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HP1 modulates the transcription of cell-cycle regulators in *Drosophila melanogaster*

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ABSTRACT

Heterochromatin protein 1 (HP1) was originally described as a non-histone chromosomal protein and is required for transcriptional gene silencing and the formation of heterochromatin. Although it is localized primarily at pericentric heterochromatin, a scattered distribution over a large number of euchromatic loci is also evident. Here, we provide evidence that *Drosophila* HP1 is essential for the maintenance of active transcription of euchromatic genes functionally involved in cell-cycle progression, including those required for DNA replication and mitosis. Depletion of HP1 in proliferating embryonic cells caused aberrant progression of the cell cycle at S phase and G2/M phase, linked to aberrant chromosome segregation, cytokinesis, and an increase in apoptosis. The chromosomal distribution of Aurora B, and the level of phosphorylation of histone H3 serine 10 were also altered in the absence of HP1. Using chromatin immunoprecipitation analysis, we further demonstrate that the promoters of a number of cell-cycle regulator genes are bound to HP1, supporting a direct role for HP1 in their active transcription. Overall, our data suggest that HP1 is essential for the maintenance of cell-cycle progression and the transcription of cell-cycle regulatory genes. The results also support the view that HP1 is a positive regulator of transcription in euchromatin.

INTRODUCTION

Chromatin in higher eukaryotes is subdivided into different functional compartments termed heterochromatin and euchromatin (1). Heterochromatin differs from euchromatin in its DNA composition, replication timing, condensation throughout the cell cycle, and its ability to silence euchromatic genes placed adjacent to or within its territory, often described as position-effect-variegation (PEV) (2).

Heterochromatin protein 1 (HP1) was the first protein identified in *Drosophila melanogaster* as a heterochromatin-associated protein (3); the corresponding gene has been cloned from a number of organisms and is highly conserved from yeast to human (4). Polytene chromosome staining showed that, in *Drosophila*, HP1 is distributed mainly in pericentric heterochromatin, telomeric heterochromatin, the banded small fourth chromosome (5–8), as well as ∼200 individual loci scattered throughout the euchromatic chromosomal arms (5). The gene encoding HP1 in *D.melanogaster*, *Su(var)2-5*, was isolated as a suppressor of PEV (9–11). The protein contains a highly conserved motif, the chromo (chromatin organization modifier) domain, similar to Polycomb (Pc), a repressor of homeotic genes (12). The association between HP1 and pericentric heterochromatin is believed to occur via the chromo domain of HP1 and the N-terminal tail of histone H3 methylated at lysine 9 (13,14), generated by histone methyltransferase-Su(var)3-9, a partner of HP1 in pericentric heterochromatin (15). The C-terminal chromo 'shadow' domain of HP1 interacts with other silencing complexes to suppress local transcriptional activity (15–18). However, studies of HP1 chromosomal distribution also showed that HP1 does not always co-localize with lysine 9 methylated histone H3 or Su(var)3-9, especially in euchromatic regions (19–21); in some cases, HP1 is found directly bound to DNA (22,23). All these features argue for distinct roles for HP1 in chromatin and in epigenetic gene regulation.

HP1 is believed to be an essential structural protein protecting the integrity of chromosomes during cell division (8,24). *Swi6*, the homolog of HP1 in fission yeast, is dispensable for survival, but its deletion results in lagging chromosomes during anaphase, and a high rate of chromosome loss (25,26). Mutations of HP1 in *D.melanogaster* result in late larval lethality, chromosome breakages/loss, telomere fusion and a high frequency of cells with abnormal anaphase (8,27). Null alleles of the HP1 functional partner in mice (*SUVAR39*) also showed various chromosomal defects (28), supporting a conserved role for heterochromatin proteins in the regulation of chromosome

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dynamics during cell-cycle progression. However, the mechanism(s) involved remains to be understood.

In this study, we utilized *Drosophila* embryonic Kc cells and an RNA interference (RNAi)-based approach to demonstrate that HP1 plays an important role at S phase and G2/M phases during the cell cycle. We further show that nearly one-third of known/predicted cell-cycle regulators require HP1 to maintain their active transcription. These genes include *MCMs*, *Orc4*, *CDC45L*, *INCENP*, *Aurora B*, *CAF1*, *Bub1*, *Bub3* and a few other cell-cycle regulators. ChIP analysis suggests that HP1 plays a direct role in their transcription. Therefore, the results of this study provide an alternative explanation for the specific role of HP1 in the regulation of chromatin dynamics and in cell-cycle progression.

