, and centrifuged for 5 min at 2,000 × g at 4°C. The supernatant was aspirated again, resuspended in 5 ml of buffer A2 (10 mM Tris HCl pH 7.5, 10 mM EDTA, 0.5 mM EGTA, 0.25% Triton X-100, 0.5 mM DTT, and complete, EDTA-free protease inhibitors [05056489001; Roche]) and centrifuged for 5 min at 2,000 × g at 4°C. The previous step was repeated one more time, and the pellet was resuspended in 4 ml of radiomunoprecipitation assay (RIPA) buffer (25 mM Tris, pH 7.5, 140 mM NaCl, 1% Triton X-100, 0.1% SDS, 0.1% Na-deoxycholate, 0.5 mM DTT, and complete, EDTA-free protease inhibitors [05056489001; Roche]) with 0.5% N-lauroylsarcosine. Samples (1.3 ml) were sonicated (Bioruptor; Diagenode) two times for 15 min (50% on/off) in 15-ml hard plastic tubes (430055; Corning) following centrifugation for 20 min at 14,000 rpm at 4°C. The supernatant was kept, and 10 μl of sonicated chromatin was reserved for gel analysis (to check sizing), and 150 μl was used as an input control. The sizing sample was reverse cross-linked at 65°C overnight with 90 μl of Tris-EDTA (TE) buffer and 3 μl of proteinase K (30 μg/ml), and 5 μl of proteinase K was used for the input sample. The remaining chromatin was diluted 5-fold with RIPA buffer and incubated overnight with the respective antibody on a Nutator at 4°C. Sixty microliters of protein A-agarose (15918-014; Invitrogen) was washed in 5 ml of RIPA buffer and centrifuged for 2 min at 1,000 rpm at 4°C. The supernatant was aspirated, and the chromatin sample was added and incubated for 2 h on a Nutator at 4°C. After centrifugation for 2 min at 1,000 rpm at 4°C, the protein A-agarose was transferred into a 1.5-ml tube, washed with 1 ml of RIPA buffer, incubated for 5 min on a Nutator at room temperature, and centrifuged for 2 min at 2,500 rpm at 4°C. The supernatant was aspirated, and the previous washing steps were repeated another seven times. Elution was performed on a Nutator at room temperature for 20 min with 300 μl of elution buffer (0.1 M NaHCO₃, 1% SDS) containing proteinase K (1 ml of elution buffer and 5 μl of 30 μg/ml proteinase K), and the sample was centrifuged for 2 min at 2,500 rpm at room temperature. The supernatant was kept, and the elution step was repeated. Elution fractions were pooled and reverse cross-linked at 65°C overnight. DNA was isolated with a Qia-gen PCR purification kit and eluted in 50 μl of H₂O, and DNA concentration was determined by a Pico Green Assay. Twenty nanograms of DNA was amplified (single-end primers; Illumina) and analyzed on a 2100 Bioanalyzer (Agilent Technologies) before being submitted to sequencing (Genome Analyzer; Illumina). ChiP-seq of eye-antenna imaginal discs. Eye imaginal discs (500) were dissected as quickly as possible in cold SFX insect medium (SH3O278.02; HyClone) and transferred into a microcentrifuge tube containing 500 μl of SFX medium, followed by centrifugation for 3 min at 300 × g at 4°C in a microcentrifuge. The supernatant was carefully removed, and 0.5 ml of 0.25% trypsin solution with EDTA (solution needs to be at 37°C) was added. After discs were preincubated for 1 min at 37°C, they were mechanically disrupted with a needle (size 25G1) by passage of the solution up and down nine times without creating bubbles. The resulting cell clumps were then incubated for another 1.5 min (maximum, 4 min) at 37°C, and the trypsinization was stopped by the addition of 1 ml of cold SFX medium. The cells were centrifuged for 5 min at 300 × g at 4°C in a microcentrifuge, and the supernatant was carefully removed. The resulting cell pellet was resuspended in 1 ml of Schneider medium with 10% fetal bovine serum (FBS) and submitted for cytometry.

Cytofluorometry and gene expression analysis. Dissociated cells (eye-antenna imaginal discs) were separated based on their green fluorescent set and their associated whole-cell extract controls were used for the input and control files, respectively. The effective genome size was configured appropriately for the fly data sets, and the P value cutoff was set to 1.00e−08 or an FDR of <1% and a fold change greater than 4. All other MACS parameters were left at default levels. Genes were called bound for Jarid2 and Su(z)12 if an enriched region occurred within 1 kb of an annotated transcript start site for any isoform of a gene from Ensembl, version 63. Based on the correlation of Jarid2 and Su(z)12 enrichment (see Fig. 4B) and random sampling of genes to verify Jarid2 and Su(z)12 occupancy, the list of Jarid2-enriched transcripts was used as the basis to define Jarid2/Su(z)12 cooccupancy.

