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Research Article

Heterochromatin protein 1 (HP1) of *Schistosoma mansoni* : non-canonical chromatin landscape and oviposition effects

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Abstract

Heterochromatin protein 1 (HP1) is widespread in several organisms playing a role in control of gene expression by heterochromatin formation and maintenance of silent chromatin. *Schistosoma mansoni* is a human parasite that is responsible for Schistosomiasis, a tropical neglected disease in the tropical and subtropical areas in the world, where the intermediate host *Biomphalaria glabrata* is present. In this study we attempted to investigate if the *SmHP1* is enriched in *S. mansoni* chromatin in cercariae larvae stage, compared with another larvae stage sporocysts and its importance for *S. mansoni* life cycle progression and parasite oviposition. We used ChIPmentation with commercial antibody ab109028 that passed in-house quality control. Our data show that *S. mansoni* HP1 enrichment is non-canonical with a peak at the transcription end sites of protein coding genes. We did not find strong differences in *SmHP1* chromatin landscapes between sporocysts and cercariae. Knock-down of *SmHP1* in schistosomula and *in vivo* experiments in mice unexpectedly increased parasite oviposition. Our results suggest that *SmHP1* may influence chromatin structure in a non-canonical way in *S. mansoni* stages and may play a role in regulation of parasite oviposition.

Keywords: Heterochromatin protein 1, HP1, ChIPmentation, cercariae, sporocysts, *Schistosoma mansoni*.

Introduction

Schistosomes are trematode parasite responsible for causing schistosomiasis. It is estimated that in 2021, approximately 251.4 million people required treatment¹. Intestinal schistosomiasis is caused by *S.mansoni*. The parasite has a complex life cycle that includes two hosts, snails of the genus *Biomphalaria* and humans, which are intermediate and definitive hosts, respectively². During its life cycle, the parasite goes through significant stage changes, and it is known that histone post-translational modifications play important roles at each stage of the life cycle. The molecular complexity of *S. mansoni* life cycle has been revealed over many years and efforts to elucidate the *S. mansoni* epigenome has given some insights about epigenetic regulation of the life cycle³⁻⁶. However, it is expected that coregulators will also be required for the maintenance of each stage. In several organisms, the heterochromatin protein 1 (HP1) acts as co-regulators and performs fundamental functions such as maintaining the silenced state of chromatin, DNA repair, among other diverse functions.

This protein is composed of the chromodomain (CD) and chromoshadow (CDS) domains and a linker region between them. CD recognizes and binds to methylated histone tails, while CDS is responsible for homo- and heterodimerization⁷. The region between the domains makes connections to nucleic acids and is quite variable between organisms. In contrast, the domains present well-conserved amino acid sequences among different species. HP1 is an important component essential for heterochromatin gene silencing. This function was described in model organisms such as cancer progression in *Homo sapiens*, *Drosophila melanogaster*, *Plasmodium falciparum*, *fission yeast* and *Arabidopsis thaliana*⁸⁻¹². Histone modifications are associated with different chromatin states and play important roles in regulating gene expression. E.g. in *P. falciparum* methylation of histone H3 at lysine 9 (H3K9) forms binding sites for the HP1 protein and is an important means of controlling gene expression^{10,13}. In *Drosophila*, there are three isoforms of HP1. While initially identified as important part of the heterochromatin through H3K9me3 binding

(<https://www.embopress.org/doi/full/10.1093/emboj/20.18.5232>)¹⁴, it was later found to be also associated with active gene expression (<https://academic.oup.com/genetics/article/219/1/iyab108/6321239>)¹⁵. Metagene profiles in (<https://academic.oup.com/genetics/article/219/1/iyab108/6321239>)¹⁵ indicated enrichment of all three HP1 around the transcription start of genes in *drosophila*.

For *S.mansoni* we showed in a previous study¹⁶ that HP1 is co-immunoprecipitated with other important DNA associated proteins such as helicases, transcription factors and methyltransferases. These results suggest an important role for HP1 in regulating gene transcription in *S. mansoni*. Geyer and colleagues also described, through *in vitro* experiments with adult worms that *SmCBX* (*Smp_179650*, *SmHP1*, *Sm Chromobox protein homolog 5*) plays a role in the parasite biology regulating oviposition¹⁷. We hypothesized that the HP1 homolog of *S.mansoni* is associated with DNA and plays a role in chromatin structure biology in the parasite life cycle. To test this hypothesis about the involvement of HP1 in the chromatin formation and maintenance in larvae stages, cercaria and sporocyst, were performed the ChIPmentation using an antibody against HP1 homolog used previously¹⁶. In addition, for investigation of the role of HP1 in parasite development, migration, fitness, and inflammatory response, we generated *SmHP1 in vivo* Knock-outs. Our results suggested that *SmHP1* may have a function to regulate epigenetic plasticity in the parasite through the increasing parasite oviposition without affecting host inflammatory response.

Results

AntiHP1 antibody ab109028 can be used for ChIP in S.mansoni

Antibody quality is an important criterion for the success and reliability of ChIP-Seq. HP1 is a conserved protein and we had shown before that antiHP1 Abcam ab109028 can be used in Western Blots with *S.mansoni*. A literature search for the use of ab109028 resulted in 32 publications (Suppl. Table

1) but only in one case it was used for ChIP-seq, and the efficiency of the antibody was not tested there. Thus, initial experiments were necessary to evaluate if the antibody was suitable for ChIP-Seq. We used a previously developed pipeline to test whether ab109028 (lot numbers GR38873387336-6 and -9) can be used for ChIP-Seq¹⁸. Essentially, the method consists of (i) performing a Western blot to assure that only the band of the expected size can be observed and (ii) doing a chromatin IP titration experiment with a constant amount of input chromatin and increasing amounts of antibody to test if saturation of available epitopes can be achieved.

We performed Western Blots on *S.mansoni* cercariae and sporocysts. We used *D.melanogaster* embryos as a positive control because in this model organism HP1 was successfully characterized by Western Blot and ChIP-Seq experiments. Expected molecular weight of *D.melanogaster* HP1 is 23.194 kD

(<https://www.uniprot.org/uniprotkb/B6UVQ8/entry>). The dimer is therefore predicted to have a molecular weight of 46 kD¹⁶. Such a band is observed in all samples in the Western blot (Figure 1) but supplementary bands can be distinguished. Apparently, under our relatively gentle extraction conditions and without beta-mercaptoethanol the dimers remained intact. We noticed that this behavior is not without precedent (e.g. <https://www.antibodies.com/fr/hp1-alpha-antibody-a88817>; <https://www.abcam.com/en-hu/products/primary-antibodies/hp1-alpha-antibody-epr5777-heterochromatin-marker-ab109028>) even for Knock-out validated antibodies (<https://www.antibodies.com/fr/hp1-alpha-antibody-a88778>). The results shown in Figure 1 agree also with previous western blotting¹⁶ which had demonstrated that *Sm*HP1 can form dimers in solution, showing a band of approximately 56 kD.