MATERIALS AND METHODS

RNAi in Kc cells

Drosophila Kc cells were routinely cultured at 25°C in Schneider *Drosophila* medium (GIBCO) supplemented with 10% fetal calf serum, 160 μg/ml penicillin, 250 μg/ml streptomycin, and 4 mM L-glutamine. Double-stranded RNA (dsRNA) of HP1 was generated by incubation of single-stranded RNA in annealing buffer (100 mM potassium acetate, 30 mM HEPES-KOH, pH 7.4, 2 mM magnesium acetate) for 3 min at 95°C and then placed in a beaker with water at 75°C and allowed to cool slowly to room temperature. The detailed procedure of RNAi was carried out according to the established protocols (http://dixonlab.biochem.med.umich.edu). Briefly, Kc cells were seeded in a six-well dish using serum-free medium at 1×10^6 cells/ml. HP1 dsRNA (5 μg/ml) was added to the cultured Kc cells. After 60 min at room temperature, 2 ml of medium containing 10% serum was added to each well and the plates transferred to 25°C for up to 8 days. Western blotting and RT–PCR were carried out using the extract/total RNA isolated from control and dsRNA-treated cells on days 2, 6 and 8.

Cell-cycle and apoptosis analysis

The procedure for flow cytometric analysis of Kc cells followed that in the manual provided with the BrdU flow kit (BD PharMingen). The cells were fed with BrdU for 4 h, then scraped and collected. Fluorescence was measured using a FACSCalibur (Becton Dickinson). Data collection and analysis were performed using CellQuest software.

Electrophoresis and immunoblotting

Cell extracts (15 μg) were fractionated by 10% SDS–PAGE, then transferred to Hybond-P PVDF membranes (Amersham) and probed with primary antibodies (CIA9), and secondary antibodies (anti-rabbit or anti-mouse horseradish peroxidase-conjugated IgG), obtained from Jackson Immunoresearch Laboratories. Enhanced chemiluminescence reagents (Amersham Pharmacia Biotech) were used for signal detection.

For the analysis of H3 ser10 phosphorylation, we used whole-cell extracts from 700 000 Kc cells (control and RNAi at day 8). Western blotting was performed using polyclonal antibodies against ser10-phosphorylated histone H3 at a dilution of 1:1000 (Upstate). Kc control cells arrested in mitosis by incubation in 25 μM colchicine (Sigma) for 24 h were also analyzed for comparison.

Immunofluorescence

Kc cells were seeded onto polylysine slides, fixed with 4% formaldehyde for 15 min and permeabilized with 0.5% Triton X-100 for 5 min. The incubation with primary antibodies was carried out in blocking solution for 1 h.

For staining of mitotic cells, the cells were permeabilized using PBST (PBS containing 0.3 % Triton X-100) and stained with polyclonal antibody against *Drosophila* Aurora B at 1:200 dilution and monoclonal mouse at anti-β-tubulin 1:300 dilution (Chemicon International) as primary antibodies. Secondary antibodies were anti-rabbit coupled with Alexa 488 (1:500) and anti-mouse coupled to Alexa 546 (1:500) (Molecular Probes, Eugene, Oregon). Images were acquired using a confocal LSM510 META microscope (Zeiss). Stacks of images were analyzed using the IMARIS 4.0 program (Media cybernetics, Carlsbad, CA).

Antibodies

Affinity-purified polyclonal antibodies of HP1 (rabbit #192 and #187, 5 μg) and 5 μg of polyclonal anti-HA antibodies (Sigma) were used in each ChIP reaction. The specificity of the HP1 polyclonal antibodies was determined using various approaches, including western blotting assay, immunofluorescence staining and immunoprecipitation to pull down HP1 (data not shown). The monoclonal antibody HP1–CIA9 (5) was used at a dilution of 1:20 in immunoblotting assays.

