Genetics and Genomics

PARP-1 cooperates with histone methyltransferase to regulate transcription

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Abstract

PARP-1 is central to transcriptional regulation under both normal and heat stress conditions, with the governing mechanisms yet to be fully understood. Biochemical and ChIP-seq-based analyses showed that PARP-1 binds specifically to active histone marks, particularly H4K20me1. PR-Set7, the sole H4K20 mono-methylase, and PARP-1 mutants undergo developmental arrest during larval-pupal shift in *Drosophila*. RNA-seq analysis showed a positive correlation between PR-Set7-and PARP-1-dependent gene expression in third-instar larvae, suggesting co-regulation during developmental phases. During heat stress, PARP-1 repositions from the *Hsp70* promoter to its gene body, facilitating gene activation. PARP-1 and PR-Set7 are essential for activating *Hsp70* and a subset of heat shock genes during heat stress, with a notable increase of H4K20me1 at their gene body. We propose that H4K20me1 plays a critical role in facilitating PARP-1 binding and the regulation of specific genes during both development and heat stress.

eLife assessment

This **valuable** study presents **convincing** evidence for an association between PARP-1 and H4K20me1 in transcriptional regulation, supported by biochemical and ChIP-seq analyses, but further validation and attempts to obtain mechanistic insights are warranted along with discussion of recent findings by others in this area.

Introduction

The chromosomes of eukaryotes are made up of chromatin, which is a complex of DNA tightly wound around nucleosomes (*1*). Nucleosomes are the basic unit of chromatin, and they are made up of about 147bp of DNA wrapped around an octamer of four core histones (H2A, H2B, H3 and H4) (*1*). Eukaryotic DNA is packed into nucleosomes to maintain higher order chromatin structure. As a consequence, DNA sequences are inaccessible to a plethora of protein effectors for biological processes such as DNA damage repair, replication and transcription (*2*). To overcome the nucleosome barrier, eukaryotes have developed several mechanisms that ensure the successful removal of nucleosomes. One of the key effectors involved in chromatin remodeling is

Poly(ADP-ribose) Polymerase 1 (PARP-1), a chromatin-associated nuclear enzyme implicated in a host of biological processes including, transcriptional regulation and DNA repair (*3*–*5*). PARP-1 catalyzes the addition of poly (ADP-ribose) moieties onto acceptor proteins by utilizing donor NAD^+ as substrate. Upon PARP-1 activation due to developmental and environmental cues or stress, PARP-1 is automodified, and it attaches negatively charged poly(ADP-ribose) polymers onto histones and other chromatin-associated proteins, thereby decondensing DNA via electro-repulsion and facilitating PARP-1 mediated biological processes (*6*, *7*). Besides PARP-1, histone modifications are essential for remodeling chromatin structure. Histones are subject to post-translational modifications, including phosphorylation, acetylation, sumoylation, ubiquitylation and ADP-ribosylation on their amino-terminal tails protruding from the nucleosome core (*2*, *8*, *9*). Modifications of these histone tails can alter chromatin structure by modifying inter-nucleosome interactions or by serving as docking sites for effectors to facilitate diverse biological outcomes depending on the histone(s) so modified (*2*, *10*).

The binding of PARP-1 to post-translationally modified histones may be necessary for PARP-1-mediated biological processes and PARP-1 targeting to chromatin. In our previous study, we showed that the phosphorylation of the histone variant H2Av directly impacts the localization, activation, and enzymatic activity of PARP-1 (*11*). Also, we previously showed that the N-terminal tails of histones H3 and H4 are needed for the enzymatic activation of PARP-1 (*12*). Taken together, there is strong evidence that histone modifications can regulate PARP-1 activity *in vivo*.

In this study, we demonstrate that PARP-1's transcriptional regulatory role under regular and heat stress conditions may be intricately tied to its interaction with specific active histone marks, notably H4K20me1, H3K4me1, H3K36me1, and H3K9me1, and that it is inhibited by repressive H3K9me2/3 marks. Through transcriptomic analysis of PR-Set7 and PARP-1 mutant *Drosophila* third-instar larvae, we uncover a high correlation in differentially expressed genes and co-enrichment of PARP-1 and H4K20me1 at a subset of these genes, underscoring the role of PR-Set7/H4K20me1 in facilitating PARP-1-mediated transcriptional regulation. Importantly, we show that PARP-1 and PR-Set7 are essential for the activation of specific heat shock genes including *Hsp70*, and the dynamic regulation of H4K20me1 during this process, further validates our hypothesis of H4K20me1 being crucial for PARP-1 binding and gene regulation under developmental and heat stress conditions.

Results

PARP-1 binds to H4K20me1, H3K4me1, H3K36me1 and H3K9me1 *in vitro* and *in vivo*

In this study, we investigated the interaction between full-length recombinant PARP-1 and various histone modifications using a histone peptide array containing modified histone peptides individually and in combination with other peptides (**Fig. 1A**). Our previous work established that PARP-1 binds to core histones (*12*). Here, we aimed to determine the specific histone marks that modulate PARP-1's affinity for chromatin. We found that PARP-1 binds specifically to spots containing H4K20me1, H3K4me1, H3K36me1, or H3K9me1 peptides (**Fig.1B**, C, D and data file S1). Specificity analysis showed that PARP-1 binding was highly specific for H3K4me1, H3K36me1 and H3K9me1 containing peptides with the highest specificity observed for H4K20me1 (**Fig.1E** and data file S1). Additionally, we explored the inhibitory effects of different histone modifications on PARP-1 binding. Our findings showed that H3K9me2/3 hindered PARP-1 binding to spots containing H3K4me1 (**Fig.1F** and Fig S1). Conversely, H3K4me1 enhanced PARP-1 binding to spots with H3K9me2/3 (**Fig.1F** and Fig S1).

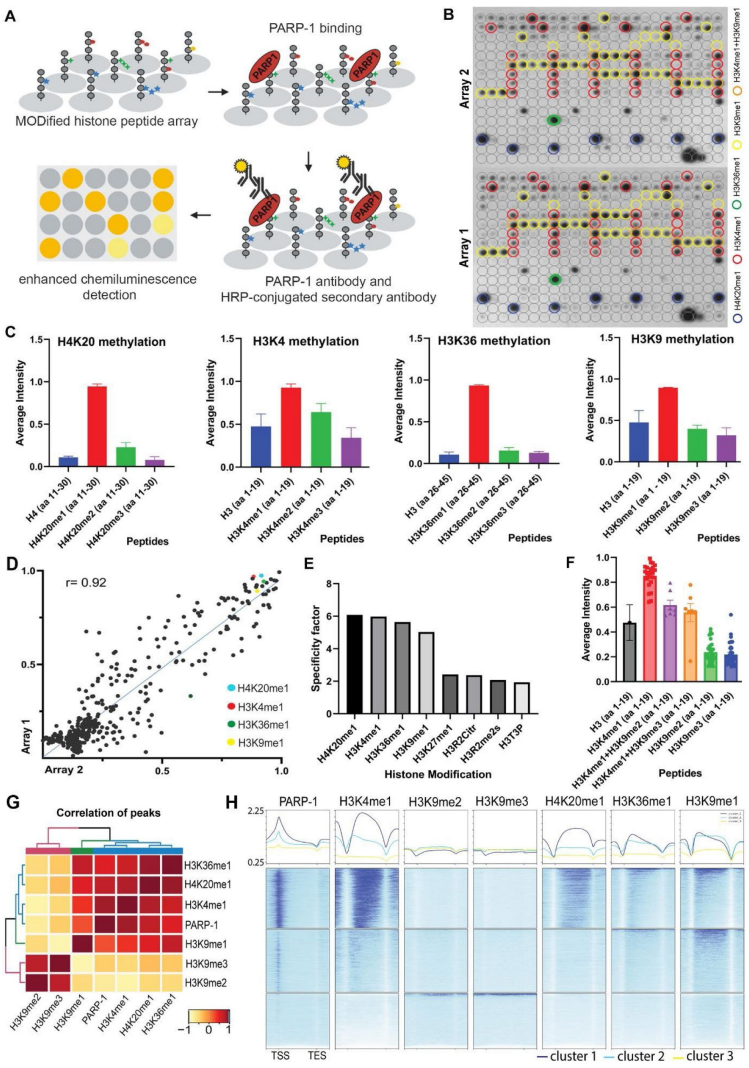

Figure 1.