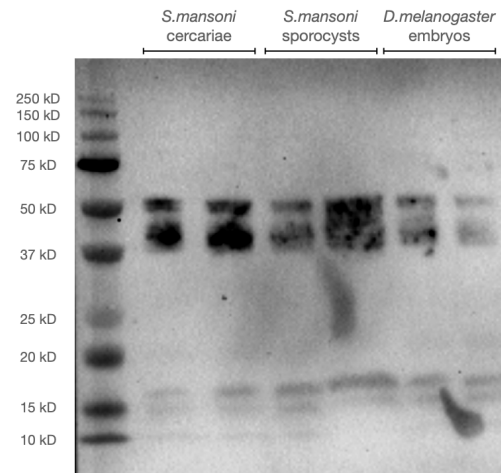
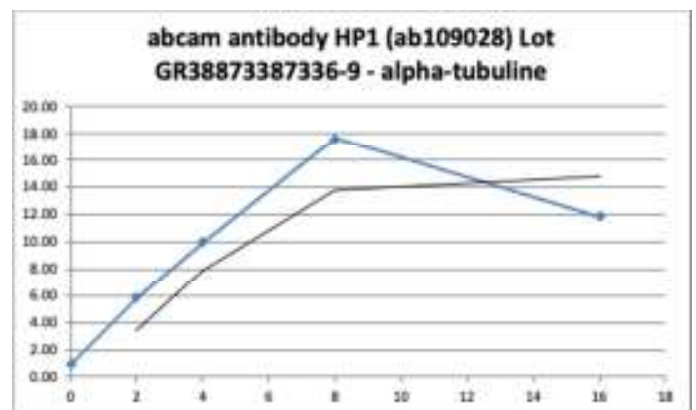


Figure 1: Western blot on crude protein extracts of *S.mansoni* cercariae and sporocysts (left and middle), and *D.melanogaster* embryos (right). All lanes in duplicates. Left lane molecular weight marker.

We then proceeded to ChIPmentation titration of the antibody using chromatin from *S.mansoni* cercariae. ChIPmentation is a streamlined method of crosslink Chromatin Immunoprecipitation (ChIP) that uses Tn5 for integration of adaptor for library amplification (Tagmentation). A constant amount of chromatin corresponding to 160,000 cells was incubated with 0, 2, 4, 8 and 16 µl of ab109028 during the ChIPmentation procedure and input recovery was measured using qPCR on 2 arbitrarily chosen loci: *Sm*-alpha-tubuline and *Sm*-28S-rDNA. Results are shown in figure 2, indicating that above 8 µl antibody saturation is achieved.



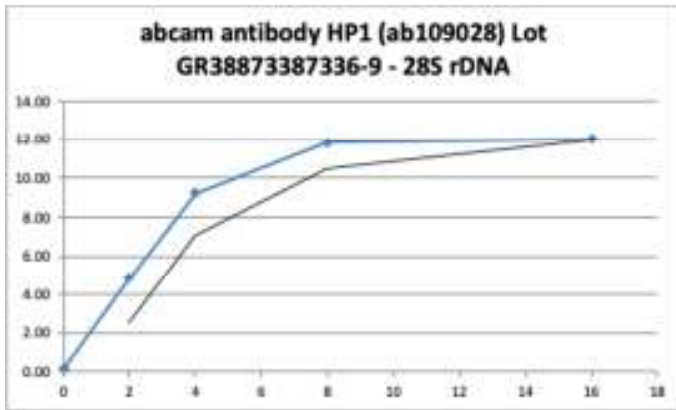


Figure 2: Results of titration of antiHP1 antibody. X-axis indicates amount of antibody in μl , Y-axis input recovery in %. Upper panel for Sm-alpha-tubuline, lower panel for Sm-28S-rDNA. Saturation is achieved with 8 μl of antibody. Blue line represents the experimental data, black line is the calculated trend line.

Based on this initial testing we decided to proceed to ChIPmentation with 8 μl of antibody.

ChIP-Seq metagene profiles in *S.mansoni* peak around transcription end sites (TES)

After having firmly established that ab109028 was suitable for ChIP we proceeded to ChIPmentation on cercariae and sporocysts of *S.mansoni*. We hypothesized that HP1 could establish a heterochromatic structure in the cercariae that are transcriptionally inactive. To investigate the distribution of HP1 with relation to known genomic features we produced metagene profiles around protein coding genes. In contrast to what was observed in other species, *Sm*HP1 shows enrichment around the TES (figure 3) in both, cercariae and sporocysts.

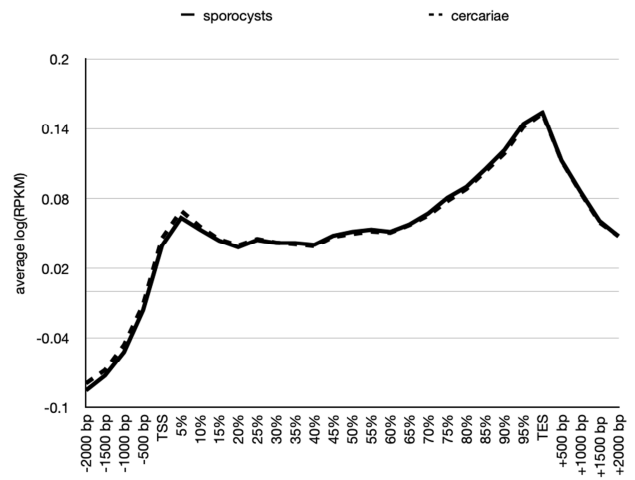


Figure 3: Metagene profiles of *S.mansoni* sporocysts (solid line) and cercariae (dotted line). X-axis : relative position around genes, Y-axis : average log(Reads Per Kilobase per Million mapped reads (RPKM)) of two replicates, TSS: Transcription start site, TES: transcription end site

This unexpected result and the fact that the current study is the first analysis of HP1 distribution and there are therefore no precedents to compare with, prompted us to test the ChIPmentation procedure with antiHP1 ab109028 on the well characterized model species *D. melanogaster*.

