JASPer controls interphase histone H3S10 phosphorylation by chromosomal kinase JIL-1 in Drosophila

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In flies, the chromosomal kinase JIL-1 is responsible for most interphase histone H3S10 phosphorylation and has been proposed to protect active chromatin from acquiring heterochromatic marks, such as dimethylated histone H3K9 (H3K9me2) and HP1. Here, we show that JIL-1’s targeting to chromatin depends on a PWWP domain-containing protein JASPer (JIL-1 Anchoring and Stabilizing Protein). JASPer-JIL-1 (JJ)-complex is the major form of kinase in vivo and is targeted to active genes and telomeric transposons via binding of the PWWP domain of JASPer to H3K36me3 nucleosomes, to modulate transcriptional output. JIL-1 and JJ-complex depletion in cycling cells lead to small changes in H3K9me2 distribution at active genes and telomeric transposons. Finally, we identify interactors of the endogenous JJ-complex and propose that JIL-1 not only prevents heterochromatin formation but also coordinates chromatin-based regulation in the transcribed part of the genome.
In mammals, several nuclear kinases contribute to phosphorylation of histone H3 at serine 10 (H3S10ph) in interphase, whereas in Drosophila melanogaster, the essential kinase JIL-1 is responsible for most of it. The significance of interphase H3S10ph is often underestimated because most H3S10 phosphorylation in asynchronous cell populations stems from mitotic chromatin, where it is deployed by Aurora B kinase. Originally, interphase H3S10ph has been associated, in combination with H3K9ac and H3K14ac, with transcriptional activation of immediate early genes upon MAPK activation. In Drosophila, interphase H3S10ph is enriched at the body of active genes. In mammal, in the extreme case of mouse embryonic stem cells (mESC), ~30% of the genome is enriched for H3S10ph in interphase.

The current model assigns JIL-1 to the protection of euchromatin from heterochromatization. According to the phospho-methyl switch model for mitotic H3S10ph, placing H3S10ph prevents H3K9 methylation and subsequent binding of heterochromatin components. JIL-1 phosphorylates various H3 peptides with different methylation states, including H3K9me2/3, with comparable efficiency, whereas histone methyltransferases of the Su(var)3-9 family are inhibited by H3S10ph. Several observations suggest that JIL-1 is important for the balance between euchromatin and heterochromatin. The Su(var)3-9 alleles of JIL-1 gene, which lead to the expression of JIL-1 truncated in its C-terminal domain (CTD), result in reduced heterochromatin spreading at euchromatin-heterochromatin boundaries. Conversely, in the JIL-1Δ2/2 null mutant, heterochromatin components spread into euchromatin. The spreading of H3K9me2 and HP1 is highest on the euchromatic part of the X chromosome in both sexes, whereas the spreading of the 7-zinc-finger protein Su(var)3-7 affects euchromatin similarly on all chromosomes. In addition, JIL-1 phosphorylates Su(var)3-9, the histone methyl transferase responsible for H3K9me2/3, suggesting a possible function for JIL-1 at constitutive heterochromatin.

JIL-1 may also play a role at telomeres, which combine features of heterochromatin and euchromatin in Drosophila. JIL-1 localizes to arrays composed of the three non-LTR retrotransposons Het-A, TART, and TAHERE (HTT) on polytene chromosomes in mutants with elongated telomeres. Transcription of HTT arrays is essential for telomere maintenance in flies, and JIL-1 is a positive regulator of retrotransposon transcription.

At the low resolution of polytene chromosomes, JIL-1 localizes to active chromatin and is enriched on the male dosage-compensated X chromosome. When the binding of JIL-1 to chromatin was studied at higher resolution using chromatin-immunoprecipitation (ChIP), conflicting results were obtained. Our early ChIP-chip study suggested that JIL-1 is found on all transcribed gene bodies and is enriched on X-chromosomal genes in male S2 cells. ChIP-seq experiments from female Kc cells suggested that JIL-1 associates to the 5′ end of euchromatic genes. Using Coexpressing recombinant FLAG-JIL-1 and untagged JASPer showed that JIL-1 was efficiently co-immunoprecipitated from embryo extracts and with similar efficiency. Coexpressing recombinant FLAG-JIL-1 and untagged JASPer yielded a stable complex. Coomassie-blue staining suggested a roughly equal stoichiometry for the recombinant and the endogenous complex.

Results

JIL-1 forms a complex with the protein JASPer. Since JIL-1 lacks a known chromatin binding domain, we hypothesized that JIL-1 is recruited to chromatin by an interaction partner. To identify such a protein, we used nuclear extracts of D. melanogaster embryos to perform preparative immunoprecipitations (IPs) using antibodies against JIL-1. A protein of ~60 kDa copurified with JIL-1 using two different JIL-1 antibodies (Supplementary Fig. 1a). Mass spectrometry analysis identified the protein as encoded by the gene CG7946 on chromosome 3R. We named this protein ‘JIL-1 Anchoring and Stabilizing Protein’ (JASPer). Consistently, reverse IP’s using antibodies against JASPer showed that JIL-1 was efficiently co-immunoprecipitated from embryo extracts and with similar efficiency (Fig. 1a).

JASPer is a well-conserved protein among Drosophila species. It has an N-terminal PWWP domain and a C-terminal LEDGF/IBD domain (Fig. 1c, Supplementary Figs. 2a, b). This PWWP-LEDGF/IBD domain architecture is found in 94 eukaryotic proteins, with mostly unknown functions, except for the PSIP1/LEDGF chromatin adapter protein, which has pleiotropic functions in HIV infection and cancer development. JIL-1 is also well conserved among distant Drosophila species (Supplementary Fig. 3), particularly in the N-terminal AGC kinase domain, the C-terminal MAPK-related domain and its CTD. The CTD is rich in proline (11%) and arginine (9%) residues and most probably intrinsically disordered. Sequence comparison revealed a prion-like domain (PrID) and putative PEST sequences, which most probably relate to lower stability of the protein because of their intrinsic disorder.