Microarray analysis and RT–PCR

Total RNA was isolated from control and HP1-depleted Kc cells at day 8 using an RNeasy kit (Qiagen). RNA labeling and microarray data analysis followed the standard protocol from Affymetrix. We used ANOVA ($P < 0.001$) to assess the expression confidence for each gene.

For RT–PCR analysis, poly(A)$^+$ mRNA was purified with the Oligotex Direct mRNA kit (Qiagen) according to the manufacturer's instructions. The purified poly(A)$^+$ RNA was reverse transcribed using the Thermoscript kit (Invitrogen). The cDNA was then used for PCR amplification for 35 cycles with gene-specific primers. PCR products were scanned after electrophoretic separation with a Typhoon Scanner, quantified using ImageQuant software (Amersham Biosciences) and normalized for amplification of the *Actin5c* transcript. The sequence of primers used for RT–PCR and ChIP analysis are provided in the Supplementary Material.

ChIP

ChIP was performed according to Orlando *et al*. (29) and the protocol provided by Upstate (www.upstate.com) with some modifications. In brief, $1–2 \times 10^8$ Kc cells were prepared and fixed in 1% formaldehyde. Nuclei were isolated according to a standard procedure in Current Protocols (http://www3.interscience.wiley.com), then resuspended in 1.7 ml of lysis buffer (50 mM Tris, pH 8.0, 10 mM EDTA, 1% SDS and protease inhibitors) and sonicated using a Branson sonifier 250. Chromatin fractions in the size range 0.2–0.8 kb were used to perform immunoprecipitation experiments. We used

5 μg affinity-purified polyclonal antibodies (#192 and #182 for HP1; HA antibody for control) and 1 ml of salmon sperm DNA/protein-A-agarose (Upstate) pre-cleared chromatin lysate in each reaction. The mixture was then rotated at 4°C overnight and the recovered beads were washed twice with 1 ml of Low salt buffer (Upstate), once with High salt buffer (Upstate), once with LiCl buffer (Upstate) and twice with TE at 4°C for 8 min. ChIP DNA was extracted according to the standard procedures (29).

RESULTS

Depletion of HP1 in *Drosophila* Kc cells

Various chromosomal defects in the cell cycle have been observed in embryos or larval tissues of *Drosophila* HP1 mutants (8,27). However, the presence of maternally loaded HP1 in embryos and the lethality of HP1 mutants at late larval stages have so far precluded a systematic study of the role of HP1 in cell-cycle regulation. Therefore, we used *Drosophila* Kc cells, a cell line derived from *Drosophila* embryos, as a model system to address this problem. HP1 transcripts were depleted using an RNAi-based approach (see Materials and Methods). The reduction in HP1 expression was measured both by RT–PCR and by western blotting analysis (Figure 1A). A significant reduction in the HP1 expression was already evident after 2 days treatment with HP1 dsRNA. Cells at day 8 showed a reduction in HP1 of ~90% (Figure 1A) and were therefore used in all subsequent experiments.

Cell-cycle progression at S and G2/M phase is altered in the absence of HP1

The impact of HP1 loss on the cell cycle of Kc cells was determined using cell-cycle profile analysis of HP1-depleted and control cells. The percentage of cells in S phase was determined by BrdU incorporation, and total DNA content by 7-amino-actinomycin (7-AAD). The results showed that the depletion of HP1 (day 8) caused a decrease in S-phase cells of at least 4-fold, and a 2-fold decrease in G2/M-phase cells (Figure 1B), although no significant effect was found at the G1 phase. In addition, depletion of HP1 caused a greater than 7-fold increase in the number of apoptotic cells. These results, therefore, confirm that HP1 is an important regulator during the cell cycle, especially at the S and G2/M phases.

Cell-cycle regulators require HP1 to maintain their active transcription

To ask whether the cell-cycle defects were due to changes in the transcription of genes functionally involved in S phase and the G2/M phase, we next assessed global changes in gene transcription following depletion of HP1. Expression profile analysis was performed using total RNA isolated from both HP1-depleted Kc cells and control Kc cells, and an Affymetrix *Drosophila* chip. For each experiment, we used total RNA isolated from two independent HP1-depleted and control samples, and at least two independent experiments were performed.