Read coverage information in the track figures was created using R by extending the reads 150 bases toward the interior of the sequenced fragment and then by computing the number of extended reads in 25-bp windows as the count of extended reads per million reads sequenced ([RPV] counts/million). The resulting coverage object was exported and visualized using the UCSC genome browser (15). Pie charts depicting locations of enriched regions are based on the transcript annotations from Ensembl, version 63, where each peak region was annotated relative to the nearest transcript. Enrichment calculations were based on the ratio of normalized read counts for the ChIP of interest divided by the normalized read counts for the associated control. Su(z)12 enrichments were computed for each transcript in Ensembl, version 63, using 100 bp on both sides of the annotated start site using the whole-cell extract levels. The ratio for H3K27me3 enrichment was computed over the entire ORF length of each transcript using the H3 normalized read counts as the control. Affymetrix probe mappings were mapped to gene identifiers. Enrichment plots consist of all annotated transcripts for each gene mapped or described. Changes in Su(z)12 were determined using calculations of enrichment ratios separately for two Jarid2 mutants and the wild type with their associated whole-cell extracts. The geometric mean of enrichment ratios was computed for the two mutants, and this value was divided by the wild-type enrichment ratio, which results in a ratio that measures gain or loss. Genes were determined to have gained Su(z)12 if the resulting ratio showed a 2-fold increase of enrichment and if the gene was bound in the Jarid2 mutant condition by Su(z)12 using ChiP-seq analysis. Genes were determined to have lost Su(z)12 if the resulting ratio showed a 2-fold decrease of enrichment and if the gene was bound in the wild-type condition by Su(z)12. Changes in H3K27me3 were also determined using calculations of enrichment ratios separately for two Jarid2 mutants and the wild type with associated H3 samples. The geometric mean of enrichment ratios was computed for the two mutants, and this value was divided by the wild-type enrichment value. The average enrichment ratio for each of the two mutants and the wild-type ratio were determined by taking the geometric mean of the wild-type and mutant enrichment ratios. Genes determined to have gained or lost H3K27me3 showed a 2-fold increase or decrease, respectively, of enrichment, and the average enrichment ratio was greater than 0.25.

Dissociation of imaginal discs. Eye imaginal discs (~500) were dissected as quickly as possible in cold SFX insect medium (SH3O278.02; HyClone) and transferred into a microcentrifuge tube containing 500 μl of SFX medium, followed by centrifugation for 3 min at 300 × g at 4°C in a microcentrifuge. The supernatant was carefully removed, and 0.5 ml of 0.25% trypsin solution with EDTA (solution needs to be at 37°C) was added. After discs were preincubated for 1 min at 37°C, they were mechanically disrupted with a needle (size 25G1) by passage of the solution up and down nine times without creating bubbles. The resulting cell clumps were then incubated for another 1.5 min (maximum, 4 min) at 37°C, and the trypsinization was stopped by the addition of 1 ml of cold SFX medium. The cells were centrifuged for 5 min at 300 × g at 4°C in a microcentrifuge, and the supernatant was carefully removed. The resulting cell pellet was resuspended in 1 ml of Schneider medium with 10% fetal bovine serum (FBS) and submitted for cytometry.

Transcriptional Regulation by Drosophila Jarid2
protein (GFP) fluorescing properties and sorted directly into TRIzol (100,000 cells per 1 ml of TRIzol). RNA (from dissociated cells) was purified based on a standard protocol from the Drosophila Genomics Resource Center (DGRC, Indiana University). The RNA pellet was resuspended in 30 μl of RNase-free water. RNA from third-instar larvae was purified by grinding 20 larvae with a plastic pestle in RLT buffer (RNeasy Kit; Qiagen). After the suspension was passed over a QIAshredder column (Qiagen), samples were processed as described by the manufacturer. The RNA was analyzed with an RNA nano-chip from Agilent.