AntiHP1 antibody ab109028 delivers canonical ChIP-Seq results in *D.melanogaster*

It might be argued that the observed metagene profiles in *S.mansoni* are due to a peculiar nature of the antibody that remained unnoticed or an experimental error in our ChIPmentation procedure. If this would hold true then our experiments would deliver results that are different from previously published data. To test this hypothesis we performed ChIPmentation experiments with *D.melanogaster* embryos, a species for which data of ChIP-Seq experiments are available. To obtain these data we used the query terms “hp1 chip D rosophila” to search the NCBI SRA and obtained 186 results belonging to 55 BioSamples. We arbitrarily choose ChIP-Seq data for WT embryo replicate 1 and 2 (NCBI SRA: SRS6795886, SRS6795887)

corresponding to ENA SRX8497063 and SRX8497065 and data for input (NCBI SRA SRS6795884, ENA SRX8497062). The NCBI SRA entry states that 2 μ g antiHP1, Developmental Studies Hybridoma Bank C1A9 had been used.¹⁹

We also downloaded ChIP-Seq data for fly heads that had been produced with the same antibody20 (NCBI BioProject PRJNA490276): ENA SRR7817540, SRR7817541, SRR7817542 for 3 ChIP-Seq replicates and ENA SRR7817573 for the input.

We then performed ChIPmentation with antibody ab109028 on fruit fly embryos under the same conditions as for our *S.mansoni* samples. After sequencing, we processed the published data and our experimental data as described for the *S.mansoni* samples. For SRA data of embryos, ChromstaR did not manage to construct a differential model probably due to relatively low enrichment of the reads. We resorted therefore to read counts distributions around metagenes ($\log(\text{RPKM})$ instead of $\log(\text{obs}/\text{exp})$, shown in figure 4). While it is interesting to note that there is a small decrease in adult flies compared to embryos at the TES (figure 5), we did not observe strong differences between the profiles generated based on the data of the two independent earlier studies and our experiment.

Figure 4: Metagene profiles of *D.melanogaster* embryos (solid line) produced in this study with antibody ab109028, and previously published data on embryos (dotted line) with antibody C1A9. X-axis : relative position around genes, Y-axis : average $\log(\text{RPKM})$ of two replicates (the values can therefore not be compared directly to y-axis of figures 3 and 4)

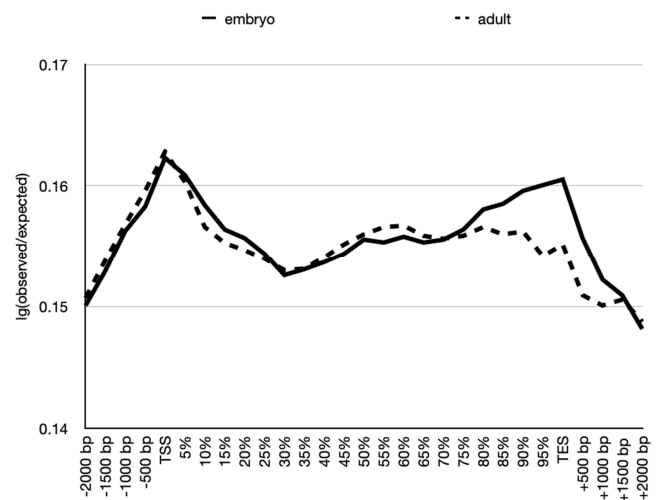
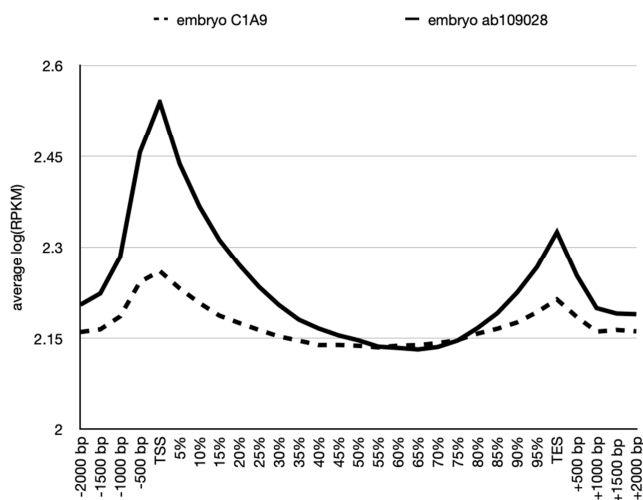


Figure 5: Metagene profiles of *D.melanogaster* embryos (solid line) produced in this study with antibody ab109028, and previously published data from another laboratory on adult flies (dotted line) with antibody C1A9. X-axis: relative position around genes, Y-axis $\log(\text{observed}/\text{expected})$ of two replicates



Therefore, we conclude that antibody ab109028 delivers canonical ChIP-seq profiles around genes with the fruit fly as a model. Consequently, we have no reason to believe that the non-canonical HP1 chromatin landscape around genes in *S.mansoni* is due to the antibody quality or the ChIPmentation procedure.

While the question was not central to our experiment we profited from the by-catch of ChIP-Seq data from *Biomphalaria glabrata* in the sporocysts ChIPmentation reads to show if this non-canonical binding pattern is schistosome specific or is a general trait of lophotrochozoans, a clade to which *Biomphalaria* and *Schistosomes* belong, and that is

evolutionary distinct from insects. Results are shown in supplementary figure 2 and indicate that *B.glabrata* metagene profiles are similar to *D.melanogaster* with enrichments in the TSS and TES of protein coding genes.

There are few regions with differential enrichment of HP1 between cercariae and sporocysts

Since we had established that both the antibody and the new ChIPmentation procedure delivered reproducible results in model organisms we proceeded to comparative analyses between *S.mansoni* live cycle stages. We used ChromstaR to identify 154 regions with differences in HP1 enrichment between cercariae and sporocysts (Suppl. File 1). However, these regions are small and visual inspection of HP1 landscape suggested that HP1 enrichment occurs over broader regions. We therefore applied another software, MACS bdgbroadcall, on combined uniquely aligned ChIP-Seq reads, independently for sporocysts and cercaria. Results are in table 1.

Table 1: Number of broad peaks in cercariae and sporocyst larvae using different p-value scores from MACS2,

	number of peaks (p-value 1e-2)	number of peaks (p-value 1e-5)
sporocysts	6406	89
cercariae	7485	222

We then used DiffBind for the identification of differential enrichment between sporocysts and cercariae. Based on the conservative peakcall at score 5 we identified 36 differentially enriched regions that separated sporocysts and cercariae into 2 clusters (figure 6).