Using a LacO-LacI targeting system in flies, we found that LacI-JIL-1 full-length and LacI-JIL-1-CTD recruited endogenous JASPer to the LacO arrays, but JIL-1-ΔCTD did not (Supplementary Fig. 1b). We further mapped the interaction by co-expression and co-purification of various derivatives. Truncations in the CTD of JIL-1 were designed according to sequence conservation in Drosophila JIL-1 homologs (Fig. 1c and Supplementary Fig. 3). Expression of the C-terminal deletion mutants of FLAG-JIL-1 (mutants a-g) with untagged, full-length JASPer showed that the minimal JASPer binding domain (JBD) encompasses 44 amino acids (982–1025) between the truncations c and d of the CTD (Fig. 1d). The JBD is rich in proline (22%), glutamic acid (16%), and aromatic residues tyrosine/phenylalanine (16%). Furthermore, it contains a stretch of 7 conserved amino acids, DFXGFE, matching the consensus motif found in proteins interacting with the LEDGF/IBD domain of PSIP1. Indeed, using various JASPer derivatives (Fig. 1c) co-expressed with full-length FLAG-JIL-1, we found that deletion of the 120 amino acids long LEDGF domain in the C-terminal half of JASPer (ALEDGF) was sufficient to abrogate binding to JIL-1 (Fig. 1e). This domain contains a high proportion of charged residues (glutamic acid/aspartic acid: 18% and arginine/lysine residues: 17%).
JASPer stabilizes JIL-1 in vivo. To understand the function of JASPer in the JJ-complex, we generated JASPer<sup>ΔΔ</sup> null allele by imprecise excision of the P-element in an appropriate EP line. The deletion encompassed the coding region of both described transcripts (Fig. 2a). Analysis of the salivary glands of homozygous JASPer<sup>ΔΔ</sup> null mutants showed that JASPer was not detectable by western blot and on polytene chromosome spreads (Fig. 2b, c). Remarkably, JIL-1 was also not detectable in the absence of JASPer. The lack of the JJ-complex in turn correlates with undetected phosphorylation of H3S10, confirming that kinase as the major source of this modification in interphase (Fig. 2d). Such a direct relationship between kinase and H3S10ph cannot be seen in an exponentially growing cell population, due to the strong dominance of mitotic H3S10ph<sup>6</sup>. As also described for the JIL-1<sup>ΔΔ</sup> hypomorph mutant<sup>14</sup>, global H3K9me2 levels were unchanged (Fig. 2b) but the mark redistributed from the euchromatic chromosomal arms, in particular of the X chromosome (Fig. 2d). Although the JASPer<sup>ΔΔ</sup> mutant mostly phenocopies JIL-1<sup>ΔΔ</sup> mutant, polytene chromosomes retain their characteristic banded pattern (Fig. 2c, d), which are lost in the JIL-1<sup>ΔΔ</sup> mutant<sup>30</sup>. This observation is consistent with the partial lethality of JASPer<sup>ΔΔ</sup> mutant (54% of expected survival, n = 1496) as compared to the lethal JIL-1<sup>ΔΔ</sup> mutant (Fig. 2e). Ablation of JASPer by RNA interference in cultured cells also led to loss of JIL-1 (Fig. 2f). JIL-1 was depleted to the same level by RNAi against jil-1 or jasper in S2 and Kc cells, suggesting that JIL-1 is unstable in the absence of JASPer. The JIL-1 transcript level was unchanged upon jasper RNAi in our RNA-seq experiments, excluding regulation at the transcription level (Fig. 2g). However, trace amounts of JIL-1 or fragments of it might still be expressed and could explain the better viability of JASPer<sup>ΔΔ</sup> mutant versus JIL-1<sup>ΔΔ</sup> mutant<sup>31</sup>.

JASPer binds nucleic acids and H3K36me3 nucleosomes in vitro. In addition to the LEDGF JIL-1-binding domain, JASPer harbors a PWWP domain at its N-terminus (Fig. 1c). PWWP domains have a positively charged surface favoring DNA binding and an aromatic pocket for methyl-lysine binding [for review<sup>32</sup>]. Conceivably, this domain is responsible for the recruitment of the JJ-complex to chromatin. As expected, recombinant JASPer had significant affinity for DNA in electrophoretic mobility shift assays (EMSA), while JIL-1 showed no detectable binding under the same conditions (Supplementary Fig. 4a). A 9-fold molar excess of JASPer shifted all DNA molecules. Apparently, several JASPer molecules can bind simultaneously one DNA molecule as at least three retarded bands appeared in the EMSA and the most retarded ones correlated with higher JASPer concentration. JASPer also bound a 123 nucleotide long RNA hairpin<sup>33</sup> in a dose-dependent manner (Supplementary Fig. 4b).
**Fig. 2** JIL-1 is unstable in absence of JASPer in the JASPer^cw2/cw2 mutant and in cell lines. 

- **a** Gene model for P-element excision in the JASPer locus to generate JASPer^cw2 allele. The mRNA isoforms RA and RB are shown below. The excised genomic portion is marked in white and EP denotes the position of the excised P-element in EP-element line GS3268.

- **b** Western blot analysis of salivary gland extracts from L3 larvae of homozygous JASPer^cw2/cw2 and JIL-1^z2/z2 mutants and wild type larvae as control. Western blots using α-JIL-1, α-JASPer, α-H3S10ph, and α-H3K9me2 antibodies are shown, western blot with α-tubulin antibody was used as loading control.

- **c** Immunofluorescence microscopy of polytene chromosome squashes from L3 larvae of homozygous JASPer^cw2/cw2 and wild type larvae as control. From left to right, staining for JASPer, JIL-1, merged images and DNA are shown. The X chromosome is marked by arrow heads. Source data are provided as a Source Data file.

- **d** Immunofluorescence microscopy of polytene chromosome spreads from L3 larvae of homozygous JASPer^cw2/cw2 and wild type larvae as control. From left to right, staining for H3S10ph, H3K9me2, merged images and DNA are shown. The X chromosome is marked by arrow head and the chromocenter is labeled with “CC”.