PARP-1 binds H4K20me1, H3K4me1, H3K36me1 and H3K9me1 in vitro and in vivo.

(**A**) Illustration of the MODified histone peptide assay (Active Motif) used to determine PARP-1 binding to histone modifications. The histone peptide array (Top, left), comprising 19mer peptides with single or up to four concurrent histone modifications, was employed to investigate PARP-1's binding affinity for histone modifications and to assess the impact of adjacent modified peptides on PARP-1 binding. This array was first blocked, then incubated with PARP-1 protein (Top, right). Subsequently, it was stained with a PARP-1 antibody and a horseradish peroxidase (HRP)-conjugated secondary antibody (Bottom, right). Visualization of PARP-1 binding was done through enhanced chemiluminescence detection and captured on X-ray film (Bottom, left). See Methods for a full description. (**B**) Signal intensity on modified histone peptide array based on incubation with PARP-1 protein. (**C**) Average intensities of PARP-1 binding to single histone peptides. (**D-E**) Reproducibility and specificity of spot intensities from modified histone peptide array duplicates. (**D**) Scatter plot showing the correlation of the average intensities of duplicate arrays. Intensities of PARP-1 binding to all peptides and spots containing single H4K20me1, H3K4me1, H3K36me1 and H3K9me1 (key) are shown. Pearson's correlation coefficient (r) is 0.92. (**E**) Bar chart showing top 8 histone modifications with the highest specificity for PARP-1 binding. The specificity factor was calculated by dividing the average intensity of spots that contain the modified histone peptide by the average intensity of spots that do not contain the peptide. (**F**) Average intensities from histone peptide array showing the inhibition of PARP-1 binding by H3K9me2/3 peptide to spots containing H3K4me1 peptides. (**G**) Spearman correlation of PARP-1, H4K20me1, H3K4me1, H3K36me1, H3K9me1 and H3K9me2/3 peaks in *Drosophila* third-instar larvae based on fraction of overlap. (**H**) Heatmaps showing k-means clustering-generated occupancy of PARP-1, H4K20me1, H3K4me1, H3K36me1, H3K9me1 and H3K9me2/3 normalized ChIP-seq signals in third-instar larvae at *Drosophila* genes. ChIP-seq signals are sorted in descending order. Upper plots show the summary of the signals

Next, we examined the association of PARP-1 with H4K20me1, H3K4me1, H3K36me1, H3K9me1 and H3K9me2/3 in *Drosophila* third-instar larvae using ChIP-seq data. PARP-1 peaks were highly correlated with H4K20me1, H3K4me1, H3K36me1, and H3K9me1 but significantly less correlated with H3K9me2/3 peaks (**Fig. 1G**). Consistently, at gene clusters, H4K20me1 and H3K4me1 were highly enriched at gene bodies in cluster I which also had the highest PARP-1 enrichment at promoters. H3K36me1 and H3K9me1 were also enriched in cluster I albeit to a lesser degree than H4K20me1 and H3K4me1 (**Fig. 1H**). In contrast, H3K9me2/3 were more enriched at clusters II and III but depleted in cluster I (**Fig. 1H**). Additionally, PARP-1 associates with H4K20me1 and H3K4me1 on *Drosophila* polytene chromosomes (**Fig.2**). Notably, H4K20me1, H3K4me1, H3K36me1 and H3K9me1 are associated with active genes while H3K9me2/3 is associated with low-expression or silent genes (*13*). Thus, our data shows that PARP-1 predominantly binds active histone marks, specifically H4K20me1, H3K4me1, and H3K36me1, while also suggesting that the binding of PARP-1 may be hindered by repressive histone marks such as H3K9me2/3.

PARP-1 and PR-Set7 co-regulate developmental gene expression programs

Next, we examined the relationship between H4K20me1 and PARP-1 during transcriptional regulation. Given that flies that lack PR-Set7, the sole methylase of H4K20 (Supplemental Fig.S2), and PARP-1 undergo developmental arrest during the larval-to-pupal transition (*14*, *15*), we hypothesized that PR-Set7/H4K20me1 and PARP-1 may regulate similar gene expression programs during development. We integrated PARP-1 and H4K20me1 ChIP-seq data in third-instar larvae and RNA-seq of *PR-Set7*^{20} and *Parp*^{C03256}mutant third-instar larvae. Our data showed that the differentially expressed genes (DEGs) in *PR-Set7*^{20} and *Parp1*^{C03256} transcriptome were highly correlated (Pearson, r = 0.79) (**Fig. 3A and B**). However, despite the global reduction of H4K20me1, and PARP-1 RNA in *PR-Set7*^{20} and *Parp1*^{C03256} mutants, respectively (*14*, *15*), there was a limited alteration in gene expression at genes where PARP-1 was co-localized with H4K20me1 (**Fig. 3C and D**). Differentially expressed genes in both *PR-Set7*^{20}and *Parp1*^{C03256} mutants and co-enriched with PARP-1 and H4K20me1 were mainly upregulated (n = 101, 72%) (**Fig. 3E**). Interestingly, among the genes co-enriched with PARP-1 and H4K20me1, those only differentially expressed in *Parp1*^{C03256} mutants were mostly upregulated (n = 94, 85%), while those only differentially expressed in *PR-Set7*^{20} mutants were mainly downregulated (n = 191, 67%), indicating that PARP-1 and H4K20me1 also have distinct functions in gene regulation at co-enriched genes (**Fig. 3E**). Gene ontology analysis of differentially expressed genes bound by PARP-1 and H4K20me1 showed that metabolic genes were upregulated while genes involved in neuron development and morphogenesis were downregulated in both *PR-Set7*^{20} and *Parp1*^{C03256} mutants (**Fig. 3F**). Overall, our results indicate H4K20me1 may be required for PARP-1 binding to preferentially repress metabolic genes and activate genes involved in neuron development at co-enriched genes.