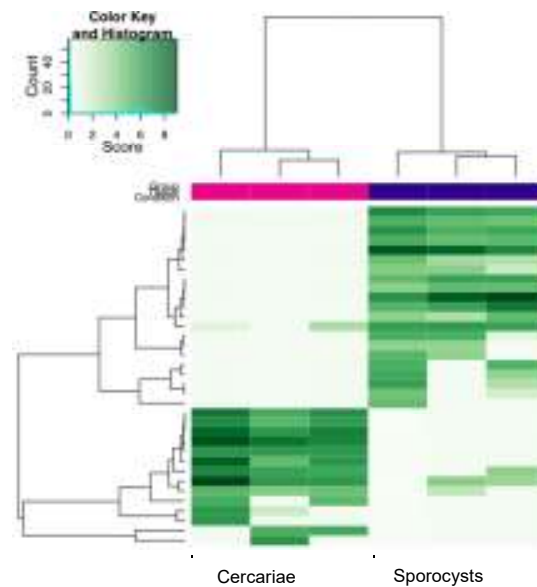


Figure 6: Clustering of Cercariae and sporocyst larvae based on differential HP1 chromatin enrichment

Since this number is relatively low, we wondered whether the number of identified differences could have been obtained by chance alone. We used bootstrapping through random segments that matched the 222 broad cercariae HP1 peaks in number and total span and used DiffBind for identification of differential enrichment. Bootstrapping through these analyses found in average 61.5 ± 6.8 differential peaks. Consequently, we conclude that the differences identified by DiffBind identified on broad regions lack the statistical power to be confident about their occurrence other than by accident.

B

In summary, our finding cannot formally exclude that there are differences between sporocyst and cercariae in HP1 enrichment, but differences are small. To establish a profile of genes which are enriched and probably regulated by SmHP1 we generated a script (supplementary file 3) to generate a gene list which were enriched by SmHP1 protein. The list gene for cercariae (Supplementary file 4) and sporocyst (Supplementary file 5) showed interesting results. For cercariae, we found several genes related to kinases proteins, which suggest the importance of phosphorylation and signaling pathways in cercariae. For sporocyst we also found kinases and other proteins with diverse functions.

In vivo knock-down of SmHP1 increases parasite's fitness in mice

Given the unexpected distribution of SmHP1 over the genome and absence of important differences between two developmental stages we wondered if SmHP1 played any role in the parasite *in vivo*. We designed an experiment to perform the knockdown of SmHP1 by RNA interference in schistosomula. Decrease of expression was small (Suppl. Figure 1) compared with control dsmCherry and wild type schistosomula. The *in vivo* results are shown in Figure 7. Parasite burden showed that parasites were able to migrate, develop and to achieve the correct destination that is portal hepatic and mesenteric veins, despite dsRNA employed and schistosomula injection. To evaluate infection success and parasite fitness, we measured granuloma and egg count/ feces gram using Kato-Katz method². The number of granulomas and area were the same for all tested

conditions, suggesting that knock-down of SmHP1 does not affect the granulomatous response from the host. Surprisingly, the parasite number of eggs/feces gram were roughly 2 times higher in the knock-down and this difference was statistically significant.

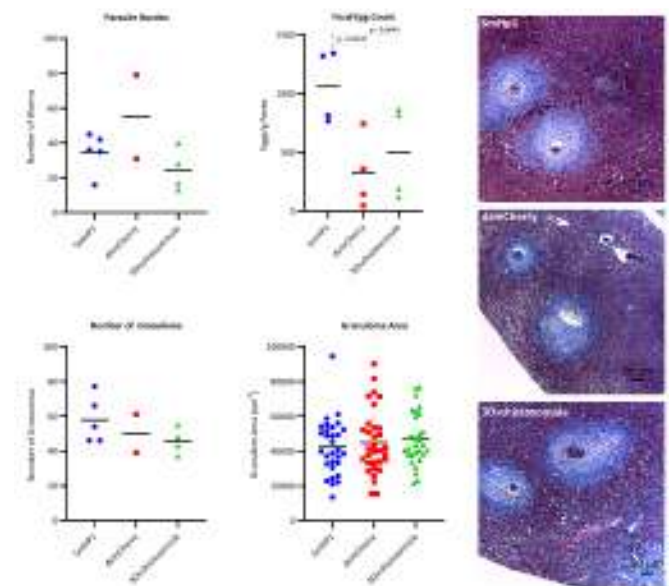


Figure 7 : Functional *in vivo* studies in mice injected with (i) dsRNA incubated HP1 3 days schistosomula (blue) compared to (ii) mock treated dsRNA gene (dsmCherry), and (iii) wild type. Parasite burden, fecal egg count, granuloma number and area were measured. Significant differences were only found for fecal egg count which increased for the knock-down parasites.

Discussion

Recent years have seen an important increase in our understanding of the chromatin structure of *S.mansoni*. However, this knowledge stems almost exclusively from work on histone modifications and DNA methylation. To widen our view to other chromatin proteins we focused here on the HP1 homologue.

Our results of antibody titration show you that antibody ab109028 is suitable for ChIP-seq

experiments on *S.mansoni*. Our report shows for the first time the HP1 profile for this species.

There are several works that have focused on transcription and translation in cercariae, absence of transcription but use of mRNA from previous stages, low rates of translational until infection and schistosomula migration when transcription is activated²³⁻²⁵. Since cercariae is transcriptionally silent and poised for transcription⁴, we reasoned that they might have a tighter heterochromatin formation and differential HP1 occupancy, but our results suggest the opposite: HP1 presence (and presumably heterochromatinization) might not be advantageous if it is necessary to rapidly activate transcription after infection.

HP1 is a conserved member of the large family of chromatin proteins, and it is believed to fulfill conserved functions in different organisms. For example, in *Drosophila* there are 5 isoforms of HP1 and it is mainly located in heterochromatic regions rich in H3K9me3¹⁹ and preferentially associated with polytene chromosomes and the X chromosome. The study also suggested that the formation of heterochromatin occurs in parallel with the transcriptional activation of the nuclei and expression of different genes²⁶. Previous work using RNA interference technology has found that HP1 silencing was directly related to chromosomal defects^{26,27}. The distribution of HP1 throughout the *S. mansoni* genome does not follow a pattern observed for other organisms suggesting that its function is also different.