- **e** Table summarizing viability of male and female JASPer^cw2/cw2 mutant flies. 

- **f** Representative western blot analysis using α-JASPer and α-JIL-1 antibodies on whole cell extracts from S2 cells (left panel) and Kc cells (right panel) after jasper or jil-1 RNAi treatment, as used for RNA-seq experiments. A cross-reacting band is marked by asterisk. Source data are provided as a Source Data file.

- **g** Bar chart showing mean log_{2} fold-change of normalized mean RNA-seq counts for JIL-1 and JASPer RNAi. Left panel, JIL-1 mRNA mean log_{2} fold-change upon jasper RNAi (S2 n = 4 and Kc n = 4). Right panel, JASPer mRNA mean log_{2} fold-change upon jil-1 RNAi (S2 n = 5 and Kc n = 4). Error bars represent standard error of the mean.
To decipher the binding specificity of JASPer and the JJ-complex for nucleosomes, we used a library of 115 different types of DNA-barcoded nucleosomes bearing different histone and DNA modifications. Recombinant, FLAG-tagged JASPer or FLAG-tagged JJ-complex were coupled to α-FLAG beads, incubated with the nucleosome library, washed and the pulled-down nucleosomes were quantified by sequencing of the associated indexes. Wild type JASPer showed high specificity towards nucleosomes bearing the single H3K36me3 modification (Fig. 3a). This modification was ~40-fold enriched in the IP relative to the unmodified nucleosome used for normalization. Mutation of two residues in the aromatic cage to alanines (Y23A and W26A) abolished specificity of JASPer and the JJ-complex for nucleosomes, that the active site-mutated enzyme is inactive and that the aromatic cage engages with the K36me3 residue.

JIL-1 is a potent kinase in vitro and phosphorylates isolated H3 peptide (amino acids 1–20) and full-length histone H3. However, the isolated kinase proved to be inactive on nucleosome arrays (>3% of the phosphorylated H3 reference) in vitro even at high molar ratios of kinase to nucleosome. To overcome this, we added 5% MNase ChIP-seq normalized coverage along representative 200 kb windows on chromosome 2 R and X in male S2 cells. HAS are marked by red bars above the gene models in gray. D box plot showing mean H3K36me3 (left panel, n = 4), JASPer (second left panel, n = 4) and JIL-1 (second right, n = 5) and MSL3 (right, n = 3) MNase ChIP-seq normalized coverage, as in c, at active (tpm > 1) and inactive (tpm ≤ 1) genes on the autosomes (n = 5785 and n = 8726, respectively) and X chromosome (n = 1214 and n = 1407, respectively) in male S2 cells. Box plot elements are defined as center line marking the median, box limits are the upper and lower quartiles, whiskers extend maximally 1.5-times the interquartile range and outliers are removed.

The JJ-complex binds H3K36me3 nucleosomes in vitro and in vivo, and is enriched on the male X chromosome. A bar chart of mean enrichment (n = 3 independent experiments with 2 different protein preparations) of nucleosome library pull-down with JASPer-FLAG (left panel) and aromatic cage mutant (right panel) relative to unmodified nucleosome, which is set to 1. Error bars represent standard error of the mean. B bar chart of mean enrichment (n = 3 independent experiments) of nucleosome library pull-down with JJ-complex (FLAG-JIL-1 and untagged JASPer) (left panel) and aromatic cage mutant (right panel) relative to unmodified nucleosome, which is set to 1, as in a. Error bars represent standard error of the mean. C genome browser profile showing mean H3K36me3 (upper panel, n = 4 independent experiments), JASPer (second upper panel, n = 4 independent experiments with 2 different antibodies), JIL-1 (second lower panel, n = 5 independent experiments with 2 different antibodies) and MSL3 (lower panel, n = 3 independent experiments) MNase ChIP-seq normalized coverage along representative 200 kb windows on chromosome 2 R and X in male S2 cells. HAS are marked by red bars above the gene models in gray. D box plot showing mean H3K36me3 (left panel, n = 4), JASPer (second left panel, n = 4) and JIL-1 (second right, n = 5) and MSL3 (right, n = 3) MNase ChIP-seq normalized coverage, as in c, at active (tpm > 1) and inactive (tpm ≤ 1) genes on the autosomes (n = 5785 and n = 8726, respectively) and X chromosome (n = 1214 and n = 1407, respectively) in male S2 cells. Box plot elements are defined as center line marking the median, box limits are the upper and lower quartiles, whiskers extend maximally 1.5-times the interquartile range and outliers are removed.
heterochromatin mark H3K9me2 slightly increased (Fig. 4 and Supplementary Fig. 8a,b). This demonstrates that JASPer per se does not need JIL-1 for H3K36me3 interaction, but its binding is enhanced on the male X chromosome in the JJ-complex. Interestingly, the loss of JASPer after depletion of JIL-1 is stronger closer to the ~300 high affinity sites (HAS) bound by the DCC along the X chromosome (Supplementary Fig. 8c). Concomitantly, the spreading of MSL3 from HAS is slightly diminished and the H4K16ac density slightly drops but independently of the distance to HAS after jil-1 RNAi. These small differences in the dosage compensation hallmark probably cannot explain the loss of JASPer enrichment. It thus appears that
JASPer’s enrichment on the male X chromosome depends directly on JIL-1.

Because the main difference between the X chromosome and autosomes is the presence of the DCC and gene-body H4K16 acetylation, the enrichment of the JJ-complex on the X chromosome may be due to functional interactions of the JJ-complex with the DCC. Direct interaction of JIL-1 with MSL1 and MSL3 subunits of the DCC has been observed in vitro, but so far no clear direct association of the two endogenous complexes has been documented (see also below). We explored the interaction between the two recombinant complexes after expression from baculovirus vectors. Extracts containing JJ-complex (FLAG-JIL-1/untagged JASPer) on the one hand and a complex (FLAG-JIL-1/untagged JASPer) on the other hand were mixed in appropriate stoichiometry and specific antibodies were used for IP (Supplementary Fig. 8d). The MSL1 antibody retrieved not only the associated MSL2 and MSL3, but also some JJ-complex. Conversely, the JIL-1 antibody immunoprecipitated MSL proteins in addition to abundant JJ-complex. This suggests that the two complexes may directly interact with each other. Altogether, the enrichment of the JJ-complex on the male X chromosome may be explained, at least in part, by a JIL-1-dependent interaction between the JJ-complex and the DCC.