PARP-1 and PR-Set7/H4K20me1 are required for the optimal expression of heat shock genes during heat stress

Next, we examined PARP-1 and H4K20me1 controlled gene expression programs during dynamic transcriptional changes. During heat shock, PARP-1 spreading from the promoter to the gene bodies of *Hsp70* facilitates nucleosomal loss which leads to transcriptional activation (*16*, *17*). Since H4K20me1 is mostly enriched in gene bodies (**Fig. 1H**), we hypothesized that H4K20me1 may be required for PARP-1 spread at *Hsp70* during heat shock. First, we examined the expression of heat shock genes before and after 30 minutes heat shock in WT, *Parp*^{C03256} *and PR-Set7*^{20}third-instar larvae. The expression of heat shock genes (*Hsp22*, *Hsp23*, *Hsp68*, *Hsp83*) including *Hsp70* were significantly reduced in *Parp1*^{C03256} *and PR-Set7*^{20} animals after heat shock (**Fig. 4A**). ChIP-seq results revealed that PARP-1 highly occupied the promoters of *Hsp23*, *Hsp68*, *Hsp70*, and *Hsp83*, as well as in the gene body of *Hsp22*, prior to heat shock (**Fig. 4B and C**). Analysis of

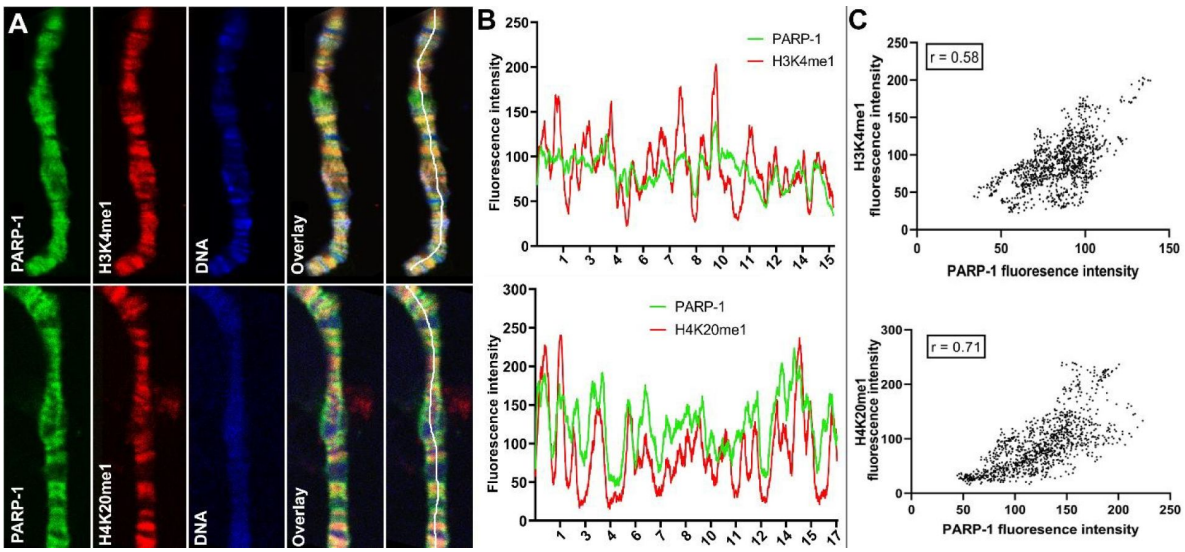

Figure 2.

PARP-1 colocalizes with H3K4me1 and H4K20me1 histone marks in polytene chromosomes.

(**A**) Immunofluorescent staining of *Drosophila* salivary gland chromosomes showing colocalization of PARP-1 with H3K4me1 and H4K20me1. White lines indicate areas of the colocalization-quantification shown in (**B**). (**B-C**) Quantification of fluorescence intensity of PARP-1, H3K4me1 and H4K20me1 at Drosophila polytene chromosomes in panel A. (**B**) Images show distribution of PARP-1 fluorescence intensity with H3K4me1 (top) and H4K20me1 (bottom) fluorescence intensity. (**C**) Images represent a scatterplot showing PARP-1 fluorescence intensity with H3K4me1 (top) and H4K20me1 (bottom) fluorescence intensity. Pearson correlation coefficients (r) are respectively 0.58 (top) and 0.71 (bottom).

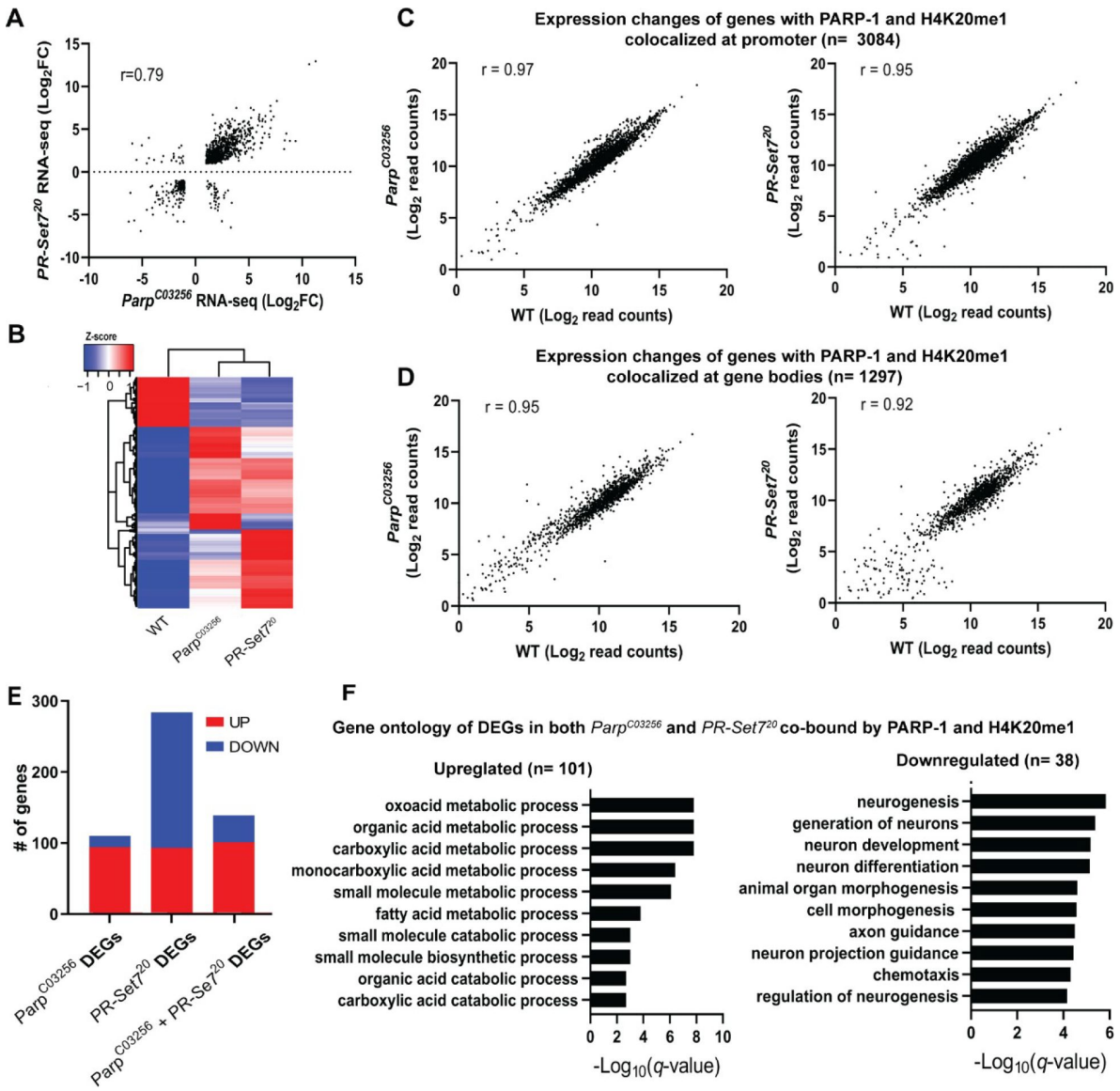

Figure 3.

PARP-1 and H4K20me1 are required for the repression of metabolic genes and activation of developmental genes at co-enriched genes.