The microarray analysis showed that loss of HP1 function in Kc cells resulted in alterations in transcription of >500 genes: ~400 genes were down-regulated and ~120 genes were up-regulated (>1.5-fold, ANOVA). The function of these genes ranged from cellular enzymes, signal transduction molecules, and membrane and cell structural proteins, to nucleic acid-binding proteins and cell-cycle regulators (Figure 2A). At the chromosomal level, the genes targeted by HP1 appeared to be distributed along all euchromatic chromosomal arms (data not shown), supporting a global role of HP1 in euchromatic gene regulation (20).

Among 60 known/predicted genes associated with DNA replication function, 15 were down-regulated in the absence of HP1 (Figure 2B). These included *McM2*, *McM5*, *McM6* and *CDC45L*, which are required for processive DNA replication and correct chromosome condensation (30–32). Other genes involved in DNA replication, such as components of the

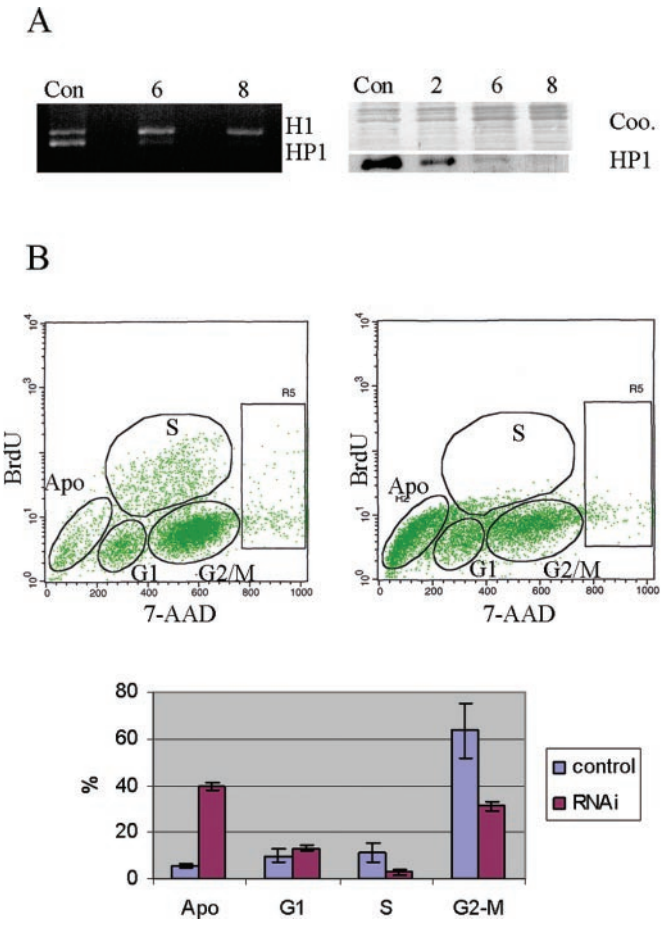

Figure 1. Depletion of HP1 alters cell-cycle progression. (**A**) Expression of HP1 after treatment with dsRNA in Kc cells. Left panel: changes in *HP1* expression after RNAi monitored by RT–PCR analysis of RNA extracted from control (Con) and RNAi-treated cells (at 6 and 8 days). Histone *H1* was used as a positive control. Right panel: western blotting with anti-HP1 antibodies (C1A9) of extracts from control (Con) and RNAi-treated cells (at 2, 6 and 8 days). Equal loading of cell extracts (15 μg of protein extract in each lane) was monitored with Coomassie blue (Coo.) staining. (**B**) Ablation of HP1 in Kc cells results in loss of cells in S and G2/M phase. Control Kc cells (left panel) and HP1-depleted Kc cells (right panel) were labeled with BrdU and 7-AAD. The fractions of cells in apoptosis (Apo), G1 phase (G1), S phase (S) and G2/M phase (G2/M) are all indicated. R5 represents over-replicated cells. Approximately 25 000 gated cell events were measured in each experiment. The comparison of cell numbers ($n = 2$) at different stages of the cell cycle in controls and cells after HP1 depletion is shown on the bottom panel of the figure. %, percentage of cells.