The JJ-complex supports expression of male X-linked genes. As we confirmed that the JJ-complex binds to active gene bodies, we wished to explore the functional consequences. To do so, we quantified the transcriptome changes by RNA-seq after RNAi depletion of JASPer or JIL-1 in male S2 and female Kc cells. PCA analysis showed that jasper and jil-1 RNAi affected overall gene expression similarly (Supplementary Fig. 9a). The per-gene analysis showed that upon jasper and jil-1 RNAi in both cell lines the transcription of many genes changed over a wide range of expression levels, with more genes being downregulated (lrd < 0.05) (Fig. 5a). The changes upon jasper and jil-1 RNAi correlate (r = 0.597 in S2 and r = 0.561 in Kc cells), indicating that depletion of the JJ-complex and of JIL-1 alone result in a similar phenotype (Supplementary Fig. 9b). Remarkably, transcription of X chromosomal genes is globally reduced upon depletion of either protein in male S2, but not in female Kc cells (Fig. 5b). We showed earlier that mapping JIL-1-dependent interphase H3S10ph in exponentially growing cells is not possible due to the overwhelming levels of mitotic H3S10ph. Instead, we monitored sequences of 126 D. melanogaster transposable elements (TEs) found that a subset of them showed an enrichment of H3K36me3 and JIL-1 in S2 cells (Fig. 6a). H3K36me3, JASPer, and JIL-1 are strongly enriched at all transposons of the HTT arrays, except for TART-C (Fig. 6b, Supplementary Fig. 11). TART-C is reduced after JIL-1 and JASPer depletion and TART-A and TART-B are additionally downregulated after JIL-1 depletion. However, we do not robustly detect expression of TAHRE. Even though we found many more significantly downregulated TEs in S2 cells, we propose that this is indirect as these TEs lack detectable H3K36me3 enrichment and JJ-complex binding (Fig. 6a, b). However, the TEs of the HTT arrays seem to be mostly active and lack H3K9me2. Upon JIL-1 depletion, we detected an increase in H3K9me2 at the TEs of the HTT arrays, except for TART-C (Fig. 6c, Supplementary Fig. 11). Concomitantly, the enrichment of JASPer is decreased at all transposons of the HTT arrays upon JIL-1 depletion (Supplementary Fig. 12), suggesting that either H3K36me3 is decreased there because of the lower expression and/or JIL-1 contributes to the enrichment of the JJ-complex at telomeres.

Altogether, we propose that TE’s of the HTT arrays acquire H3K36me3 when they are transcribed and recruit the JJ-complex to maintain their active state at least in part by preventing heterochromatization.

The JJ-complex associates with other chromatin complexes. To elucidate the interaction network of the JJ-complex, we immuno-precipitated JASPer with various antibodies under stringent conditions from embryo extracts and identified associated proteins by mass spectrometry. We identified 69 statistical significantly enriched proteins (p-value < 0.05 and fold-change > 4) (Fig. 7a, Supplementary Data 1). The five most enriched GO terms associated to these proteins include ‘chromatin remodeling’, ‘protein acetylation’, ‘chromatin organization’, and
'transcription from RNA Pol II promoters' and its regulation (Fig. 7b). Among the most enriched interacting proteins we found BOD1, Dpy-30L1, Rbbp5, and Set1, subunits of the Set1/COMPASS complex mediating promoter-proximal H3K4 dimethylation and trimethylation [for review47]. Dpy-30L1 and Rbbp5 are common subunits of the different COMPASS complexes, containing one of the three histone methyltransferases Set1, Trx, and Trl in flies. Interestingly, BOD1/CG5514 had not been described in the D. melanogaster Set1/COMPASS complex but is a specific subunit of the Set1B/COMPASS complex in humans48,49. The next most represented interactors were the related PBAP and Brm remodeling complexes with e(y)3, polybromo, Bap170, Bap111, and Snr1 (Fig. 7a and Supplementary Fig. 13). Further subunits of the PBAP/Brm complex and other subunits of remodeling complexes were also enriched, though below statistical significance of this experiment (Fig. 7b). Furthermore, we found the heterochromatin components Su(var)3-7 and Su(var)205 (HP1) significantly enriched (Fig. 7a), which are known to genetically interact with JIL-1,14,15. Several published interactors of JIL-1, like Chromator50 or MSL1 and MSL345 were not detected or not significantly enriched, possibly because of more dynamic association. Among the subunits of the DCC, only MOF was detected together with other subunits of the alternative MOF-containing NSL (non-specific-lethal) complex (Fig. 7a). NDF (nucleosome destabilizing factor) which was found associated with JIL-1 by mass spectrometry after cross-linking51 was also enriched (Fig. 7a). NDF has recently been shown to destabilize nucleosomes in front of the transcribing polymerase, but

![Fig. 5 JIL-1 and JASPer depletion in cells modulates the transcriptional output of genes, especially on the male X chromosome. a MA-plot showing mean log2 fold-change of RNA-seq counts upon jasper RNAi versus control (upper panel, \(n = 4\)) and jil-1 RNAi versus controls (lower panel, \(n = 5\)) against mean RNA-seq counts for robustly detected genes at autosomes (left, chromosomes 2L, 2R, 3L, and 3R, \(n = 6833\)) and X chromosome (right, \(n = 1441\)) in male S2 cells (left site). Statistically significant differentially expressed genes between RNAi and control conditions (fdr < 0.05) are marked in red and the number of significant genes is indicated on the plot. On the right, mean log2 fold-change of RNA-seq counts upon jasper RNAi versus control (upper panel, \(n = 4\)) and jil-1 RNAi versus controls (lower panel, \(n = 4\)) against mean RNA-seq counts for autosomal genes (left, chromosomes 2L, 2R, 3L, and 3R, \(n = 7144\)) and X chromosomal genes (right, \(n = 1509\)) in female Kc cells (left site). b Density plot showing mean log2 fold-change of RNA-seq counts upon jasper RNAi versus controls (\(n = 4\)) and jil-1 RNAi versus controls (\(n = 5\)) at genes in male S2 cells, in left panel, as in a. X chromosomal genes (\(n = 1441\)) are marked with solid line and autosomal genes (chromosomes 2L, 2R, 3L, and 3R, \(n = 6833\)) with dashed line and jasper RNAi additionally in orange. Right panel, mean log2 fold-change of RNA-seq counts upon jasper RNAi and jil-1 RNAi versus controls (\(n = 4\) each) at genes in female Kc cells. X chromosomal genes (\(n = 1509\)) and autosomal genes (chromosomes 2L, 2R, 3L, and 3R, \(n = 7144\)).