(**A**) Scatterplot plot showing correlation of differentially expressed genes (DEGs) in *Parp*^{C03256}and *PR-Set7*^{20} (Pearson's r = 0.79). (**B**) Heatmap showing the normalized read counts of DEGs in both *Parp*^{C03256} and *PR-Set7*^{20}. Normalized read counts are shown as row z-scores. (**C-D**) Dot plots showing transcriptional changes of genes co-enriched with PARP-1 and H4K20me1 in *Parp*^{C03256} and *PR-Set7*^{20} compared to WT at promoters (**C**) and gene bodies (**D**). (**E**) Summary of DEGs in *Parp*^{C03256} and *PR-Set7*^{20} and both mutants that were co-enriched with PARP-1 and H4K20me1. (**F**) Gene ontology of upregulated (left) and downregulated (right) DEGs in both *Parp*^{C03256} and *PR-Set7*^{20} mutants that were co-enriched with PARP-1 and H4K20me1.

H4K20me1 enrichment showed low levels across the gene bodies of *Hsp22*, *Hsp68*, and *Hsp70* (group A) but high levels at gene bodies of *Hsp23* and *Hsp83* (group B) before heat shock (**Fig.4B** and C). Based on these findings, we hypothesized that an increase in H4K20me1 enrichment in the gene bodies of group A genes would facilitate PARP-1 binding after heat shock, while H4K20me1 enrichment remains unchanged in group B genes thereby facilitating PARP-1 binding and spreading. To our surprise, H4K20me1 levels increased at the gene bodies of group A genes (low H4K20me1 before heat shock) (**Fig. 4D**) but decreased significantly at group B genes (high H4K20me1 before heat shock) after heat shock (**Fig. 4E**). These findings suggest that dynamic changes in H4K20me1 enrichment may regulate the expression of heat shock genes during heat shock and indicate that H4K20me1 may not be necessary for PARP-1 binding and PARP-1-mediated activation of group B genes during heat shock.

Mutating PR-Set7 methyl transferase disrupts PARP-1 binding to chromatin and diminishes poly(ADP-ribosyl)ation levels during heat shock stress

Our results showed that prior to heat shock, there was no effect on PARP-1 binding in both *WT* and *PR-Set7*^{20} background animals to chromatin (**Fig. 5A**). However, after heat shock, we observed that PARP-1 binding was concentrated at specific loci in WT animals, while it was diminished from chromatin in PR-Set7^{20} animals (**Fig. 5A**). These findings suggest that PR-Set7/H4K20me1 plays a crucial role in mediating PARP1 binding to chromatin during heat stress, thus, controls PARP1-dependent processes in chromatin. Previous studies showed that heat shock results in pADPr accumulation at the heat shock-induced puffs during PARP-1-dpendent transcriptional activation (*4*,*7*,*11*,*16*,*17*,*37*). Therefore, we tested if heat-shock treatment alters pADPr accumulation in *PR-Set7*^{20} animals. As expected, the pADPr level increased by 26.1 times after heat-shock treatment in wild type animals, while in *PR-Set7*^{20}mutants the level of pADPr increased only 2.3 folds (**Fig. 5B**). This observation implies that PR-Set7/H4K20me1 plays a key role in regulating PARP-1 upon environmental stresses such as heat shock.

Discussion

This study illuminates the intricate interactions between PARP-1 and diverse histone modifications, with a particular emphasis on the influence of histone marks on the chromatin binding propensity of PARP-1. We discovered an elevated binding affinity of PARP-1 towards specific active histone modifications such as H4K20me1, H3K4me1, H3K36me1, and H3K9me1 in vitro (**Fig.1B** and C). Intriguingly, our results also suggest that the repressive histone modifications, H3K9me2/3 may potentially hinder PARP-1 binding (**Fig.1F**). The association between PARP-1 peaks and distinct histone marks in *Drosophila* third-instar larvae, as revealed through ChIP-seq data, corroborates the significance of these histone marks in PARP-1 binding. This is predominantly apparent for H4K20me1, H3K4me1, H3K36me1, and H3K9me1, while H3K9me2/3 demonstrated a lesser correlation (**Fig.1G**), further validating the repressive role of these marks.

We additionally examined the potential functional consequences of PARP-1's interaction with these histone marks, particularly H4K20me1. Mono-methylation of histone H4 on lysine 20 (H4K20me1), catalyzed by PR-SET7, is evolutionarily conserved from yeast to humans (*18*, *19*). H4K20me1, similar to PARP-1, is enriched at highly active genes (*20*–*26*). Also, H4K20me1 is critical for transcriptional regulation, maintenance of genome integrity, cell cycle regulation and double-strand break (DSB) DNA damage repair, all biological processes in which PARP-1 plays a role (*5*, *19*, *27*–*31*). Hence, histone modifications such as H4K20me1 may act to regulate the activity of key chromatin remodelers such as PARP-1.

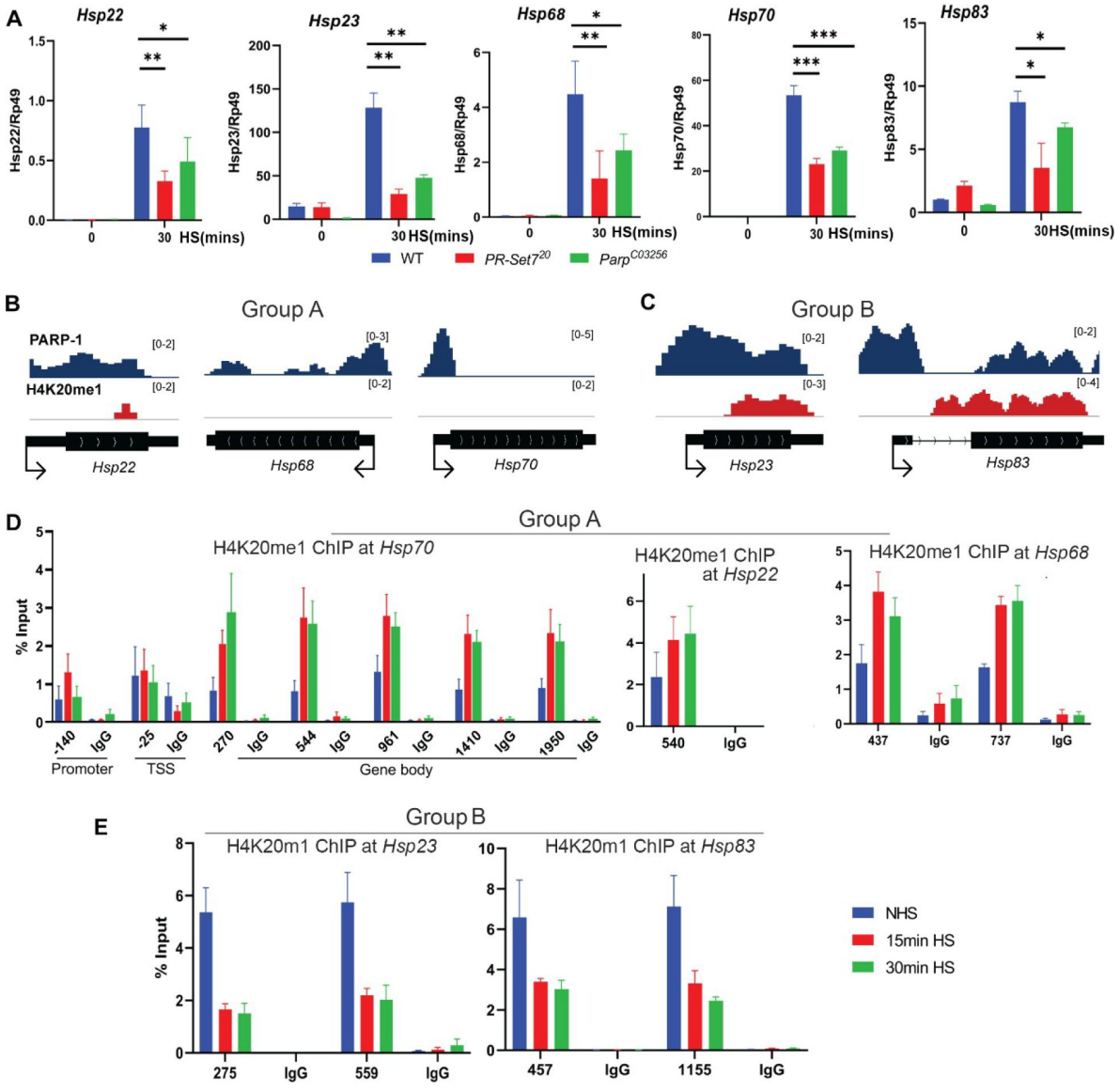

Figure 4.