Affymetrix Drosophila 2 arrays were analyzed in R, version 2.11.1, using the packages affy (10), version 1.26.1, and limma (46), version 3.4.3. Normalization was done using robust multiarray averaging (RMA). Annotation information for the probes was taken from Ensembl, version 63. Differentially expressed genes were called with an unadjusted P value of <0.05 and fold change of at least 1.5 in either direction. Bound probes for MA (where M is the intensity ratio and A is the average intensity for a dot in the plot) plots from fly larvae expression values display bound gene lists for either Su(z)12 or Jarid2 from larval ChIP-seq analysis; eye disc MA plots display bound probes from the Su(z)12 gene list from eye disc ChIP-seq. Bound probes for MA plots from fly larvae expression values display bound gene lists for either Su(z)12 or Jarid2 from larval ChIP-seq analysis; eye disc MA plots display bound probes from the Su(z)12 gene list from eye disc ChIP-seq. Bound probes for MA plots from fly larvae expression values display bound gene lists for either Su(z)12 or Jarid2 from larval ChIP-seq analysis; eye disc MA plots display bound probes from the Su(z)12 gene list from eye disc ChIP-seq.

Microarray data accession number. Sequencing and expression data sets described in this study have been deposited at the National Center for Biotechnology Information Gene Expression Omnibus under accession number GSE36039.

RESULTS
In order to purify endogenous Jarid2 complexes, we used recombinant technology to construct a fly line that contains a FLAG-HA sequence at the N terminus of the Jarid2 ORF (see Materials and Methods). The resulting FLAG-HA-Jarid2 line was able to rescue lethality of the two strongest available Jarid2 mutants, Jarid2MB00996 and Jarid2e03131 (data not shown; see also Fig. S1A to C in the supplemental material). Also, colocalization studies on polytene chromosomes displaying only partial overlap between E(z) and Jarid2 (Fig. 1A) demonstrate that in the tagged line Jarid2 is not mislocalized. FLAG immunopurification was performed on extracts from 6- to 18-h-old Drosophila embryos to isolate Jarid2 and any associated proteins (Fig. 1B to D). However, full-length FLAG-HA-Jarid2 appears to be highly unstable as only a truncated form of FLAG-HA-Jarid2 could reproducibly be detected on a silver-stained SDS-PAGE gel (Fig. 1B, lane 2, asterisk) or in the

FIG 1 Purification of an endogenous Jarid2 complex in Drosophila. (A) Colocalization of Jarid2 and FLAG-HA-Jarid2 on polytene chromosomes from third-instar larvae showing overlap of endogenous Jarid2 with FLAG-HA-Jarid2. (A) Merged image of Jarid2 and HA antibody channels. (A′) Jarid2 antibody labeling. (A′′) HA antibody labeling. (A′′′) DAPI (4,6-diamidino-2-phenylindole) staining. (B) Silver staining of FLAG immunopurification of Jarid2. Lane 1, wild-type control FLAG purification; lane 2, Jarid2 FLAG purification. A degraded product of FLAG-HA-Jarid2 is marked with an asterisk. (C) Western blot analysis from nuclear embryonic extracts of wild-type control and FLAG-Jarid2 before and after FLAG purification. Lane 1, nuclear embryonic extract of the FLAG-HA-Jarid2 line labeled with anti-HA (upper panel) and anti-E(z) (lower panel); lane 2, nuclear embryonic extract from the FLAG-HA-Jarid2 line labeled with anti-HA (upper panel) and anti-E(z) (lower panel); lane 3, Eluate of wild-type control labeled with anti-HA (upper panel) and anti-E(z) (lower panel); lane 4, eluate of FLAG-HA-Jarid2 line labeled with anti-HA (upper panel) and anti-E(z) (lower panel). (D) MudPIT analysis of the Jarid2 FLAG purification. All major components of PRC2, including E(z), Su(z)12, Esc, and Caf1 along with Jing, the Drosophila homolog of human AEBP2, could be copurified. The relative abundance of the purified components is depicted by distributed normalized spectrum abundance factor (dNSAF) values on the y axes. Components of the wild-type control are blotted in red, and components of the Jarid2 purification are shown in blue. (E) Colocalization of E(z) and FLAG-HA-Jarid2 on polytene chromosomes displaying only partial overlap between E(z) and Jarid2. (E) Merged image of E(z) and HA antibody channels. (E′) E(z) antibody labeling. (E′′) HA antibody labeling. (E′′′) DAPI staining.
when Jarid2 was overexpressed, but no changes in H3K27me3 (C, arrow) are observed when Jarid2 is overexpressed, but no changes in H3K27me3 (C, arrow) can be detected in wild-type controls. (D) H3K27me1 levels are not affected under Jarid2-overexpressing conditions (arrow). (E) Filipase-catalyzed induction of Jarid2 mutant clones (no GFP expression) with the eye-specific eyeless (ey) promoter. Wild-type tissue is marked in green (GFP expression). (E') Jarid2 mutant clones display increased levels of H3K27me3 (two representative clones are outlined and highlighted by arrows). (F) Expression of E(z)-RNAi in the posterior part of the wing imaginal disc under the control of the en promoter. GFP expression in green marks the posterior part where E(z)-RNAi is expressed. (F') H3K27me3 is strongly reduced in the posterior compartment (white arrow). Genotypes: en-GAL4 UAS-GFP, UAS-Jarid2 (Jarid2^{AEBP2}) (A to D), ey-FLP; Jarid2^{AEBP2} FRT80B/ubi-GFP FRT80B (E), and en-GAL4 UAS-GFP, UAS-E(z)-RNAi (F).