We speculated that the JJ-complex and NDF may have redundancy in their functions on transcription. Therefore, we compared the expression changes in male S2 cells after RNAi depletion of either JASPer or NDF alone or in combination. Although the depletion efficiency is only partial for NDF (Supplementary Fig. 13a), the combined depletions showed no increased variance, and the expression changes after JASPer or NDF depletion show only weak correlation (r = 0.39, Supplementary Fig. 13c). Although the JJ-complex and NDF co-localize to active genes marked by H3K36me3, they seem to not have redundant roles in the regulation of steady state mRNA levels.
In summary, we found that the JJ-complex associated with Set1/COMPASS and several nucleosome remodelling complexes. These interactions provide links for understanding the regulation of chromatin structure and function through the JJ-complex.

**Discussion**
We showed that JIL-1 kinase forms as stable complex with a so far uncharacterized protein encoded by CG7946. We named the protein JASPer (JIL-1 Anchoring and Stabilizing Protein). Together the proteins form the JASPer/JIL-1 (JJ)-complex (Fig. 1), which is the major form of JIL-1 kinase in vivo, since JIL-1 is unstable in the absence of JASPer (Fig. 2). The interaction is mediated by a short stretch of conserved residues within JIL-1’s CTD containing a conserved FxGF motif and the LEDGF domain of JASPer. This interaction mode seems to be conserved throughout the animal kingdom, since the human JASPer ortholog PSIP1 (or LEDGF/p75) binds via its LEDGF/IBD (Integrase Binding Domain) domain to various interaction partners, including HIV integrase, MLL1-MENIN complex and IWS1 containing the conserved FxGF motif. These interactions may also trigger deleterious targeting. For example, PSIP1 is hijacked by the HIV integrase to ensure integration of the viral genome in active chromatin, or PSIP1 mis-targets the MLL1 fusion in mixed-lineage leukemia (MLL), inducing malignant transformation. Interestingly, the stability of the interaction with MLL1 is regulated through phosphorylation. We found similar proteins mixed-lineage leukemia (MLL), inducing malignant transformation. We found similar proteins mixing with MLL1 to the JJ-complex through stringent IP-MS conditions. The most prominent interactors, Dpy-30L1, BOD1, Rbbp5, and Set1 are subunits of the Set1/COMPASS complex, which is related to the human MLL complexes. Several subunits of the PBAP/Brm complex, as well as other remodeling complexes are also enriched with the JJ-complex and contribute to the most enriched GO term (Fig. 7).

We suggest that JASPer drives the targeting of JIL-1 to active chromatin through its PWWP domain. The protein binds DNA and RNA, as well as H3K36me3 nucleosomes in vitro. We propose that the recruitment of the JJ-complex to the body of active genes enriched in H3K36me3 (Fig. 3) is the main recruitment mode of JIL-1 kinase to chromatin, but we do not exclude that additional binding modes are relevant at promoters and enhancers as described earlier. Those binding modes could implicate interactions with other chromatin complexes, RNA or DNA. Recently, the protein PWWP2A protein was described to bind...
H2A.Z-containing nucleosomes at the 5’ end of transcribed genes, as well as active gene bodies decorated with H3K36me3 using two different binding modules.

The same targeting principle by JASPer binding via its PWPD domain to H3K36me3 may be used to recruit JIL-1 to telomeric HTT transposons (Fig. 6). However, it is not clear if those transposons acquire H3K36me3 through the Set2-dependent methylation associated with elongating RNA Pol II, as coding genes do or by another mechanism.

The recombinant JJ-complex has a strong kinase activity towards S10 on isolated H3 in vitro but the efficiency of phosphorylating H3S10 in nucleosomes is very low (Supplementary Fig. 4). H3K36me3 is essential to bring JIL-1 to active chromatin, but is not sufficient to unleash its kinase activity upon nucleosomes in vitro. We speculate that JIL-1 may need to be activated by specific signals generated within chromatin or downstream of a signaling pathway, similarly to its orthologous kinases MSK1/2 (for review see ref. 50). However, the nucleosome may not be the physiological substrate for JIL-1. During the course of transcription, nucleosomes are disassembled and histones associate with various chromatin marks and HP1 spread from the chromocenter especially to the X chromosome in male cells in a JIL-1- and dosage dependent manner (Fig. 4).

Fig. 4). H3K36me3 is essential to bring JIL-1 to active chromatin, future goals will be to unravel the signaling events that lead to activation of the JJ-complex, its non-histone substrates and role in modulating histone structure and function.