The choice of *Drosophila* as a comparative model is justified by the fact that there are no previous ChIP-seq studies for SmHP1 in *S. mansoni*. Although *Drosophila* is an organism evolutionarily distant from *S. mansoni*, the functions of this protein are conserved among organisms with link to the trimethylation of lysine 9 in histone H3 (H3K9me3) to maintain the silenced state of chromatin. This binding has been shown to be important for HP1 to perform its functions and studies have shown this binding of HP1 to the epigenetic mark H3K9me3 in

yeast²⁸, *Drosophila*²⁹⁻³¹, mammals^{32, 33, 11} and *Plasmodium*^{10, 34, 13}.

The conservation of HP1 domains among organisms is a strong indication of conservation of function and, although SmHP1 is conserved and has similarities with other HP1 proteins, it is noteworthy that in *S. mansoni* this protein exerts different functions. In *S. mansoni*, SmHP1 can be found in almost the entire genome, exhibiting some genes differentially expressed in the stages studied. In addition to HP1 binding to H3K9me3 in many organisms, this protein also binds to the epigenetic mark H3K27me3 to produce repressive effects on gene transcription. Thus, the study of SmHP1 was based on the theory that SmHP1 participated in transcription silencing in cercariae and, for the purposes of evolutionary conservation of the protein function, we suggest that SmHP1 had the same mechanism of action already studied in other organisms.

Immunoprecipitation and mass spectrometry data from a previous study¹⁶ showed that SmHP1 immunoprecipitates with the PRMT1 protein, an arginine methyltransferase. This protein is highly homologous to the vertebrate PRMT1 enzyme and is responsible for methylating histone H4 and plays a role in nuclear receptor-mediated chromatin remodeling³⁵. These results, together with the fact that no methyltransferases responsible for H3K9 methylation were found co-immunoprecipitated with SmHP1, corroborate the narrative that SmHP1 plays different functions in *S. mansoni* based on the methylation of histones other than H3.

In addition, we performed a script that showed a gene list that matched with the SmHP1 in cercariae and schistosomula. The gene list uncovered several genes for protein kinases which are probably regulated by HP1 in cercariae and sporocyst. Recently protein kinases functions were reviewed in Schistosomes³⁶. These proteins were involved in several homeostasis processes in the parasite. Moreover, transcriptome analysis was reviewed and uncovered several genes which were expressed in cercariae and in the whole life cycle³⁶. Taken together, our results and the transcriptomes analysis may suggest the importance of kinases for cercariae homeostasis and life cycle progression of *S. mansoni*.

Our *in vivo* findings indicate a an increase of oviposition. Even with a moderate decrease of *SmHP1* expression it was possible to observe an increase of oviposition. This goes in line with earlier results where alteration of oviposition was shown in *in vitro* adult worms' culture with siRNA against *SmCBX/SmHP1* (Smp_179650). In this work, it was also demonstrated that *SmCBX/SmHP1* interacts with putative methylated DNA binding protein *Smmdb2/3* colocalizing together in schistosome neoblasts and reproductive tissues, suggesting a role in the reproductive organs of the parasite¹⁷.

Our results are another step towards the better understanding of the role of *SmHP1* in chromatin structure, gene expression and parasite fitness. It shows that results from model organisms, albeit tremendously useful in many cases, cannot be simply extrapolated to any other organism. *SmHP1* seems to play according to rules that are yet to be discovered.

Materials And Methods

Extraction of sporocysts and cercariae

Sporocysts were obtained from *Biomphalaria glabrata* strain BgGUA, 6 months infected with *S.mansoni* strain SmDFO. Dissection was performed at room temperature. The snails were placed individually in large petri dishes and water was added. With the help of smaller glass plates, the snails were crushed, and fragments and tissues were removed. The sporocysts were transferred to new petri dishes and the membrane surrounding them was removed. Then, the hepatic pancreas was removed, and two samples were obtained for each snail. While care was taken to dissect sporocysts that were free of host tissue, contaminations with *Biomphalaria* tissues could not be avoided completely. Sporocyst samples were stored at -20°C

Cercariae strain *SmVEN* were obtained from snails *B. glabrata*. The snails were placed in pots of water and exposed to artificial light for 2 hours. Next, the Cercariae were counted and divided into aliquots with 1000 Cercariae each. They were then stored at -20°C.

Experiments were done according to the national ethics standards. The IHPE animal facility holds

agreement number F6613601 and has authorization APAFIS #39910-2022121915564694 v2 for the routine production and shared use of *S.mansoni* larvae.

ChIPmentation *S.mansoni* cercariae, sporocysts, *B.glabrata* and drosophila

ChIPmentation Kit for Histones (Diagenode, Cat. No. C01011009) was used. Approximately 1000 cercariae, 1 sporocyst and a corresponding amount of drosophila embryos were used for each ChIPmentation reaction. Samples were thawed, resuspended in 500 µL of HBSS, crushed for 1 minute with a sterile pestle, and then left at room temperature for 3 minutes. After this, 13.5 µL of 37% formaldehyde was added and gently homogenized for 10 minutes at room temperature. To stop the cross-link reaction, 57 µL of glycine from the Diagenode kit was added and left under agitation for 5 minutes. We then proceeded according to the suppliers manual. Chromatin was disrupted by sonication using the Bioruptor Pico with 5 cycles of 30s ON and 30s OFF. ChIPmentation was done on an IP-Star pipetting robot according to the pre-established protocol with the modification of washing time to 20 minutes. Antibody titration was done as described in¹⁶ with 0, 2, 4, 8 and 16µl antibody to obtain the saturating quantity and finally 8 µL of antibody HP1 AbCam (ab109028, lot GR38873387336-9) were used for each reaction. Input libraries were generated as described earlier and optimal number of library amplification cycles was determined by qPCR as described in the same protocol³⁷.

Primers and low molecular weight fragments were removed from the libraries with AMPure beads on the IP-Star and the quality and quantity of the sequencing libraries were checked with a BioAnalyzer High Sensitivity DNA Assay. Sequencing was done by the BioEnvironnement core facility on an Illumina NextSeq 550 as 75 bp single-end reads.