To determine the role of Jarid2 in PRC2 function in an in vivo system, we studied the effect of Jarid2 overexpression (Fig. 2A to D) and of Jarid2 mutations (Fig. 2E) on H3K27 methylation, the mark implemented by PRC2. Overexpression of Jarid2 was confirmed by a Jarid2 antibody (Fig. 2A). The white arrow points out the posterior compartment where Jarid2 is overexpressed (Fig. 2A). Overexpression of Jarid2 results in reduction of H3K27me3 (Fig. 2E). Jarid2 mutant clones display increased levels of H3K27me3, and chromosomal aberrations are evident on the protein level as assessed by Western blotting (see Table S1 in the supplemental material). Quantitative RT-PCR experiments confirmed a very strong decrease of Jarid2 mRNA in Jarid2 mutant animals that were used to create mutant clones in eye-antenna imaginal discs (see Fig. S1A and B in the supplemental material) compared to wild-type controls (see Fig. S1A and B). This is also evident on the protein level as assessed by Western blotting (see Fig. S1C). Even though a global increase and reduction in H3K27me3 could be reproducibly detected under both Jarid2 mu-
In order to assess a possible role of Jarid2 in recruiting PRC2 complexes to chromatin, we compared Jarid2 (see Fig. S2A to D in the supplemental material), Su(z)12 (Fig. 3A to D) and H3K27me3 patterns on polytene chromosomes in the wild type (Fig. 3A, E, and I; see also Fig. S2A), Jarid2 mutant (Fig. 3B, F, and J; see also Fig. S2B), and under Jarid2-overexpressing (Fig. 3C, G, and K; see also Fig. S2C) and E(z) mutant (Fig. 3D, H, and L; see also Fig. S2D) conditions. Jarid2 is widely distributed in wild-type animals (see Fig. S2A) and is completely lost from chromatin in Jarid2 mutant animals (see Fig. S2B). Surprisingly, Su(z)12 binding to chromatin (compare Fig. 3A and B) and H3K27me3 levels (compare Fig. 3E and F) are not detectably affected in Jarid2 mutants. Similarly, the pattern of Pc (compare Fig. 3I and J), a core component of Polycomb repressive complex 1 (PRC1), is not considerably altered in Jarid2 mutants. This seems to suggest that, at least in Drosophila, Jarid2 is not globally required for recruitment of the PRC2 or PRC1 complex to Polycomb response elements (PREs).

Upon overexpression, Jarid2 binding to chromatin is strongly enhanced (see Fig. 3C’ in the supplemental material), and in some cases, the polytene chromosomes appear shortened, bloated, and poorly banded, indicating defects in higher-order chromosome compaction (Fig. 3C; see also Fig. S2C). In other cases this bloating phenotype is weaker (Fig. 3K) or not distinctly