**Methods**

**Cell culture and RNAi** S2-DSRC (DGRC stock # 181), Kc167 (DGRC stock # 1) cells were cultured in Schneider’s Drosophila Medium (Thermo Fisher), supplemented with 10% heat-inactivated Fetal Bovine Serum (Sigma-Aldrich), 100 units/ml penicillin and 0.1 mg/mL streptomycin (Sigma-Aldrich) at 26°C. RNAi against target genes in S2 and Kc cells for ChIP-seq was performed for 7 days in 1 or 2 flasks (75 cm²) seeded with 12 million cells and treated with 50 µg dsRNA/flask after a wash in serum free medium. Fresh medium was added at day 5 to sustain growth. For RNAi against target genes in S2 and Kc cells for RNA-seq, cells were washed with serum-free medium and 10 µg dsRNA per 10⁶ cells at a concentration of 10 µg/mL in serum-free medium (10⁶ cells in 6-well plate) was added, incubated for 10 min at room temperature (RT) with slight agitation and further 50 min at 26°C. Two volumes of complete growth medium were added and cells were incubated for 3 days at 26°C. At day 3, cells were split, reseeded and retreated as at day 1. Cells were incubated for further 4 days at 26°C. dsRNA was generated from PCR products obtained using the following forward and reverse primers (separated by comma):

**jasper RNAi #1:** TTAATACGACTCACTATAGGGAGAATGTCCCCTATACTAGGTTA,
TTAATAGCCTACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATAGCCTACTATAGGGAGAAGGAGGAGGTGTTAGT;

**jasper RNAi #2:** TTAATACGACTCACTATAGGGAGACAGCAGCGTCGg,
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**jil-1 RNAi #1:** TTAATAGCCTACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**jil-1 RNAi #2:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**gfp RNAi:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**gtt RNAi:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**pca RNAi:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**cag RNAi:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**ttt RNAi:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**tag RNAi:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**ndf RNAi #1:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**ndf RNAi #2:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**dcd RNAi:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**dcr RNAi:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**sca RNAi:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**cag RNAi:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**cag RNAi:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**cag RNAi:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;

**cag RNAi:** TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
TTAATACGACTCACTATAGGGAGAAGGAGGAGGTGTTAGT;
inactivated Fetal Bovine Serum (Sigma-Aldrich), 100 units/mL penicillin, and 0.1 mg/mL streptomycin (Sigma-Aldrich) at 26 °C. S2E cells (Thermo Fischer) were cultured in S900 II SFM (Thermo Fischer), supplemented with 10% heat-inactivated Fetal Bovine Serum (Sigma-Aldrich), 0.1 mg/mL gentamicin (Sigma-Aldrich) at 26 °C.

Recombinant protein expression and purification. For purification of GST-JASPer fusion protein, the coding sequence of JASPer (CG7946-RA) from EST clone LDL23804 was cloned into pGEX-4T2. GST-JASPer was expressed in E. coli Rosetta 2 (DE3) (Merck) and purified using Glutathione Sepharose High Performance beads (GE Healthcare) for antibody generation. For all biochemical assays, we used the baculovirus expression system in Sf21 cells. For purification of recombinant JASPer and aromatic cage mutant (Y23A and W26A) by FLAG-tag affinity chromatography, the coding sequence of JASPer was directly fused to a C-terminal coding sequence of FLAG affinity tag and cloned into pBDM under control of the polychadin promoter22. For dual expression of the JJ-complex, we amplified twice with 20 bed volumes each of Lysis Buffer, Wash Buffer (Lysis Buffer with 1 M NaCl, 1 mM MgCl2, 10% (v/v) glycerol, 0.05% (v/v) Triton-X-100, 1 mM DTT, 0.5 mM EDTA, 4 °C and subsequently 0.6 bed volumes of Elution Buffer with PI were added and end-over-end rotation for 1 h at 4 °C and subsequently 0.6 bed volumes of Elution Buffer with PI were added and end-over-end rotation for 1 h at 4 °C. Beads were pelleted (4 °C, 5 min, 500 g) and supernatant was removed. Beads were washed twice with 20 bed volumes each of Lysis Buffer, Wash Buffer (Lysis Buffer with 1 M NaCl) and finally twice with 20 bed volumes Elution Buffer (Lysis Buffer with 200 mM NaCl). For protein elution, beads were incubated with 0.2 bed volumes of Elution Buffer containing 5 mg/mL FLAG peptide (Sigma-Aldrich) for 10 min at 4 °C and subsequently 0.6 bed volumes of Elution Buffer with PI were added and incubated with end-over-end rotation for 2 h at 4 °C. The elution step was repeated and elution fractions were combined and concentrated if needed. Protein concentrated proteins were used using BSA stand-alone SADS-PAGE with Coomassie brilliant blue G250 staining. Protein samples were flash-frozen in aliquots in liquid nitrogen and stored at −80 °C. For nuclease IP, buffer was exchanged by adding 9 volumes of Exchange Buffer 1 (30 mM HEPES pH 7.6, 500 mM NaCl, 1 mM MgCl2, 10% (v/v) glycerol, 0.05% (v/v) Triton-X-100, 1 mM DTT, 0.5 mM EDTA, 2.5 mM L-Aspartate), and concentrating and resuspending in 30 WMCO Amicon Ultra-15 (Merck) to the starting volume. The proteins were then digested in 9 volumes of Exchange Buffer 2 (Exchange Buffer 1 with 200 mM NaCl) and concentrated with 30 WMCO Amicon Ultra-15 (Merck).

Electro mobility shift assay. EMSA with dsDNA was performed as described in ref. 1, with slight modifications. In brief, binding reactions containing 70 mM 40 bp C5-slabelled dDNA (CCTGGA GAATCCCGGCTGGCGCCGCCTCAATTGGTCTGTA) in Binding buffer (50 mM HEPES pH 7.6, 50 mM NaCl, 10% (v/v) glycerol, 2 mM MgCl2, 10% (v/v) BSA) were incubated for 10 min at RT. EMSA with RNA was performed as described in ref. 1, with 2.5 mM 123 nt 3′-labeled RNA2-123 RNA in EMSA buffer (25 mM HEPES pH 7.6, 100 mM KCl, 3 mM MgCl2, 1 mM DTT, 5% (v/v) glycerol, 100 µg/mL yeast RNA (Sigma) for 15 min at 20 °C. The protein/DNA/RNA complexes were resolved by native PAGE (4% gel in 0.5x TBE running buffer).

Generation of JASPer null mutant fly line. The JASPer null allele cw2 was isolated in a screen for imprecise excisions from the EP-element line GS2368 from the Kyoto Stock Center using standard techniques14 and as previously described15. The approximate breakpoint locations determined by PCR-analysis are shown in Fig. 2c.