Bioinformatic Analyzes

Analyzes were carried out on a local Galaxy instance (<http://bioinfo.univ-perp.fr>). First, the quality of the sequences were checked by FastQC/MultiQC, adapter sequences were removed with Trim Galore! and reads were aligned to version 9 of the *S. mansoni* genome (*schistosoma_mansoni.PRJEA36577.WBPS17.genomic.fa*) with permission from <https://parasite.wormbase.org/>³⁸ using Bowtie2 evoking sensitive end-to-end. Uniquely aligned reads were retained using the Bowtie tag": "XS:". PCR duplicates were removed with SamTools RmDup. The number of aligned unique reads was downsampled to 4.7 Mio reads per library using Picard Tools (Suppl. table 2). Differential analysis was done with ChromstaR²¹ with bin size 1000 bp and step size 500 bp.

Annotations came from *schistosoma_mansoni.PRJEA36577.WBPS17.genes.gff3*. For metagene analysis, the gene feature was retained and 4898 genes of the forward strand were used.

Differential chromatin states were detected with ChromstaR and default parameters.

Peakcalling was performed also with MACS2 and default parameters, followed by MACS2 bdgbroadcall, with 2 and 5 as –cutoff value. The score in a bedGraph file, which the --cutoff applies to, represents the level of enrichment of the ChIP-seq signal at each genomic position relative to the background. Score 5 means pvalue 1e-5 which is calculated during the initial peak calling process. Scores between 2 and 5 are frequently used. A cutoff of 2 is a relatively lenient threshold a cutoff of 5 is more stringent, thus reducing the number of peaks but increasing their reliability. Differential HP1 enrichment was detected by DiffBind.³ IGV was used for visual inspection.

The same procedures were applied to *D.melanogaster* data based on *dmel_r6.06_FB2015_03_gtf_dmel-all-r6.06.gff3* and the corresponding genome fasta file. Details in Suppl. table 2.

For analysis of *B.glabrata*, the by-catch of the sporocyst ChIPmentation experiments was used, *i.e.* biomphalaria chromatin that had accidentally been immunoprecipitated as contaminants of the sporocyst

preparation and that represented roughly 25% of the total reads. Alignment was done against all contigs ≥ 5 kb of VectorBase Biomphalaria-glabrata-BB02_SCAFFOLDS_BglaB1.fa, and gene annotations were used from Biomphalaria-glabrata-BB02_BASEFEATURES_BglaB1.6.gff3. The list of genes was generated with the script on the supplementary file 3.

***In vivo* studies**

In vivo study was carried out with 15 female Balb mice divided into 3 groups. Ethical statement at the University of Campinas was under number CEUA protocol (Comissão de Ética no Uso de Animais # 6064-1/2022). Each group contained 5 animals and each animal was infected with 100 3-day-old schistosomula (Groups 1-3). The quantity of schistosomula and cercariae per animal was determined according to the study by Vilar and collaborators⁴. The infection of the groups was subcutaneous, however, in groups 1 and 2, represented by SmHP1 and dsmCherry, respectively, the schistosomula were previously incubated in culture for 3 days with dsRNA. The third group was also kept in culture for 3 days, however, in the absence of dsRNA. For each group, 100 schistosomula were inoculated per animal. Schistosomula was conducted as previously described⁴. Briefly, BH (Belo Horizonte) lineage of the *S. mansoni* was used and schistosomula were transformed by tail break and several RPMI washes for tail removal. After 3 washes, 200 schistosomula were counted and distributed equally in culture plates with 2ml of Medium 169 (Atená, Biotechnology, Campinas, Brazil), described by⁴, supplemented with hormones and fetal bovine serum, 30 μ g of dsRNA and kept in CO₂ atmosphere at 37°C for 3 days.

All dsRNAs were done using the T7RiboMAX Express RNAi System (Promega, Belo Horizonte, Brazil). Specifically, the oligonucleotides for amplification HP1 gene (T7 + Forward TAATACGACTCACTATAGGGTGTGGAAGAG TCAGCTGGT and T7 + reverse TAATACGACTCACTATAGGGAGGGTTCGATT TTCAGGTGTG), containing T7 promotor for

dsRNA synthesis. Gene expressions were evaluated in the QuantStudio qPCR System (Thermo), using the HP1 primers (Forward primer (CGTCACTCAGTTCAGACAGC) e reverse primer (CTCTTCCACACTCACGGGTA)) and endogenous SmEIF4E (Smp_001500) as described previously⁴³, and using the 3 days-schistosomula condition as gene expression calibrator⁴⁴. Infected animals with wild type and dsRNA were weighted and Kato Katz²⁰ were done one week before perfusion. Perfusion was done as described⁴⁵. For histology, liver tissues were fixed in 10% formaldehyde and fixed in paraffin block and tissue slices were cut and colored by Masson's trichrome. All granulomas present in 10 random fields of the histological section of each animal were quantified. The images were captured using a photomicroscope using Leica® LAS EZ4 HD software. The total area of the granuloma was measured using ImageJ 1.53t software.

End Matter

Author Contributions and Notes

F.J.C and C.G designed research, C.G. N.S.T. M.V.B. A.R. and T.M.F.M. performed research, C.G and MSG analyzed ChIP-seq data, N.S.T. and M.B.V. analyzed *in vivo* results; and F.J.C., C.G and NST wrote the paper.

The authors declare no conflict of interest.

Data availability

Sequencing raw data are available at NCBI SRA under BioProject accession number PRJNA1090249.

Acknowledgments

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Supplementary table 1: previous use of antiHP1 antibody Abcam ab109028