Dynamic H4K20me1 enrichment regulates the expression of heat-shock genes during heat shock.

(**A**) Expression of *Hsp22, Hsp23, Hsp68, Hsp70* and *Hsp83* in WT, *Parp*^{C03256}and *PR-Set7*^{20} third-instar larvae before and after 30 mins heat shock. Data shown are from 3-5 biological replicates. ***P < 0.001, **P < 0.01, *P < 0.05 (Unpaired t-test; Two-tailed). Integrative Genome Viewer (IGV) tracks showing normalized ChIP-seq tracks of PARP-1 and H4K20me1 in third-instar larvae at (**B**) *Hsp22, Hsp68, Hsp68, Hsp70* and (**C**) *Hsp22, Hsp83* before heat shock. Mononucleosome ChIP-qPCR in WT showing enrichment of H4K20me1 at (**D**) *Hsp70, Hsp22, Hsp68* and (**E**) *Hsp23* and *Hsp83* before heat shock (NHS), after 15 mins heat shock and 30 mins heat shock. Primers used spanned the *Hsp70* locus and the gene bodies of *Hsp22, Hsp23, Hsp68, Hsp70* and *Hsp83*. Data shown are from 3 biological replicates.

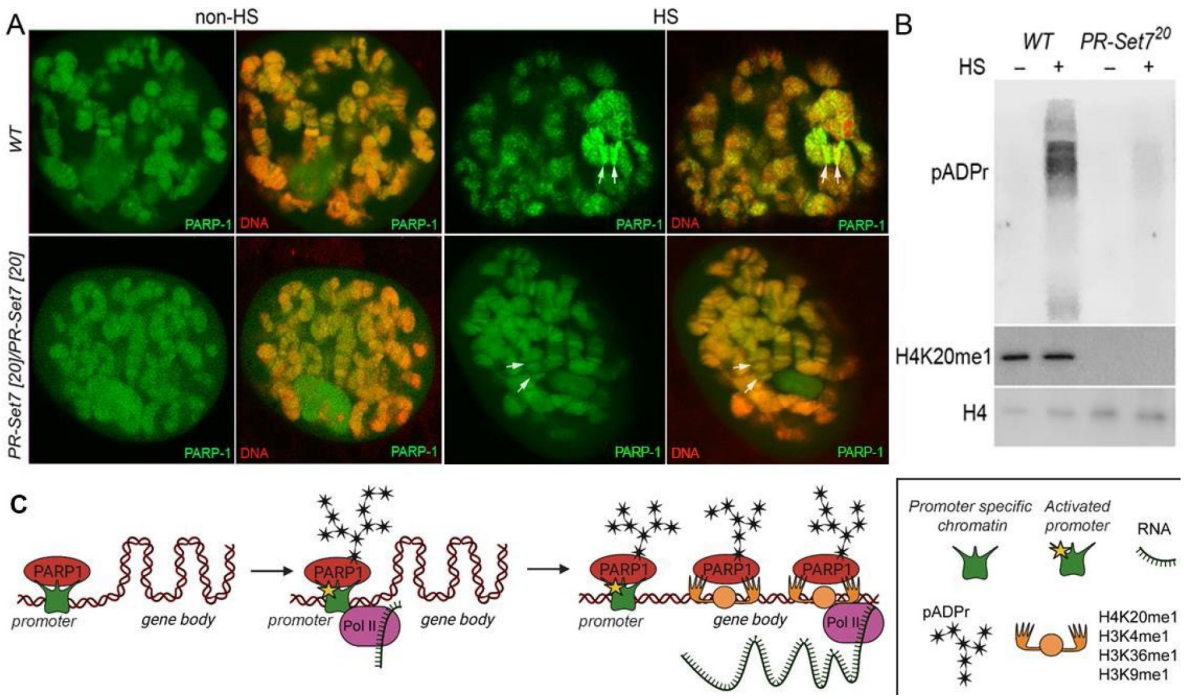

Figure 5.

Mono-methylated histones controls PARP-1 binding along gene body to regulate transcription.

(**A**) PR-Set7/H4K20me1 is required for holding PARP-1 in chromatin during HS. PARP-1 (green) protein recruitment to Hsp70 locus (Arrows) in salivary gland polytene nuclei in wild type (WT) and PRSte7 mutant (PRSet7[20]) third instar larvae. A single life salivary gland polytenized nucleus of wandering third instar larvae is shown for each genotype. Wild type genotype – UASt::PARP-1-EYFP, GAL4[Mz1087.hx]; PRSet7 mutant genotype – UASt::PARP-1-EYFP, GAL4[Mz1087.hx]; PRSet7[20]. (**B**) Equal amounts of lysates from the wild-type expressing PARP-1-EYFP (*WT*) and PR-Ste7[20] mutants expressing PARP-1-EYFP (*PR-Ste7^{20}*) grown at 22°C or heat shocked at 37°C for one hour at the third-instar larvae stage were subjected to immunoblot analysis using mouse anti-pADPr (10H), anti-H4K20me1 and anti-histone H4 (loading control) antibodies. (**C**) **Model of PARP-1 regulation by histone modifications.** PARP-1 binds to a nucleosome that carries the H2A variant (H2Av) at the promoter region. Upon developmental triggers or heat shock-induced phosphorylation of H2Av, PARP-1 is activated (*11*). Activated PARP-1 fosters a transcription start site (TSS) that is more accessible, thereby enabling the binding of RNA Polymerase II (Pol II) and initiation of transcription (*26*). Following this, the distribution of PARP-1 is further enhanced by active mono-methylated forms of H4K20, H3K4, H3K36, or H3K9. Each of these histone modifications can interchangeably facilitate this function. The spreading of PARP-1 to the gene body contributes to the loosening of chromatin in this region (*7*, *16*, *17*, *49*). Consequently, this facilitates the transition of Pol II into a productive elongation phase, leading to the generation of a mature transcript.

Our transcriptomic analysis from *PR-Set7*^{20} and *Parp1*^{C03256}mutant third-instar larvae implies that PR-Set7/H4K20me1 might be crucial for PARP-1's role in transcriptional regulation. Remarkably, differentially expressed genes (DEGs) in *PR-Set7*^{20} and *Parp1*^{C03256} mutants exhibited a high degree of correlation (**Fig.3A**), although we noticed minimal alteration in gene expression where PARP-1 was co-localized with H4K20me1 across the genome (**Fig.3C** and D). This suggests that H4K20me1 might only be essential for PARP-1-mediated binding and gene regulation at a select group of genes, specifically during metabolic gene repression and developmental gene activation. Our results also propose that PARP-1 and PR-Set7/H4K20me1 might have separate roles in gene regulation at co-enriched genes.