Antibodies. Polyclonal antibodies against JIL-1, a-JIL-1 R69, and R70 were described in ref. 14 and Hope in ref. 20. GST-JASPer (1-475) was used to generate polyclonal antibodies (α-JASPer GP13 and GP14) in guinea pigs (Eurogentech), as well as the monoclonal (E. Kremmer) antibodies α-JASPer 6F7 and 4D6. α-NDF was a kind gift from J. Kadonaga2 and GST-MSL3 was used to generate polyclonal chitin-binding domain (CBM)-MSL3 in guinea pig (Pineda Antikörper-Service).α The following commercially available antibodies were used: α-H3K36me3 (Abcam, ab9050), α-FLAG (Sigma, F3161), α-H3K9me2 (Abcam, ab1220), α-H3 (Cell Signaling, 9715), α-H3S10ph (Cell Signaling, 9701), α-H4 (Abcam, ab10158), α-H4K16ac (Millipore, 07-329), and α-Tubulin (Sigma-Aldrich; T9026), and α-LacI (Millipore, 05-503). For western blotting, antibodies against LacI were either determined experimentally (polyosomal sera: 1/500-1/5000; monoclonal culture supernatants: 1/5-1/10). All antibody dilutions in PBS 3% BSA were reused several times. For detection either the infra-red based Odyssey system (Li-Cor) or the ECL based chemiluminescence system with Chemidoc Touch (Bio-rad) were used.

Immunofluorescence microscopy of polytene chromosomes. Immunofluorescence microscopy analysis of polytene chromosome squash preparations was performed as described in ref. 74. LacI-tagged JIL-1 constructs and the Lac operon insertion line P113 was described in refs. 31,65. These lines include: LacI-JIL-1-FL, LacI-JIL-1-CTD, and LacI-JIL-1-ACD. GALA-expression was driven by recombinant line with Sgs3-GAL4 and da-GAL4 drivers obtained from the Bloomington Stock Center. Antibody labeling protocols were as in ref. 77. DNA was visualized by staining with Hoechst 33258 (Molecular Probes) in PBS. The appropriate species-specific and isotype-specific Texas Red-conjugated TRITC-conjugated, and FITC-conjugated secondary antibodies (Cappel/ICN, Southern Biotech) were used (1:200 dilution) to visualize primary antibody labeling. Monoclonal 4% w/v polyacrylamide gels and purified albumin gel. Epifluorescence optics were used to examine the preparations on a Zeiss Axioskop microscope. Images were obtained and digitized using a Spot CCD camera. Photoshop (Adobe) was used to pseudocolor, image process, and merge images. Non-linear adjustments were performed for some of the Hoechst labeling for the best chromosomal visualization.

JASPer identification. Nuclear extract from fly embryos were prepared from 12 h embryo collections as described in ref. 75. For preparative immunoprecipitation (IP), 300 µg nuclear embryo extract 0–12 h at a concentration of 3 mg/mL in HEMG100 buffer (25 mM HEPES pH 7.6, 100 mM KCl, 10% (v/v) glycerol, 0.1 mM EDTA, 12.5 mM MgCl2) were used per IP. Protein A and Protein G beads (5 µL) (GE Healthcare) were washed with HEMG100. The diluted extract was pre-cleared with 15 µL (30 µL 50% slurry) Protein A/Protein G beads mix by incubating with end-over-end rotation for 1 h at 4 °C. Beads were pelleted and supernatant was directly used for IP. For IP, the reaction was added to 15 µL (30 µL 50% slurry) Protein A/Protein G beads mix by incubating with end-over-end rotation for 1 h at 4 °C. Beads were washed and supernatant was directly used for IP. For IP, the reaction was added to 15 µL (30 µL 50% slurry) Protein A/Protein G beads mix by incubating with end-over-end rotation for 1 h at 4 °C. Proteins were eluted with 150 µL HEMG100 supplemented with 0.5% (m/v) α-N-lauroylsarcosine with end-over-end rotation for 1 h at 4 °C. Proteins were separated by 4–20% gradient SDS-PAGE, stained with Coomassie brilliant blue G250 staining and the most prominent band was cut out for mass spectrometry analysis.

Immunoprecipitation from embryo extracts. Nuclear extract from fly embryos was prepared as described in ref. 76. For each IP, 400 µg of extract was diluted to 1 mg/mL in BBN buffer (10 mM Tris/Cl pH 8.0, 140 mM NaCl, 1 mM EDTA, 1% (v/v) Triton X-100, 0.1% (v/v) Na deoxycholate, 0.1% (v/v) IGEPAL-CA-360, 0.5 mM DTT) supplemented with Complete EDTA-free Protease Inhibitor Cocktail (Sigma-Aldrich). Protein G beads (GE Healthcare) were washed thrice with 10 bed volumes BBN buffer. The diluted extract was pre-cleared with 10 µL (20 µL 50% slurry) Protein G beads by incubating with end-over-end rotation for 1 h at 4 °C. Beads were pelleted and supernatant was directly used for IP. For IP, the reaction was added to 15 µL (30 µL 50% slurry) Protein A/Protein G beads mix by incubating with end-over-end rotation for 1 h at 4 °C. Beads were washed and supernatant was directly used for IP. For IP, the reaction was added to 15 µL (30 µL 50% slurry) Protein A/Protein G beads mix by incubating with end-over-end rotation for 1 h at 4 °C. Proteins were eluted with 150 µL HEMG100 supplemented with 0.5% (m/v) α-N-lauroylsarcosine with end-over-end rotation for 1 h at 4 °C. Proteins were separated by 4–20% gradient SDS-PAGE, stained with Coomassie brilliant blue G250 staining and the most prominent band was cut out for mass spectrometry analysis.