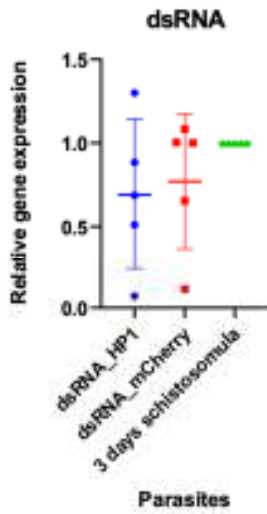
Designation of target	Reference	Technique	Cat# (Abcam)
HP1 α	https://doi.org/10.1101/2024.02.07.579285	Cell Tension	ab109028
CBX5	https://doi.org/10.1021/acsomega.1c05381	Chemiprecipitation	ab109028
HP1 α	https://doi.org/10.1101/2023.11.09.566494	ChIP-seq	ab109028
HP1 α	https://doi.org/10.17615/p7cp-z032	Co-Immunoprecipitation	ab109028
HP1 α	https://doi.org/10.1038/s41556-022-01008-5	eCLIP-seq	ab109028
HP1 α	https://doi.org/10.1016/j.ccell.2019.08.006	Immunoblots	ab109028
HP1 α	https://doi.org/10.7554/eLife.63972	Immunoblotting and immunostaining	ab109028
HP1 α	https://doi.org/10.15252/embr.202051041	immunoblotting, immunofluorescence, cytometry	ab109028
HP1 α	https://doi.org/10.1080/15384101.2018.1471313	Immunocytochemistry	ab109028
HP1 α	https://doi.org/10.1016/j.theriogenology.2024.01.003	Immunofluorescence	ab109028
HP1	https://doi.org/10.1016/j.bpj.2020.04.001	Immunofluorescence	ab109028
HP1 α	https://doi.org/10.1038/s41467-023-38118-z	Immunofluorescence	ab109028
HP1 α	https://doi.org/10.1186/s13059-021-02455-3	Immunofluorescence	ab109028
HP1 α	DOI: 10.1126/sciadv.abq4831	Immunofluorescence	ab109028
HP1 α	https://doi.org/10.1016/j.dnarep.2023.103592	Immunofluorescence	ab109028
HP1 α	https://doi.org/10.1111/cpr.13566	Immunofluorescence	ab109028
HP1 α	https://doi.org/10.1091/mbc.E18-04-0256	Immunofluorescence	ab109028
HP1 α	https://doi.org/10.1101/2023.07.17.549271	Immunofluorescence	ab109028
HP1 α	https://doi.org/10.1016/j.celrep.2023.112329	Immunofluorescence	ab109028
HP1 α	https://doi.org/10.1016/j.cell.2022.12.013	Immunofluorescence	ab109028
HP1 α	https://doi.org/10.1101/2023.12.21.572889	Immunofluorescence	ab109028

HP1α	https://doi.org/10.1096/fj.201700753R	Immunofluorescence Western Blotting	and	ab109028
HP1α	https://doi.org/10.1016/j.stemcr.2017.12.016	Immunofluorescence Western Blotting	and	ab109028
HP1α	https://doi.org/10.3390/cancers12102833	Immunohistochemistry		ab109028
HP1α	https://doi.org/10.1101/2023.12.03.569804	Immunoprecipitation Western blotting	and	ab109028
CBX5	https://doi.org/10.1186/s12935-020-1157-7	Western Blotting		ab109028
HP1	https://doi.org/10.1007/s43032-021-00500-x	Western Blotting		ab109028
HP1	https://doi.org/10.1093/nar/gkab249	Western Blotting		ab109028
CBX5	https://doi.org/10.1186/s12885-023-11108-6	Western Blotting		ab109028
HP1	10.18632/genesandcancer.84	Western Blotting		ab109028
HP1α	https://doi.org/10.18632/genesandcancer.84	Western Blotting		ab109028
HP1α	https://doi.org/10.1016/j.celrep.2021.10.9694	Western Blotting immunoprecipitation	and	ab109028

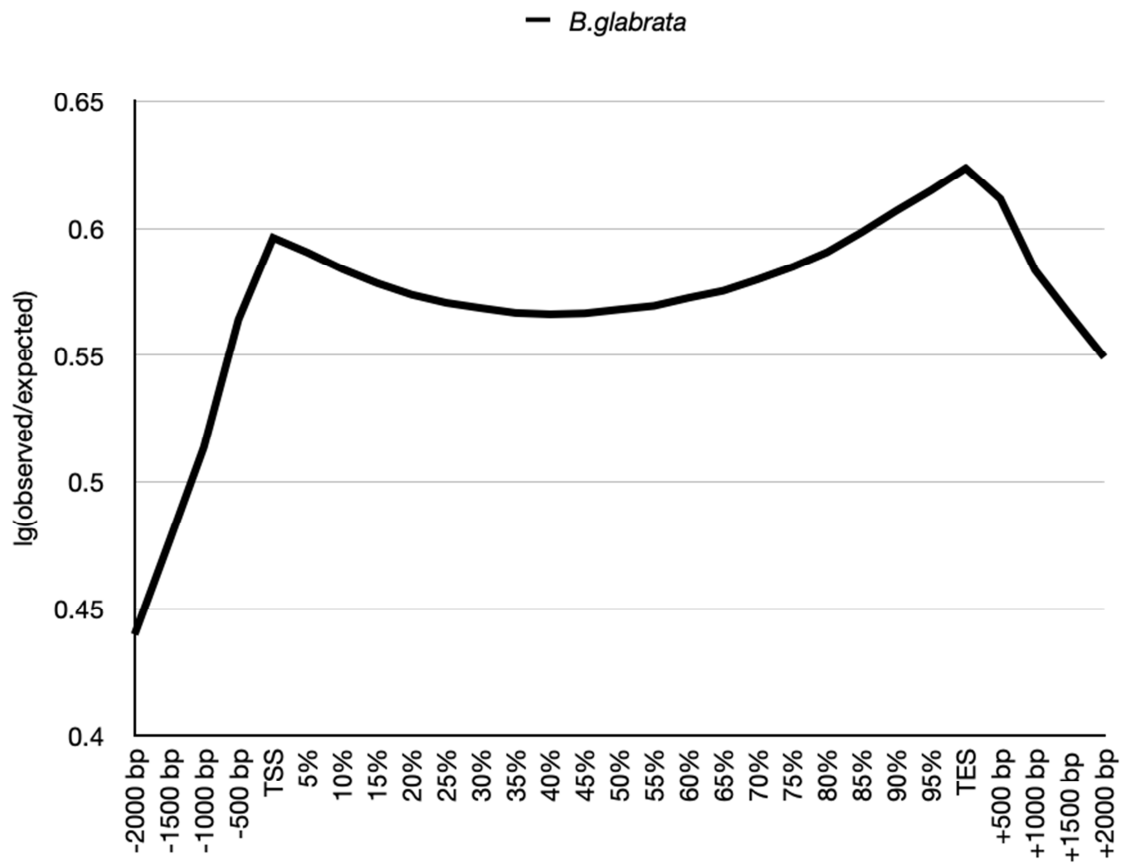
Supplementary table 2: Detailed sequencing and alignment data