In this study, we have demonstrated that PARP-1 is essential for the activation of *Hsp22, Hsp23,* and *Hsp83* during heat shock (**Fig.4A**). Importantly, the expression of these heat shock genes significantly decreased in *PR-Set7*^{20} mutants following heat shock (**Fig.4A**), which shows a role for PR-Set7/H4K20me1 in the heat shock response. We have also observed that H4K20me1 regulation is dynamic in the gene bodies of PARP-1- and PR-Set7-dependent heat shock genes. Specifically, at genes that show low or no H4K20me1 enrichment prior to heat shock, H4K20me1 levels increase post-heat shock (**Fig.4B** and D). Conversely, at genes with high levels of H4K20me1, the level of H4K20me1 decreases following heat shock (**Fig.4C** and D). Previous studies have shown that H4K20me1 occupancy can either inhibit or facilitate transcriptional elongation, depending on the biological context (*24*, *32*–*35*). As such, we propose that for group A genes, which show a significant increase in H4K20me1 enrichment after heat shock, H4K20me1 may facilitate the spread of PARP-1 in their gene bodies, thus enhancing transcriptional activation. In contrast, the removal of H4K20me1 at group B genes, which exhibit a significant reduction of H4K20me1 at their gene bodies, may enable transcriptional elongation. Thus, an unidentified H4K20me1 demethylase and PR-Set7's non-catalytic functions may be required to facilitate transcriptional activation of group B heat shock genes during heat stress.

Notably, *Drosophila* embryo expressing an unmodifiable H4K20 (H4K20A) and a catalytically-inactive trr mutant (monomethylase of H3K4) were still able to mature into adult flies (*15*, *36*), demonstrating that H3K4me1 and H4K20me1 are dispensable for transcription during development. This finding contrasts our hypothesis that H4K20me1 or H3K4me1 might modulate PARP-1 binding at PARP-1-dependent genes during development. Importantly, our data also revealed that PARP-1 binds to H3K36me1 and H3K9me1. Consequently, we suggest that H3K36me1 and H3K9me1 could potentially substitute the role of H3K4me1 and H4K20me1 in controlling PARP-1 binding at PARP-1 dependent genes (**Fig. 5C**).

Materials and methods

Drosophila strains and genetics

Flies were cultured on standard cornmeal-molasses-agar media at 25°C. The transgenic stock pP{w1, UAST::PARP1-EYFP} has been previously described (38). The *Parp1*^{C03256}hypomorph mutant was generated in a single pBac-element mutagenesis screen (39). The *PR-Set7*^{20} null mutant was generously provided by Dr. Ruth Steward (15). Wandering third-instar larvae was used for all experiments. *W*^{1118}strains were used as controls for ChIP-seq and RNA-seq and termed WT as appropriate. We used TM6B and TM3-GFP to isolate *Parp1*^{C03256} and *PR-Set7*^{20} homozygous mutants respectively.

Histone peptide array

The Modified Histone peptide array (Active Motif) was blocked by overnight incubation in the tween 20 containing tris buffered saline (TTBS) buffer (10 mm Tris/HCl pH 8.3, 0.05% Tween-20 and 150 mm NaCl) containing 5% non-fat dried milk at 4°C. The membrane was then washed once with the TTBS buffer, and incubated with 4.0µgm PARP-1 (Trevigen) in PARP binding buffer (10

mm Tris-HCl, pH 8, 140 mm NaCl, 3 mm DTT, and 0.1% Triton X-100) at room temperature for 1 h. The membrane was washed in the TTBS buffer and incubated with anti-PARP antibody (SERUTEC at 1:500 dilution) for 1 h at room temperature in blocking buffer (5% milk in TTBS). The unbound antibody was washed three times with TTBS, and the membrane was incubated with horseradish peroxidase-conjugated anti-mouse antibody (Sigma, 1:2500) in TTBS for 1 h at room temperature. Finally, the membrane was submerged in ECL developing solution (GE Healthcare) and the image was captured on X-ray film. Typical exposure times were 0.5–2 min. The images were analyzed using an in-house program (Array Analyze, available at *https://www.activemotif.com/catalog/668/modified-histone-peptide-array*).

Drosophila salivary gland polytene chromosome immunostaining

Preparation and immunostaining of polytene chromosome squashes were performed exactly as described (40). The primary antibody used was anti-GFP (Living Colors, #JL-8, 1:100), H3K4me1 (Abcam, 1:50) and H4K20me1 (Abcam, 1:100) and the secondary antibody used was goat anti-mouse Alexa-488 (Molecular Probes, 1:1500) and goat-anti-rabbit Alexa-568 (Molecular Probes, 1:1500). DNA was stained with TOTO3 (1:3000). Slides were mounted in Vectashield (Vector Laboratories, Burlingame, CA).

Chromatin immunoprecipitation and sequencing

PARP-1-YFP ChIP-seq has been previously described (41). Briefly, 75 third-instar larvae were collected, washed with 1ml 1X PBS, and homogenized in lysis buffer (200ul 1X protease inhibitor cocktail, 250ul PMSF, 800ul 1X PBS, 1ul Tween 20). Crosslinking was achieved by adding 244.5ul of 11% formaldehyde for a final concentration of 1.8%, and quenched with 500mM glycine. The pellet was resuspended in sonication buffer (0.5% SDS, 20mM Tris pH 8.0, 2mM EDTA, 0.5mM EGTA, 0.5mM PMSF, 1X protease inhibitor cocktail) and sonicated to fragment chromatin. The supernatant was then collected after pelleting. This was pre-cleared, incubated with anti-GFP antibody (TP-401, Torrey Pines Biolabs), and immunoprecipitated chromatin was collected using Protein A agarose beads. Sequential washing was performed using low salt buffer, high salt buffer, LiCl wash, and TE buffer washers. Bound chromatin was eluted using 250ul ChIP elution buffer (1% SDS, 100mM NaHCO3) and reverse-crosslinked. The eluates were treated with RNase A and proteinase K, and DNA was extracted via phenol-chloroform extraction and ethanol precipitation. Sequencing was carried out at Novogene.

ChIP-seq analysis

The quality of FASTQ files (raw reads) were checked using FastQC (version. 0.11.9) and adapters were removed with fastp (42). Trimmed FASTQ files were aligned to the *Drosophila* genome (dm6) using Bowtie2 to generate bam files (43). Unmapped and low-quality reads were discarded from bam files (<=20 mapQuality) using BamTools (44). Duplicate reads were identified and removed from mapped reads using Picard MarkDuplicates (*http://broadinstitute.github.io/picard/*). Deeptools MultiBamSummary was used to determine reproducibility of ChIP-seq reads. MACS2 was used to call peaks against pooled Input/control using default settings except narrowPeaks were called for PARP-1 and broadPeaks were called for H3K4me1 (gapped), H3K9me1, H4K20me1, H3K36me1, H3K9me2, H3K9me3. Peaks were annotated to genomic features with ChIPseeker (45). Pairwise correlation of peaks was determined using Intervene (46). MACS2 bedGraph pileups were used to generate normalized coverage of ChIP-seq signals using Deeptools bigWigCompare by computing the ratio of the signals (IP vs Control/Input) using a 50bp bin size. Deeptools multiBigwigSummary was used to determine genome-wide signal correlation using a 10kb bin size. Deeptools plotHeatmap was used to create gene-centric enrichment profiles using scaled region mode. Gene clusters were determined via K means function (n=3) using Deeptools suite (47).