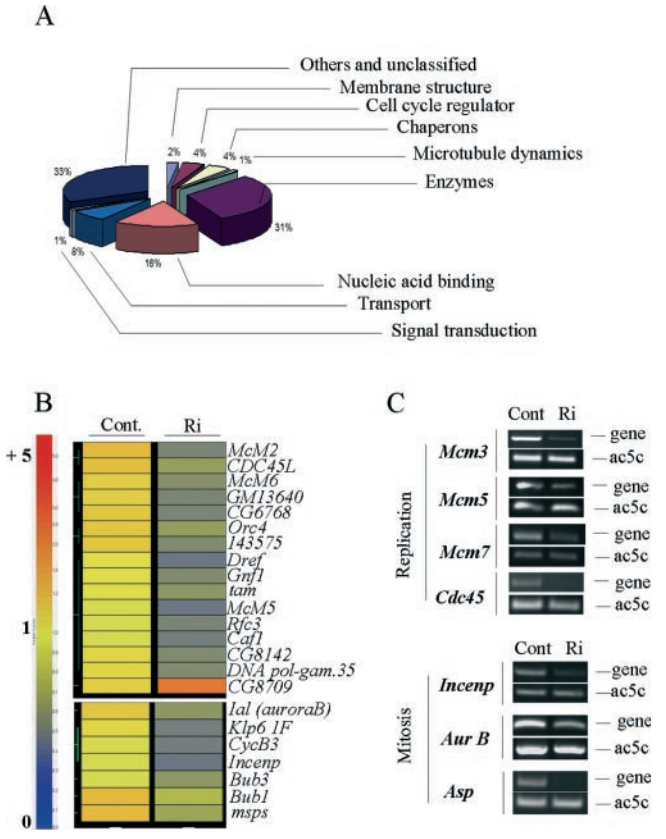

Figure 2. (**A**) Functional clusters of HP1-regulated genes in *Drosophila*. The molecular functions and the percentage of the total for each group are indicated. (**B**) Changes in the expression of genes essential for DNA replication (upper panel) and mitosis (lower panel) after RNAi treatment (Ri) compared with control cells (Cont.). The names of the genes are indicated. Expression levels are indicated by a color scale, with light blue/gray, indicating low expression and yellow/red higher expression (0- to 5-folds). (**C**) RT–PCR confirmation of the change in the expression of selected genes after HP1 ablation in Kc cells. Expression of the genes in controls (Cont.) and after RNAi (Ri) is shown. The expression level of *Actin 5c* (ac5c), used as an internal control, is also indicated.

origin recognition complex (Orc)—*Orc4*, *Caf1*, *Gnf1*, *Dref1*, *DNA polymerase-γ* and *Tam*—were also down-regulated (Figure 2B). Aurora B and inner centromere protein (INCENP), known to be required for kinetochore assembly, chromosome condensation and bipolar chromosome attachment during mitosis (33), also showed a reduction in transcription. A similar loss of transcription was observed in *Bub1* and *Bub3* (Figure 2B), encoding mitotic checkpoint control proteins (34,35). Loss function of Bub1 has been shown to cause chromatin bridges to extend between the two separating groups of chromosomes, and extensive chromosome fragmentation in anaphase cells (35).

We confirmed the changes in the transcription of cell-cycle regulators using semi-quantitative RT–PCR, which gave results consistent with the microarray analysis. In addition, cell-cycle regulator genes, such as *McM3*, *McM7* and *Asp* (abnormal spindle), were also confirmed to be down-regulated (Figure 2C). Collectively, these results demonstrate that HP1 is indeed involved in the regulation of transcription of cell-cycle regulators.