**FIG 3** The role of Jarid2 in recruitment of Polycomb repressive complexes 2 and 1 and modulation of H3K27me3 on chromatin. (A to L) Squashes of polytene chromosomes from salivary glands of wild-type, Jarid2 mutant, Jarid2-overexpressing (UAS-Jarid2), and E(z) mutant third-instar larvae labeled with various antibodies. Panels show individual and merged images of antibody and DAPI channels. Panels A’ to L’ reflect the antibody-only channel, and panels A’ to D’ show results of Su(z)12 antibody labeling. (A’) Su(z)12 binding pattern in wild-type controls. Su(z)12 localization is not changed in Jarid2 mutants (B’) but is lowered in Jarid2-overexpressing (C’) and E(z) mutant (D’) animals. (E’ to H’) H3K27me3 antibody labeling. (E’) H3K27me3 binding pattern in wild-type controls. H3K27me3 is not visibly changed in Jarid2 mutants (F’). A reduction of H3K27me3 can be observed when Jarid2 is overexpressed (G’) or E(z) is removed (H’). (I’ to L’) Pc antibody labeling. (I’) Pc binding pattern in wild-type controls. No effects on Pc localization can be observed in either the Jarid2 mutants (J’) or under Jarid2-overexpressing (K’) or E(z) mutant (L’) conditions. Genotypes: Oregon R (A, E, and I), Jarid2MB00996 (B, F, and J), act-GAL4/UAS-Jarid2 (Jarid2LA00681) (C, G, and K), and E(z) TS [E(z)61, temperature shifted to 29°C for 24 h] (D, H, and L).
FIG 4 Genome-wide localization patterns by ChIP-seq of H3K27me3, Su(z)12, and Jarid2 in wild-type and Jarid2 mutant tissue. (A) Pie charts showing the genome-wide distribution of Jarid2 and Su(z)12 peaks in wild-type larvae. Gene localizations are represented as indicated on the figure. (B) The scatter plot shows the correlation of Jarid2 occupancy and Su(z)12 occupancy for each transcript from Ensembl, version 63, measured as the maximum normalized fragment count within 50 bp of the TSS. Highlighted in red are transcripts that fall within the lower quartile of Jarid2 occupancy (the correlation of Jarid2 occupancy and Su(z)12 occupancy for each transcript from Ensembl, version 63, measured as the maximum normalized fragment count genome-wide distribution of Jarid2 and Su(z)12 peaks in wild-type larvae. Gene localizations are represented as indicated on the figure. (B) The scatter plot shows the correlation of Jarid2 occupancy and Su(z)12 occupancy for each transcript from Ensembl, version 63, measured as the maximum normalized fragment count genome-wide, only comparatively few genes show significant changes in Su(z)12 binding (Fig. 4C, red tracks) in Jarid2-overexpressing larvae, both Su(z)12 recruitment (compare Fig. 3A’ and C’) and H3K27me3 levels (compare Fig. 3E’ and G’) are negatively affected. Pc localization on polytene chromosomes was marginally reduced when Jarid2 was overexpressed (compare Fig. 3I’ and K’).

The concept that PRC2 is generally not required for recruitment of PRC1 was further confirmed when E(z)61 mutants were analyzed (4). Upon a temperature shift to 29°C for 24 h, E(z)61 mutant third-instar larvae show almost a complete loss of Su(z)12 from polytene chromosomes (compare Fig. 3A’ and D’) and consequently H3K27me3 is lost (Fig. 3H’). Nevertheless, Pc recruitment is not appreciably altered (compare Fig. 3I’ and L’). This strongly supports a PRC2-independent recruitment of PRC1 to chromatin (see also references 29 and 30) and furthermore suggests that at least in vivo the H3K27me3 mark is not the primary determinant for Pc binding, in contrast to previous proposals based largely on in vitro data (9, 26). The absence of E(z) does not impair Jarid2 localization on polytene chromosomes (compare Fig. S2A’ and D’ in the supplemental material), suggesting that Jarid2 can be recruited to chromatin independently of PRC2.

To address whether Jarid2 affects recruitment of PRC2 at a molecular level, ChIP-seq experiments for Jarid2, Su(z)12, and H3K27me3 in the wild type and Jarid2 mutants were performed. Most Jarid2 and Su(z)12 peaks are found at transcription start sites (TSSs) (Fig. 4A; see also Fig. S3A in the supplemental material). Of the peaks that were detected upstream, downstream, or inside genes, some could define TSSs of novel transcription units. Of the peaks that were detected upstream, downstream, or inside genes, some could define TSSs of novel transcription units. Additionally, a small group of genes, which display high Su(z)12 occupancy and low Jarid2 occupancy (Fig. 4B, highlighted in red). Interestingly, in this class, 7 out of 30 genes are Hox genes (see Table S2 in the supplemental material). The binding patterns of Jarid2 and Su(z)12 on the Bithorax complex illustrate this finding (Fig. 4C). Occupancy of canonical PRC2 components such as Su(z)12 is usually very high on this locus and was distributed in several peaks (Fig. 4C, red tracks). At many of these highly occupied Su(z)12 sites, Jarid2 occupancy is relatively low (Fig. 4C, compare red with green tracks). This indicates a fundamentally different Jarid2 binding behavior than that of canonical PRC2 members for some of the major PRC2 target genes such as Hox genes.