Mononucleosome chromatin immunoprecipitation

We collected 60 WT wandering Drosophila third-instar larvae and rinsed them in 1XPBS twice. For heat shock experiments, larvae were heat-shocked in a 1.5ml DNA LoBind tube for 15mins and 30mins in a water bath at 37°C. Larvae were then homogenized using a pellet pestle homogenizer in 500ul ice-cold buffer A1 (60 mM KCl, 15 mM NaCl, 4 mM MgCl2, 15 mM 4-(2-Hydroxyethyl)piperazine-1-ethanesulfonic acid (HEPES) (pH 7.6), 0.5% Triton X-100, 0.5 mM DTT, 1×EDTA (Ethylene Diamine Tetraacetic Acid)-free protease inhibitor cocktail (Roche 04693132001). The larvae were then crosslinked by adding formaldehyde to a 1.8% concentration and incubating for 5mins at RT on a rotator. Crosslinking was stopped by adding glycine (final concentration was 0.125 M) at room temperature for 5 min on a rotator. The mixture was centrifuged at 2000g for 5 min at 4°C, and the supernatant was discarded. The pellet was washed as follows: once with 500µl A1 buffer, and once with 500µl A2 buffer (140 mM NaCl, 15 mM HEPES (pH 7.6), 1 mM EDTA, 0.5 mM ethylene glycol tetraacetic acid (EGTA), 1% Triton X-100, 0.1% sodium deoxycholate, 0.5 mM DTT, 1×EDTA-free protease inhibitor cocktail (Roche 04693132001)). For each wash, the tube was shaken for 1 min and centrifuged as before, and the supernatant was discarded. The pellet was resuspended in 500µl A2 buffer + 0.1% sodium dodecyl sulfate (SDS) and incubated on a rotating wheel at 4°C for 10 min. Then the mixture was centrifuged at 16 000g for 5 min at 4°C and the supernatant was discarded. The pellet was washed with 500µl MNase digestion buffer (10 mM Tris–HCl (pH 7.5), 15 mM NaCl, 60 mM KCl, 1 mM CaCl2, 0.15 mM spermine, 0.5 mM spermidine, 1×EDTA-free protease inhibitor cocktail) and centrifuged at 16 000g for 10 min at 4°C. The nuclei were resuspended in 500µl MNase digestion buffer and 400 U MNase (Worthington, LS004797) was added and incubated at 37°C for 20 min. The MNase digestion was terminated on ice by adding EDTA to a final concentration of 10 mM and kept on ice for 10 min. The mixture was centrifuged at 16 000g for 10 min at 4°C. The supernatant was discarded. The pellet was resuspended in 500µl A3 buffer (140 mM NaCl, 15 mM HEPES (pH 7.6), 1 mM EDTA, 0.5 mM EGTA, 1% Triton X-100, 0.1% sodium deoxycholate, 0.1% SDS, 1×EDTA-free protease inhibitor cocktail) and incubated for 30 min on a rotator at 4°C. The mixture was then centrifuged at 16 000g for 15 min at 4°C. The supernatant containing mononucleosomal fragments was transferred to a new DNA LoBind tube, then 20ul of mixture was used to check fragment size prior to immunoprecipitation. The mixture was then made up to 2ml with IP buffer (0.5% SDS, 20mM Tris, pH 8.0, 2mM EDTA, 0.5mM EGTA, 0.5mM PMSF, protease inhibitor cocktail). The mononucleosomal chromatin was pre-cleared and 800ul was incubated with 5ul anti-H4K20me1 antibody (abcam, 9051) or 5ul Rabbit IgG control at 4°C overnight and 80ul was kept as input. The immunoprecipitated chromatin was then collected with pre-washed Protein A agarose beads for 2 hours. The beads were sequentially washed with the following buffers: 1 low salt buffer wash (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCL pH 8.0, 150mM NaCl), 3 high salt buffer washes (0.1% SDS, 1% Triton X-100, 2mM EDTA, 20mM Tris-HCL pH 8.0, 500mM NaCl), 1 LiCL wash (2mM EDTA, 20mM Tris-HCl pH 8.0, 0.25M LiCl, 1% NP-40) and 2 TE buffer washers before elution. Bound chromatin on beads was eluted twice at room temperature using 250ul of freshly prepared ChIP elution buffer (1% SDS, 100mM NaHCO3) for 15 minutes and reverse-crosslinked overnight. The eluates were then treated with RNase A and proteinase K prior to DNA extraction via phenol-chloroform extraction and ethanol precipitation.

RNA sequencing

RNA was isolated from 10 wandering third-instar larvae (3 biological replicates per genotype; *WT*, *PR-Set7*^{20} and *Parp1*^{C03256}) using RNeasy lipid tissue mini kit (Qiagen). RNA samples were flash-frozen in liquid nitrogen and sent to Novogene for library preparation and sequencing. mRNA was purified from total RNA via poly-T oligo beads. Libraries were prepared using Ultra II RNA library kit (NEB) and samples were sequenced on the NovaSeq 6000 platform (Illumina) at Novogene.

RNA-seq analysis

Paired-end reads were quality-checked using FastQC and reads were mapped to the Drosophila genome (dm6) using RNA STAR (48). Reads per annotated gene was counted using featureCounts (49). Differential expression analysis was performed with DESeq2 (50), with Log2 fold change

of at least 1 (absolute) considered significant (FDR < 0.05).

Quantitative RT-PCR

For heat shock experiments, 10 third-instar larvae were heat-shocked in 1.5ml tube in a water bath at 37°C for 30 min. Total RNA was then isolated for each biological replicate (3) using an RNeasy Lipid Tissue Mini kit (Qiagen). DNA contamination was removed using TURBO DNA-free (Ambion). RNA concentration was measured using an Agilent 2100 BioAnalyzer (Agilent Technologies) in combination with an RNA 6000 Nano LabChip (Agilent Technologies). Real-time PCR assays were run using StepOnePlus Real-Time PCR System (Applied Biosystems).

Primers used for Hsp70 ChIP-qPCR were gotten from (37, 51). Primers used for Hsp22, Hsp23, Hsp68 and Hsp83 qPCR were gotten from (51). Primer sequences are listed in **data file S2**.

PR-Ste7[20] mutants validation using qRT-PCR

Total RNA was isolated from 10 third-instar larvae for each biological replicate (3) using an RNeasy Lipid Tissue Mini kit (Qiagen). DNA contamination was removed using TURBO DNA-free (Ambion). RNA concentration was measured using an Agilent 2100 BioAnalyzer (Agilent Technologies) in combination with an RNA 6000 Nano LabChip (Agilent Technologies). Real-time PCR assays were run using StepOnePlus Real-Time PCR System (Applied Biosystems). The following primers were used: PR-Set7 (Forward) CGCACAATAGGAGTTCCC (Reverse) CCTCATCGTCCAGTTTCAG and Rp49 (Forward) GCTAAGCTGTCGCACAAAT (Reverse) GAACTTCTTGAATCCGGTGGG.

PARP-1-EYFP re-localization in chromatin during heat shock

Larvae were grown at 20°C. Heat shock was performed by placing third instar wandering larvae individually in a 1.5ml Eppendorf tube with a small hole in the cap to let the larvae breath. The tube was placed in a Marry bath at 37°C for 30 minutes. After heat shock, the larvae were briefly washed in 1X PBS then immediately dissected in 1X supplemented Grace's insect media (Gibco). Salivary glands were mounted in 1X supplemented Grace's insect media (Gibco) containing Draq-5 DNA dye (Life technologies) at a 1/5000 dilution.

Western Blot Analysis

The following antibodies were used for immunoblotting assays:anti-PR-Set7 (Rabbit, 1:1000, Novus Biologicals, 44710002), anti-H4K20me1 (Rabbit, 1:1000, Abcam, ab9051), anti-H4 (Mouse, 1:1000, Abcam, ab31830).