Mass spectrometry and data analysis. Whole IPs were used for trypsin digestion and mass spectrometry (IP/MS) identification of binding partners. For LC/MS/MS purposes, desalted peptides were injected in an Ultimate 3000 RSLCnano system
Nucleosome pull-down. Nucleosome library preparation, pull-down experiments and data analysis were performed as described in ref. 35. For pull-down reaction, 1.5 pmol of JASPer was used for JASPer-FLAG wt and aromatic cage mutant and for wt and aromatic cage mutant of JF-complex and pre-coupled to 5 μL FLAG-M2 beads (Sigma-Aldrich) (10 μL 50% slurry) in Binding buffer (20 mM Tris/Cl pH 7.5, 50 mM NaCl, 5 mM EDTA, 0.1% (v/v) TWEEN 20). The protein pre-coupled to beads was incubated with 1.38 pmol nucleosome library containing 115 nucleosome types (12 fmol per nucleosome type) in a total of 200 μL Binding buffer for 4 h at 4 °C with end-over-end rotation. Beads were washed four times with 20 bed volumes (200 μL) Binding buffer and DNA eluted by Proteinase K digestion and purified using a QIAGEN PCR purification kit for further library preparation and sequencing.

ChIP-seq. ChIP-seq on MNase-digested chromatin and sonicated material was performed as previously described66,67. For spike-in ChIP-seq on MNase-digested chromatin in combination with mild sonication, S2 cells (∼3 × 106 cells) after RNAi were harvested and cross-linked with 1% formaldehyde for 8 min by adding 1 ml 10x fixing solution (50 mM HEPES pH 8.0, 100 mM NaCl, 1 mM EDTA, 0.5 mM EGTA) with 10% formaldehyde [16% formaldehyde solution (w/v) methanol-free (Thermo Fischer)] per 10 mL culture at RT. The reaction was stopped by adding 125 mM glycine and incubating for 10 min on ice. Cells were washed twice in PBS and snap-frozen in liquid N2. For nuclei isolation, cells were rapidly thawed and resuspended in PBS supplemented with 0.5% (v/v) Triton-X 100 and Complete EDTA-free Inhibitor Cocktail (Covaris, Woburn, MA) (10−5 cells/mL). Nuclei were processed as described for S2 cells without RNAI treatment, relative to S2 cells were added, volume was adjusted to 7 × 105 cells/mL and cells incubated for 15 min at 4 °C with end-over-end rotation. Nuclei were collected by centrifuging at 4 °C for 10 min at 2000g and washed once in PBS. For chromatin fragmentation, nuclei were resuspended at 4 °C for 10 min at 2000g, washed and resuspended in RIPA (10 mM Tris/Cl pH 8.0, 140 mM NaCl, 1 mM EDTA, 1% (v/v) Triton-X 100, 0.1% (v/v) SDS, 0.1% (v/v) DOC) supplemented with PI and 2 mM CaCl2 at 7 × 105 cells/mL and digested in 1 mL aliquots by adding 0.6 U MNNase (Sigma Aldrich), resuspended in EX50 at 0.6 U/mL65, and incubated at 37 °C for 35 min with slight agitation. The reaction was stopped by adding 0.5 mM EGTA and resuspending. Digested chromatin was sheared with Covaris AFA S220 using 12 × 12 tubes at 50 W peak incident power, 20% duty factor and 200 cycles per burst for 8 min at 5 °C. Subsequent steps were performed as described in ref. 66. Libraries were prepared with NEBNext Ultra II DNA Library Prep Kit for Illumina (NEB, E7845) and analyzed with the 2100 Bioanalyzer with DNA 1000 kit (Agilent). Libraries were sequenced on HiSeq 1500 (Illumina) instrument yielding typically 20–25 million 50 bp single-ended reads per sample at the genomics facility.

RNA-seq. For RNA-seq, 2 million S2 cells or Kc cells after RNAi treatment were resuspended in Trizol and RNA was purified using the RNeasy Mini Kit (QIA-GEN). Afterwards, 1 μg purified total RNA’s was used for RNA depletion using Ribo-Zero Gold rRNA Removal Kit (Illumina, MRZG 12324) or NEBNext rRNA Depletion Kit (NEB, E6310). Library preparation was done according to the manufacturer’s instructions with NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB, E7760) and analyzed with the 2100 Bioanalyzer with DNA 1000 kit (Agilent). Libraries were sequenced on HiSeq 1500 (Illumina) instrument yield typically 15–20 million 50 bp paired-ended reads per sample at the genomics facility.

NGS data analysis. Sequencing data were processed using SAMTools version 1.3.1β8, BEDTools version 2.2.09β, R version 3.5.1 (http://www.r-project.org) and Bioconductor version 3.8 (http://www.bioconductor.org) using default parameters for function calls, unless stated otherwise.

Reading processing. Sequence reads were aligned to the D. melanogaster release 6 reference genome (BDGP6). D. virilis flyBase release r1.07_FB2018.05 reference genome or to D. melanogaster transposon sequence set version 9.4.1 (BDGP), including only D. melanogaster transposons (n = 126), using Bowtie version 1.1.2β9 (parameter –m1 for D. melanogaster genome and transposon) for ChIP-seq and STAR version 2.6β0 (parameters –quantMode TranscriptomeSAM GeneCounts, –outSAMstrandField none, –outFilterMultimatchNmax 1) and CountTransposable element quantification of RNA-seq data was performed using RSEM version 1.3.0β (parameters –bam, –paired-end, –forward-prob 0).
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Author contributions
C.R. conceived this study and performed experiments. C.A. performed MNase and sonication ChIP-seq experiments and all bioinformatics analysis also with support from T.S. C.W. generated and characterized the cw2 mutant line with help from J.G., W.C. did the LacO-LacI targeting experiments with support from Y.L., and J.J. and K.M.J. supervised the work and secured funding. G.P.D. performed mononucleosome library experiments and F.W. generated the mononucleosomes and arrays for the kinase assays in T.W.M.’s lab. S.K. prepared recombinant proteins for all in vitro assays and RNA-seq libraries under the supervision of C.R. and spike-in ChIP-seq experiments under supervision of C.A. S.M. studied RNA binding by JASPer. P.B.B. secured funding and established collaborations. All authors analyzed data. C.R. and C.A. wrote the paper with contributions from all authors.

Competing interests
The authors declare no competing interests.

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