Sample	Supplier Name	file name	reads R1	adapter trimmi ng	Aligne- ment rate	after pick unique and RmDup	in %	Down- sample to
Ce 1	cercaria ChIP HP1 - replica 1	Ce1_S25_L001_R1_001 _fastq_gz	27,584,702	100%	85.75%	8,539,009	30.9 6%	4,700,000
Ce 2	cercaria ChIP HP1 - replica 2	Ce2_S26_L001_R1_001 _fastq_gz	31,243,784	100%	90.78%	10,362,799	33.1 7%	4,700,000
Ce 3	cercaria ChIP HP1 - replica 3	Ce3_S27_L001_R1_001 _fastq_gz	30,792,782	100%	81.45%	8,857,714	28.7 7%	4,700,000
Ce-In	input cercariae undiluted Tn5	InNa_S31_L001_R1_00 1_fastq_gz	14,441,656	100%	96.04%	9,316,089	64.5 1%	4,700,000
Sp 1	sporocyst ChIP HP1 - replica 1	Sp1_S28_L001_R1_001 _fastq_gz	34,896,078	100%	59.46%	7,340,487	21.0 4%	4,700,000
Sp 2	sporocyst ChIP HP1 - replica 2	Sp2_S29_L001_R1_001 _fastq_gz	35,630,630	100%	61.87%	7,967,706	22.3 6%	4,700,000
Sp 3	sporocyst ChIP HP1 - replica 3	Sp3_S30_L001_R1_001 _fastq_gz	29,439,162	100%	44.01%	4,719,547	16.0 3%	4,700,000
Sp-In	input tagmentation library sporocysts SmDFO in BgGUA	Sp- In_S7_L001_R1_001_fa stq_gz	188,264,06 4	100%	57.44%	16,001,570	8.50 %	4,700,000
Dm2	D.melanogaster embryos HP1 ChIPmentation rep 2	Dm2_S9_L001_R1_001 _fastq_gz	157,680,68 8	100%	83.54%	18,515,265	11.7 4%	16,000,00 0

Dm3	D.melanogaster embryos HP1 ChIPmentation rep 3	Dm3_S10_L001_R1_001_fastq.gz	185,909,252	100%	87.05%	22,123,566	11.90%	16,000,000
Dm-In	D.melanogaster embryos HP1 Input	Dm-In_S11_L001_R1_001_fastq.gz	173,301,784	100%	95.84%	20,385,745	11.76%	16,000,000
Dm-adult1	D.melanogaster adults HP1 ChIP rep 1 from SRA	SRR7817540.fastq.gz	27,849,221	100%	91.26%	12,676,124	45.52%	10,000,000
Dm-adult2	D.melanogaster adults HP1 ChIP rep 2 from SRA	SRR7817541.fastq.gz	30,240,617	100%	91.42%	15,525,830	51.34%	10,000,000
Dm-adult3	D.melanogaster adults HP1 ChIP rep 3 from SRA	SRR7817542.fastq.gz	37,549,684	100%	89.03%	10,173,605	27.09%	10,000,000
Dm-input	D.melanogaster adults HP1 Input from SRA	SRR7817573.fastq.gz	23,583,211	100%	95.44%	17,566,290	74.49%	10,000,000
Dm-embryo1	D.melanogaster embryo HP1 ChIP rep 1 from SRA	SRR11952662.fastq.gz	19,894,682	100%	94.63%	12,376,186	62.21%	10,000,000
Dm-embryo2	D.melanogaster embryo HP1 ChIP rep 2 from SRA	SRR11952664.fastq.gz	46,050,252	100%	91.61%	25,623,510	55.64%	10,000,000
Dm-embryo-In	D.melanogaster embryo HP1 Input from SRA	SRR11952661.fastq.gz	27,346,764	100%	95.32%	19,767,614	72.29%	10,000,000
Sp 1	sporocyst ChIP HP1 - replica 1	Sp1_S28_L001_R1_001_fastq.gz	34,896,078	100%	24.34% on B.glabrata	5,387,400	15.44%	4,600,000
Sp 2	sporocyst ChIP HP1 - replica 2	Sp2_S29_L001_R1_001_fastq.gz	35,630,630	100%	20.29% on B.glabrata	4,600,990	12.91%	4,600,000
Sp 3	sporocyst ChIP HP1 - replica 3	Sp3_S30_L001_R1_001_fastq.gz	29,439,162	100%	31.97% on B.glabrata	6,069,096	20.62%	4,600,000
Sp-In	input tagmentation library sporocysts SmDFO in BgGUA	Sp-In_S7_L001_R1_001_fastq.gz	188,264,064	100%	31.93% on B.glabrata	39,540,484	21.00%	4,600,000



Supplementary Figure 1. Relative gene expression of dsRNA schistosomula cultures compared to expression of wild type schistosomula. The gene expression was calculated by delta CT method³⁴, using wild type schistosomula as gene expression calibrator and the endogenous gene was SmEIF4E (Smp_001500) as described previously³³



Supplementary Figure 5: Metagenes profiles of *B.glabrata* adults, X-axis : relative position around genes, Y-axis log(observed/expected) of three replicates

Supplementary file 3 – script

```
#!/bin/bash

# Step 1: Filter genes longer than 200 nucleotides and extract relevant columns
#awk '{if ($3-$2>200){print $0}}' MACS2_bdgbroadcastcall_on_combined_Cer-D-U_cut-off_5.bed | cut -f1,2,3 | sort -k1,1 -k2,2n >
genes_sorted_fran.bed
awk '{if ($3-$2>200){print $0}}' MACS2_bdgbroadcastcall_on_combined_Sp-D-U_cut-off_5.bed | cut -f1,2,3 | sort -k1,1 -k2,2n >
genes_sorted_fran.bed
echo "Filtered peaks longer than 200 nucleotides and saved to genes_sorted_fran.bed"

# Step 2: Select gene-related lines from the annotation file and extract relevant columns
awk '{if ($3 == "gene"){print $0}}' schistosoma_mansoni.PRJEA36577.WBPS17.annotations.gff3 | cut -f1,4,5,9 | sort -k1,1 -
k2,2n > genes_all.bed
echo "Selected gene-related lines and saved to genes_all.bed"
echo "Linhas relacionadas aos genes selecionadas e salvas em genes_all.bed"

# Step 3: Perform intersection to obtain regions that overlap between both files
multiIntersectBed -i genes_sorted_fran.bed genes_all.bed -header -names A B | awk '{if ($5 == "A,B"){print $0}}' | cut -f1,2,3 >
resultnew.bed
echo "Performed intersection and saved the overlapping regions to resultnew.bed"
echo "Interseção realizada e regiões sobrepostas salvas em resultnew.bed"

# Step 4: Add gene annotation related to each region
bedtools intersect -a genes_all.bed -b resultnew.bed -wb | cut -f1,2,3,4 > FINAL.bed
echo "Added gene annotation and saved to FINAL.bed"
echo "Anotação gênica adicionada e salva em FINAL.bed"
```