HP1 is required for Aurora B distribution and histone H3 phosphorylation

INCENP is localized to the centromeric region of chromosomes at metaphase and the spindle midzone at anaphase, which then targets Aurora B, a kinase essential for histone H3 ser10 phosphorylation, to these sites (36). Loss of function of both these 'chromosomal passenger' proteins causes abnormal chromosomal segregation at metaphase, as well as certain cytokinesis defects (36,37). The loss of transcription of both *INCENP* and *Aurora B* after depletion of HP1, therefore, raised the possibility that localization of Aurora B (Figure 3 and data not shown) may be altered. Staining of HP1-depleted Kc cells with anti-Aurora B antibodies indeed revealed an altered localization of Aurora B and, in a number of cases, a complete loss of Aurora B (Figure 3A). Consistent with the loss function of Aurora B, the spindles in the metaphase cells were also disorganized, with a large number of cells showing an altered prometaphase chromosome alignment (Figure 3A). Some showed extensive chromosome fragmentation (Figure 3B), or the presence of a third spindle pole-like structure as indicated by beta-tubulin (Figure 3A). At telophase, we observed defective separating cells with an extra cell envelope-like structure without nuclei (Figure 3A). Chromatin bridges or lagging chromatids at telophase were also evident in some cells (Figure 3C); however, in some cases, localization of Aurora B appeared not to be affected, arguing that other pathways are possibly involved.

We next analyzed changes in histone H3 serine 10 phosphorylation, since the loss of transcription of *INCENP* is known to affect localization of Aurora B (33), which is essential in the regulation of histone H3 phosphorylation (36). Total cell extracts from HP1-depleted Kc cells were analyzed by western blotting (Figure 3D). The results indeed showed a severalfold reduction in H3 ser10 phosphorylation after depletion of HP1, consistent with the functional disruption of INCENP and Aurora B in the absence of HP1.

HP1 directly targets genes encoding cell-cycle regulators in euchromatin

To test whether the loss of transcription of genes involved in DNA replication and mitosis was a direct effect of the loss of HP1, we performed a ChIP analysis to determine whether HP1 is physically associated with these genes. Chromatin lysates from formaldehyde-fixed Kc cells were sonicated into small chromatin fragments (0.2–0.8 kb) and immunoprecipitated with polyclonal antibodies against *Drosophila* HP1. As a control, we used a mock precipitation (beads only) and polyclonal antibodies against HA. Our ChIP results showed that known transposable elements distributed in heterochromatin, such as *F-element*, *TART* and *1360* (7,38), were all enriched in HP1 binding (Figure 4), which is also consistent with a previous study (20).

Using the same ChIP DNA material, we then attempted to determine whether HP1 was enriched in genes involved in DNA replication. Primers were designed to cover the promoter regions of selected genes. The results showed that *McM3*, *McM5* and *Tam* were all enriched in HP1 binding (Figure 4). However, *McM7* appeared to be HP1-negative, although its