Despite substantial colocalization of Jarid2 and Su(z)12 genome-wide, only comparatively few genes show significant changes in Su(z)12 binding (Fig. 4C, red tracks) in Jarid2 mutants, confirming our observations on polytene chromosomes. No general effects on Su(z)12 binding were apparent when wild-type Jarid2 mutant tissues in eye imaginal discs were compared (data not available).}

observable (Fig. 3G). Similar bloating phenotypes have been described for chromatin remodelers such as Imitation SWI (Iswi) (5), and it has been shown that mutants of another chromatin remodeling factor, the trithorax group protein Kismet (Kis), globally affect H3K27 methylation (47). This phenotype is interesting in the light of a recent report which suggests a genetic interaction between yet another chromatin remodeler, brahma (brm), and Jarid2 (7), providing further evidence for a possible role of Jarid2 in regulating higher-order chromatin structure. Interestingly, in Jarid2-overexpressing larvae, both Su(z)12 recruitment (compare Fig. 3A’ and C’) and H3K27me3 levels (compare Fig. 3E’ and G’) are negatively affected. Pc localization on polytene chromosomes was marginally reduced when Jarid2 was overexpressed (compare Fig. 3I’ and K’).

The concept that PRC2 is generally not required for recruitment of PRC1 was further confirmed when E(z)61 mutants were analyzed (4). Upon a temperature shift to 29°C for 24 h, E(z)61 mutant third-instar larvae show almost a complete loss of Su(z)12 from polytene chromosomes (compare Fig. 3A’ and D’) and consequently H3K27me3 is lost (Fig. 3H’). Nevertheless, Pc recruitment is not appreciably altered (compare Fig. 3I’ and L’). This strongly supports a PRC2-independent recruitment of PRC1 to chromatin (see also references 29 and 30) and furthermore suggests that at least in vivo the H3K27me3 mark is not the primary determinant for Pc binding, in contrast to previous proposals based largely on in vitro data (9, 26). The absence of E(z) does not impair Jarid2 localization on polytene chromosomes (compare Fig. S2A’ and D’ in the supplemental material), suggesting that Jarid2 can be recruited to chromatin independently of PRC2.
shown). Nonetheless, in a few cases, a significant increase or reduction (\(\geq 2\) or \(\leq 0.5\)) of Su(z)12 occupancy in Jarid2 mutants can be observed (Fig. 4C, red tracks, black arrows). In contrast to the mammalian findings, Su(z)12 occupancy can also be increased on certain genes in Jarid2 mutants (Fig. 4C, red tracks, left arrow). Neither genes with reduced Su(z)12 occupancy (Fig. 4C, red tracks, right arrow; see also Fig. S3B in the supplemental material) nor genes with increased Su(z)12 occupancy (Fig. 4C, red tracks, left arrow; see also Fig. S3C) displayed any significant changes (\(\geq 2\) or \(\leq 0.5\)) in H3K27me3 enrichment in Jarid2 mutants (Fig. 4C, blue tracks) or represented genes with no or low H3K27me3 enrichment (see Fig. S3B and C, upper two tracks). Although genes with changes in Su(z)12 occupancy and the majority of other genes [Su(z)12/Jarid2 bound and unbound] do not generally display significant changes (\(\geq 2\) or \(\leq 0.5\)) in H3K27me3 enrichment, a relatively big group of genes shows a very weak but statistically observable increase in H3K27me3 in Jarid2 mutant versus wild-type larvae (see Fig. 3D, dashed box). Likewise, the sum of these small increases in H3K27me3 enrichment of a large group of genes might be the reason why H3K27me3 is globally increased in Jarid2 mutant eye-antenna imaginal disc clones (Fig. 2E).