Gene ontology (GO)

Gene ontology terms were determined using g:profiler (FDR<0.05) (52).

Statistical analysis

Results were analyzed using the indicated statistical test in GraphPad Prism (9.4.0). Statistical significance of ChIP-seq peaks were determined using MACS2. Q-value cutoffs for RNA-seq and GO analysis were determined with DESeq2 and G:profiler respectively.

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Author contributions

G.B, G.B and N.L performed the experiments. G.B analyzed the data.

G.B and A.T wrote the manuscript. G.B and A.T reviewed and edited the manuscript. G.B and A.T supervised the project.

Competing interests

The authors declare they have no competing interests.

Data and materials availability

All data generated or analyzed during this study are included in this article. No custom code was generated in this study. ChIP-seq and RNA-seq data generated in this study are available on the GEO database (Accession no: GSE217730 and GSE222877). The following public *Drosophila* third-instar larvae ChIP-seq (ENCODE) were used in this study: GSE47282 (H3K4me1), GSE47254 (H4K20me1), GSE47249 (H3K36me1), GSE47289 (H3K9me1), GSE47260 (H3K9me2) and GSE47258 (H3K9me3).

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Reviewer #1 (Public Review):

The study investigates the role of PARP-1 in transcriptional regulation. Biochemical and ChIP-seq analyses demonstrate specific binding of PARP-1 to active histone marks, particularly H4K20me, in polytene chromosomes of Drosophila third instar larvae. Under heat stress conditions, PARP-1's dynamic repositioning from the Hsp70 promoter to its gene body is observed, facilitating gene activation. PARP-1, in conjunction with PR-Set7, plays a crucial role in the activation of Hsp70 and a subset of heat shock genes, coinciding with an increase in H4K20me1 levels at these gene loci. This study proposes that H4K20me1 is a key facilitator of PARP-1 binding and gene regulation. However, there are several critical concerns that are yet to be addressed. The experimental validation and demonstration of results in the main manuscript are scant. Recent developments in the area are omitted, as an important publication hasn't been discussed anywhere in the work (PMID: 36434141). The proposed mechanism operates quite selectively, and any extrapolations require intensive scientific evidence.

Major Comments:

1. PARP1 hypomorphic mutant validation data must be provided at RNA levels as the authors have mentioned about its global reduction in RNA levels.

2. The authors should provide immunoblot data for global Poly (ADP) ribosylation levels in PARP1 hypomorphic mutant condition as compared to the control. They must also provide the complete details of the mouse anti-pADPr antibody used in their immunoblot in Figure 5B.

3. PR-Set7 mutant validation results should be provided in the main manuscript, as done by the authors using qRT-PCR. Also, immunoblot data for the PR-set7 null condition should be supplemented in the main manuscript as the authors have already mentioned their anti-PR-Set7 (Rabbit, 1:1000, Novus Biologicals, 44710002) antibody in the materials and methods section.

4. The authors have probably missed out on a very important recent report (PMID: 36434141), suggesting the antagonistic nature of the PARP1 and PR-SET7 association. In light of these important observations, the authors must check for the levels of PR-Set7 in PARP1 hypomorphic conditions.

5. Also, the results of the aforementioned study should be adequately discussed in the present study along with its implications in the same.

6. Gene transcriptional activation requires open chromatin and RNA polymerase II binding to the promoter. Since, differentially expressed genes in both PR-Set7 null and PARP1 hypomorph mutants, co-enriched with PARP-1 and H4K20me1 were mainly upregulated, the authors should provide RNA polymerase II occupancy data of these genes via RNA-Pol II ChIP-seq to further attest their claims.

7. As discussed in Figure 4, the authors found transcriptional activation of group B genes even after a significant reduction of H3K20me1 in their gene body after heat shock. Given the dynamic equilibrium shift in epigenetic marks that regulate gene expression and their locus-specific transcriptional regulation, the authors should further look for the enrichment of other epigenetic marks and even H4K20me1 specific demethylases such as PHF8 (PMID: 20622854), and their cross-talk with PARP1 to further bridge the missing links of this tale. This will add more depth to this work.

- https://doi.org/10.7554/eLife.91482.1.sa1

Reviewer #2 (Public Review):

Summary:
This study from Bamgbose et al. identifies a new and important interaction between H4K20me and Parp1 that regulates inducible genes during development and heat stress. The authors present convincing experiments that form a mostly complete manuscript that significantly contributes to our understanding of how Parp1 associates with target genes to regulate their expression.

Strengths:
The authors present 3 compelling experiments to support the interaction between Parp1 and H4K20me, including:

1. PR-Set7 mutants remove all K4K20me and phenocopy Parp mutant developmental arrest and defective heat shock protein induction.
2. PR-Set7 mutants have dramatically reduced Parp1 association with chromatin and reduced poly-ADP ribosylation.
3. Parp1 directly binds H4K20me in vitro.

Weaknesses:

1. The histone array experiment in Fig1 strongly suggests that PARP binds to all mono-methylated histone residues (including H3K27, which is not discussed). Phosphorylation of nearby residues sometimes blocks this binding (S10 and T11 modifications block binding to K9me1, and S28P blocks binding to K27me1). However, H3S3P did not block H3K4me1, which may be worth highlighting. The H3K9me2/3 "blocking effect" is not nearly as strong as some of these other modifications, yet the authors chose to focus on it. Rather than focusing on subtle effects and the possibility that PARP "reads" a "histone code," the authors should consider focusing on the simple but dramatic observation that PARP binds pretty much all mono-methylated histone residues. This result is interesting because nucleosome mono-methylation is normally found on nucleosomes with high turnover rates (Chory et al. Mol Cell 2019)- which mostly occurs at promoters and highly transcribed genes. The author's binding experiments could help to partially explain this correlation because PARP could both bind mono-methylated nucleosomes and then further promote their turnover and lower methylation state.
2. The RNAseq analysis of Parp1/PR-Set7 mutants is reasonable, but there is a caveat to the author's conclusion (Line 251): "our results indicate H4K20me1 may be required for PARP-1 binding to preferentially repress metabolic genes and activate genes involved in neuron development at co-enriched genes." An alternative possibility is that many of the gene expression changes are indirect consequences of altered development induced by Parp1 or PR-Set7 mutants. For example, Parp1 could activate a transcription factor that represses the metabolic genes that they mention. The authors should consider discussing this possibility.

3. The section on the inducibility of heat shock genes is interesting but missing an important control that might significantly alter the author's conclusions. Hsp23 and Hsp83 (group B genes) are transcribed without heat shock, which likely explains why they have H4K20me without heat shock. The authors made the reasonable hypothesis that this H4K20me would recruit Parp-1 upon heat shock (line 270). However, they observed a decrease of H4K20me upon heat shock, which led them to conclude that "H4K20me may not be necessary for Parp1 binding/activation" (line 275). However, their RNA expression data (Fig4A) argues that both Parp1 and H40K20me are important for activation. An alternative possibility is that group B genes indeed recruit Parp1 (through H4K20me) upon heat shock, but then Parp1 promotes H3/H4 dissociation from group B genes. If Parp1 depletes H4, it will also deplete H4K20me1. To address this possibility, the authors should also do a ChIP for total H4 and plot both the raw signal of H4K20me1 and total H4 as well as the ratio of these signals. The authors could also note that Group A genes may similarly recruit Parp1 and deplete H3/H4 but with different kinetics than Group B genes because their basal state lacks H4K20me/Parp1. To test this possibility, the authors could measure Parp association, H4K20methylation, and H4 depletion at more time points after heat shock at both classes of genes.

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