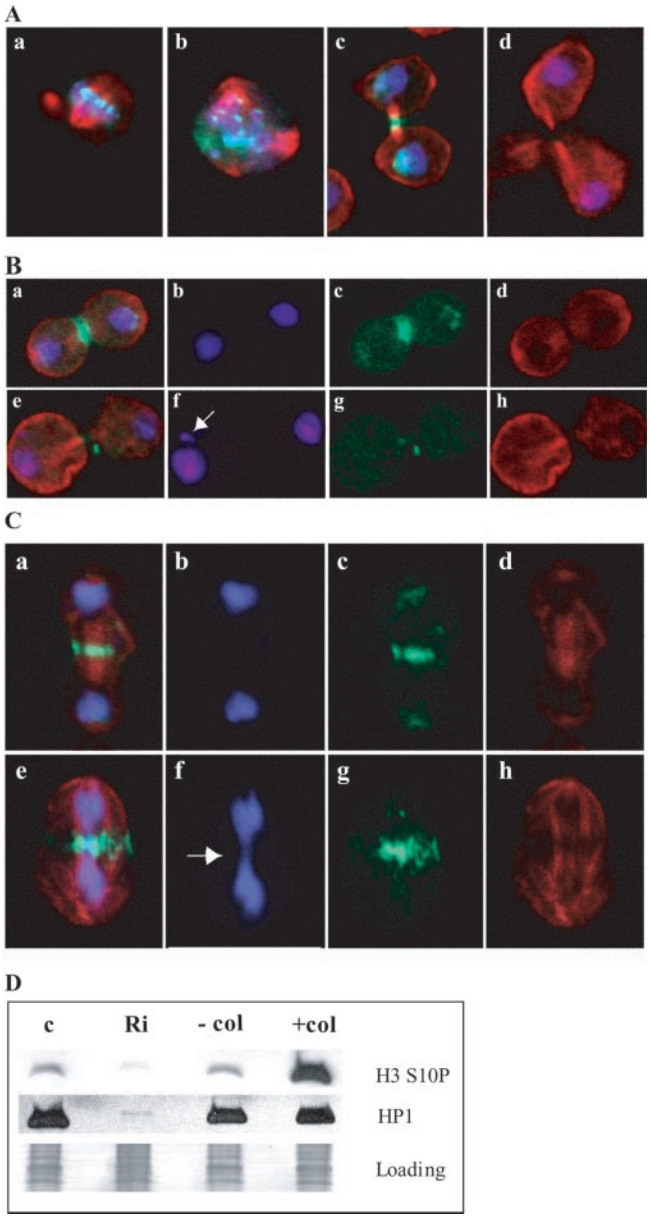

Figure 3. (**A**) Ablation of HP1 causes defects at metaphase (*a*, *b*) and telophase (*c*, *d*). Control cells at metaphase and telophase are shown in *a* and *c*; *b* and *d* show cells after HP1–RNAi. Microtubules are indicated by anti-beta-tubulin (red), anti-Aurora B (green), and DNA is indicated by DAPI (blue). (**B**) Abnormal telophase in HP1-depleted cells. Control cells are shown in *a–d*. HP1-depeleted cells are shown in *e–h*. The white arrow in *f* indicates chromosome loss/chromatin breakage. (**C**) Defective anaphase in HP1-depleted cells. Control cells are shown in *a–d*. HP1-depleted cells are shown in *e–h*. The white arrow in *f* indicates a chromatin bridge at anaphase. (**D**) Depletion of HP1 causes loss of H3 ser10 phosphorylation. Total cellular proteins from HP1–RNAi cells (Ri) and control cells (**C**) were used for western blotting using antibodies recognizing ser10-phosphorylated histone H3. Extracts from cells treated with colchicine (+Col) or not (−Col) were used as controls.

transcription was also affected by the loss of HP1 function. Genes essential for mitosis, such as *Aurora B*, were also HP1-positive (Figure 4). These results demonstrate that these cell-cycle regulator genes are directly targeted by HP1 in their promoter regions.

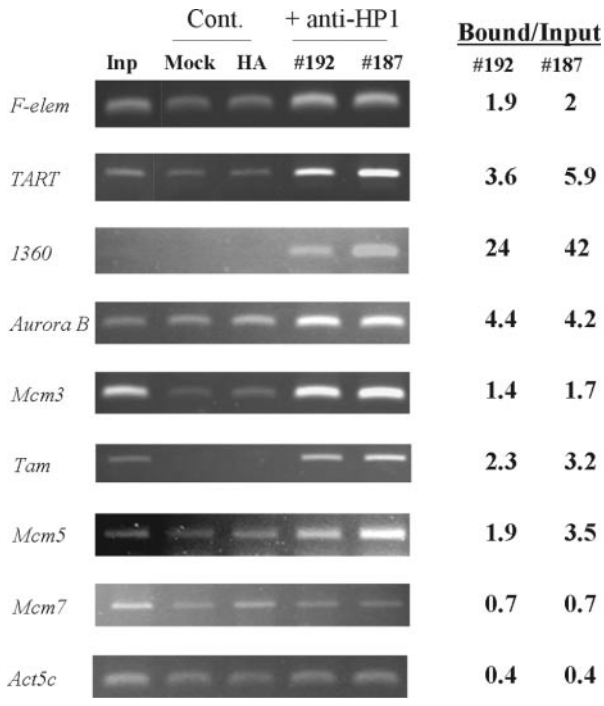

Figure 4. HP1 is physically associated with genes encoding cell-cycle regulators. Enrichment of HP1 in the genes tested was determined using PCR amplification of ChIP DNA precipitated with control and anti-HP1 antibodies (#192 and #187). Primers were designed to cover the promoter region of the genes (see Supplementary Material). The PCR products from the input (Inp) DNA are shown in the lane to the left of the gel. 1:300 dilution was used for genes *Aurora B*, *McM3*, *Tam*, *McM5*, *McM7* and *Act5c*. 1:1500 dilution used for *F-element*, *TART* and *1360* because of their high copy number in the genome. The PCR products from the controls (mock beads and anti-HA) and that from ChIP DNA using anti-HP1 are shown in agarose gels. The names of the individual gene or heterochromatin repeats analyzed are indicated. PCR amplification was performed for 35 cycles. The ratio between the intensities of the PCR products of HP1 ChIP DNA and input DNA is indicated on the right.