To gain further insight as to how Jarid2 regulates the expression of its target genes, we performed gene expression analyses from wild-type, Jarid2 mutant, and E(z)-RNAi larvae (Fig. 5; see Fig. S4 and S5 in the supplemental material). A total of 204 Jarid2/Su(z)12 cobound genes showed at least a 1.5-fold gain (Fig. 5A, highlighted in blue), and 136 Jarid2/Su(z)12 cobound genes had a more than 1.5-fold loss (Fig. 5A, highlighted in red) in expression in Jarid2 mutant larvae. Genes with a significant increase/reduction in Su(z)12 localization and/or increased/reduced H3K27me3 enrichment in Jarid2 mutants (\(\geq 2\) or \(\leq 0.5\)) did not generally correlate with gene expression changes (data not shown). Not all Jarid2/Su(z)12 cobound genes that are transcriptionally changed in Jarid2 mutants are also modified in E(z)-RNAi larvae (Fig. 5B) and vice versa (see Fig. S4A and B). We find evidence for coregulation of PRC2 target genes by Jarid2 and E(z) (Fig. 5C and D show individual examples), confirming a role for Jarid2 in PRC2-mediated regulation of transcription. Jarid2 and E(z) only not appear to function in maintenance of transcriptional repression (Fig. 5C) but are also possibly involved in activation of common target genes (Fig. 5D). However, the possibility exists that a gene might be cobound by Jarid2/Su(z)12 and yet would not directly be transcriptionally controlled by these factors. Despite the binding of Jarid2/Su(z)12, a change in transcription in the corresponding mutant/RNAi animal could be a secondary consequence of other genes that are under direct control of Jarid2/Su(z)12. Generally, Jarid2/Su(z)12 cobound genes that show transcriptional changes in Jarid2 mutants and/or E(z)-RNAi larvae do not correlate with changes (\(\geq 2\) or \(\leq 0.5\)) in Su(z)12 occupancy (Fig. 5E) and/or changes in H3K27me3 enrichment (Fig. 5F) in Jarid2 mutants. We also identified Jarid2/Su(z)12 cobound genes that are regulated by Jarid2 independently of canonical PRC2 members [E(z)] (see Fig. S4C and D) and target genes solely controlled by canonical PRC2 members [E(z)] independently of Jarid2 (see Fig. S4E and F).

It needs to be stressed that both ChIP-seq experiments and gene expression analyses have been carried out in larvae which represent a mixed population of cells. This could prevent the identification of genes that are regulated by Jarid2 in a tissue-specific manner. Alterations in Su(z)12 occupancy and H3K27me3 enrichment might be masked because only a subpopulation of cells is controlled by Jarid2. Therefore, we also performed ChIP-seq studies for Su(z)12 occupancy and gene expression analyses in eye-antenna imaginal discs (see Fig. S5A and B in the supplemental material). Similar to the results obtained in larvae, gene expression changes in Jarid2 mutant eye-antenna imaginal disc clones usually do not correlate with altered Su(z)12 occupancy (see Fig. S5B). Taken together, these results imply a role for Jarid2 in PRC2-dependent and -independent regulation of transcription. Our data support a role for Jarid2 and PRC2 in maintenance of transcriptional repression (Fig. 5C) and suggest the possibility for both Jarid2 and PRC2 to function in transcriptional activation or elongation (Fig. 5D). For most of its target genes, Jarid2 seems to carry out its role in transcriptional regulation independently of changes in H3K27me3 or PRC2 recruitment. However, the general tendency implies that most Jarid2/PRC2-coregulated genes have relatively low levels of H3K27me3 (Fig. 5E).

**DISCUSSION**

In this study, we describe the purification of a Jarid2 complex in *Drosophila*. Consistent with previous results in mammalian systems, we find that Jarid2 is a component of PRC2 (Fig. 1) (19, 22, 32, 33, 42, 53). We provide evidence that in imaginal discs (Fig. 2) and on polytene chromosomes (Fig. 3), Jarid2 is required to fine-tune global H3K27me3 levels. Jarid2 might accomplish this by modulating the activity of the core complex [E(z), Su(z)12, Esc, and Caf1]. Our data indicate that Jarid2 could play an inhibitory role in the implementation of H3K27me3 as Jarid2 mutant imaginal disc clones display a global increase (Fig. 2E) and as overexpression of Jarid2 results in a reduction in H3K27me3 (Fig. 2B and 3G). Despite having a JmJC domain, Jarid2 has been predicted (16) and reported (22, 42) to be catalytically inactive as a histone demethylase. Therefore, it is unlikely but not impossible that it could function in this manner toward H3K27me3, thereby countering PRC2 activity. Even if Jarid2 would be inactive as a histone demethylase, it might still be able to bind to chromatin and prevent spreading of the H3K27me3 mark, such as opposing a possible positive spreading effect of Esc (EED in mammals) (24).

Furthermore, even though Jarid2 could be purified with the PRC2 core members (Fig. 1) and its occupancy generally correlates very well with canonical PRC2 components such as Su(z)12 (Fig. 4B), it does not appear to play a significant role in regulating PRC2 recruitment in a physiological context, as assessed by our Jarid2 mutant animal studies (Fig. 3 to 4). Apparent differences with published mammalian studies, which imply a major role for Jarid2 in recruitment of PRC2, could be explained by variation in the mechanisms employed or by the fact that the recruitment of PRC2 in ES cells generally differs from that in differentiated tissues. For example, PREs have been known to be highly effective in recruiting PRC2 to target sites in *Drosophila* (36, 43). In mammals, attempts have been made to identify functionally analogous sequences but with only limited success (45, 49). Indeed, it seems more likely that the recruitment of PRC2 in mammals not only requires specific sequences but is also more dependent on additional factors (proteins and RNA), which might explain why PRC2 recruitment is more strongly affected in Jarid2-depleted cells and why PRC1 recruitment in some instances appears to be dependent on PRC2 (H3K27me3) (2). However, our data in *Drosophila* salivary glands and that of other groups suggest that recruitment of PRC2 (and methylation of H3K27) is not a prerequisite for targeting of PRC1 (Fig. 3L) (29, 30), and the generality of
this mechanism is also increasingly questioned in the mammalian system (35). Nonetheless, when Jarid2 is overexpressed in Drosophila, changes in chromosome compaction can be observed (Fig. 3C; see also Fig. S2C in the supplemental material). Under these conditions, Jarid2 extensively occupies the chromosomes and negatively affects Su(z)12 localization and H3K27me3 (Fig. 3C and G). It is possible that increasing Jarid2 levels beyond a certain physiological level might interfere with...
PRC2 integrity. Larger amounts of Jarid2 might alter the stoichiometry of the PRC2 subunits, resulting in destabilization of the PRC2 complex on chromatin.

Jarid2 also behaves differently from other canonical PRC2 members in Drosophila, as is evident from its binding pattern on certain Hox genes (see Table S2 in the supplemental material). Here, occupancy of PRE sites by canonical PRC2 members is one of the highest in the whole genome (Fig. 4C, red tracks). In contrast, Jarid2 displays relatively low occupancy on many of these loci (Fig. 4C, compare red with green tracks), implying a minor or different function for Jarid2 in controlling transcription of these well-described PRC2 targets. It is also possible that at these loci Jarid2 has a more transient association or even that it is less accessible to interact with the antibodies that we have generated. However, our findings are also in agreement with modifier screens that have been performed in Drosophila to identify major regulators of Polycomb group-mediated phenotypes but that were unable to capture Jarid2 (11).

Additionally, our data suggest that Jarid2 appears to control PRC2-dependent transcription (Fig. 5; see also Fig. S4 in the supplemental material), although not necessarily in the same way as expected for canonical PRC2 members. For example, in contrast to the mammalian findings (19, 22, 32, 42, 53), we observe that PRC2-mediated transcriptional regulation by Jarid2 in Drosophila is generally independent of changes in Su(z)12 occupancy (Fig. 5E; see also Fig. S5B) and does not correlate with changes in H3K27me3 enrichment (Fig. 4C and 5F). However, it needs to be stressed that most Jarid2/PRC2 cobound genes with altered expression patterns in jarid2 mutants and E(z)-RNAi larval contain no or low levels of H3K27me3 (Fig. 5F), which is in contrast to the mammalian system where PRC2 components are usually found only at genes with high H3K27me3 enrichment (2, 21). Nonetheless, in Drosophila, genes with high H3K27me3 enrichment exist that change in transcription in Jarid2 mutants and E(z)-RNAi animals (Fig. 5F), demonstrating that H3K27me3 is not necessarily instructive for transcriptional repression per se. To date most of the evidence ascribing to H3K27me3 the role of a repressive mark is based on correlation from the observation that PRC2 components colocalize with H3K27me3 and that the respective genes seem to be transcriptionally silenced. Our data imply that this might generally be the case but that there are also exceptions to the rule. That certain H3K27me3 patterns can also be connected to transcriptionally active genes in mammals has just recently been reported (51).

Finally, our results imply that Jarid2 and PRC2 are not only involved in maintenance of gene repression (Fig. 5C) but could also function in active transcriptional processes such as transcriptional activation of elongation (Fig. 5D). This is in agreement with previous reports (13, 28, 29, 31) and demonstrates that PRC2 has cellular functions that extend beyond what we have learned from its role at Hox genes. Importantly, our studies also suggest that despite a very good correlation of Jarid2 and Su(z)12 occupancies (Fig. 4B), Jarid2 might function in transcriptional repression and activation independently of the canonical PRC2 complex [E(z)] (see Fig. S4C and D in the supplemental material) and vice versa (see Fig. S4E and F). This distinction in target genes between Jarid2 and canonical PRC2 components [E(z)] provides additional confirmation that Jarid2 in some respects behaves fundamentally differently than the canonical PRC2 complex. Together with the varied functions proposed for Jarid2 in mammals (12), our studies highlight the diverse aspects of Jarid2 function in PRC2-mediated gene regulation.

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