DISCUSSION

In this study, we used microarray and RT–PCR techniques to demonstrate that transcription of cell-cycle regulators is misregulated in the absence of HP1. Certain defects in S phase may be a direct consequence of the loss of transcription of DNA replication genes such as *McM2*, *McM5*, *McM6*, *CDC45L*, *Orc4* and others, since these genes have been functionally implicated in the initiation of DNA replication and/or the progression of replication forks (39). Depletion or mutation of these genes has been shown to result in DNA damage (32), the blockage of replication forks (39), increased chromosome loss/genome instability, and defective condensation (30).

The reduction in the number of cells in G2/M phase may be a consequence of the reduction in transcription or functional disruption of *INCENP*, *Aurora B*, *Bub1*, and *Bub3* (34,36). Chromosome segregation defects, such as chromosome fragmentation and chromatin bridges in anaphase/telophase cells, and certain cytokinesis defects in HP1-depleted cells, mimic the phenotype of cells with loss function of INCENP, Aurora B or Bub1 (35–37). The mislocalization of Aurora B in the absence of HP1 is also consistent with the loss of transcription and functional disruption of *INCENP* (37), and the reduction in *Aurora B* transcription may be partially responsible for the

observed chromosomal defects, including loss of histone H3 phosphorylation at serine 10.

HP1 is also known to physically interact with certain components of replication complexes such as ORCs and MCMs (30,40,41), with the inner centromere protein INCENP (42) and the chromatin assembly factor CAF1 (43) promoting delivery of HP1 to heterochromatin sites (44). Loss of HP1 is, therefore, expected to cause disruption to such HP1-associated complexes, and will partially contribute to the chromatin/chromosomal defects in HP1 mutants (8,27) and HP1-depleted Kc cells. It is therefore well possible that the loss of transcription of these cell-cycle regulator genes, and consequent disruption of HP1 functional complexes or heterochromatin structure, all contributed to the cell-cycle defects observed.

The ChIP assay supports the hypothesis that the loss of transcription of cell-cycle regulator genes is a direct effect of the lack of HP1. *Aurora B*, *McM3*, and *McM5* were all bound by HP1 at their promoter regions, although other cell-cycle regulators, such as *McM7*, were HP1-negative, implying that the altered transcription in these genes might be a secondary effect of the loss of HP1.

A previous study in *Drosophila* Kc cells (20) employed an approach based on the ectopic expression of a fusion protein of HP1 with a prokaryotic DNA adenine methyltransferase and identified a number of methylated targets in the genome. In this study, *MCM3* and *MCM5* were not found to be methylated, indicating lack of association with HP1. On the other hand, heterochromatin repeats, such *F-element* and *1360*, were consistently found to be HP1-enriched both here and in the previous study. It remains to be determined whether these discrepancies are due to the different experimental systems used. However, we note that the previous study was performed using a cDNA array, while we observe binding of endogenous HP1 at the promoter of these genes. Similarly, another study using chromatin immunoprecipitation in larvae also showed few HP1-positive genes that were not detected in Kc cells by the Dam ID approach (21).

A large number of genes affected by the loss of *D.melanogaster* HP1 in larval tissues (21) seem to be different from that in embryonic Kc cells. The change in the transcription of *Aurora B* and few cell-cycle regulators reported in this study is also not found among the HP1-affected genes at larval stage (21). This may be due to specific role(s) of HP1 in different stages of development. Alternatively, it is also possible that the impact of HP1 in the transcription of cell-cycle regulators in proliferating cells is underestimated when performing the analyses on larval tissues, and thus on mixed populations of both proliferating and differentiating/differentiated cells.

HP1 is generally known as a transcriptional repressor, as supported by several lines of evidence: silencing of a euchromatic reporter gene in heterochromatin requires HP1 (10,11), tethering of HP1 next to a euchromatic reporter gene causes silencing (45), and the repression of genes within euchromatic region 31 bound by HP1 is relieved in the absence of HP1 (46). In contrast, genes in heterochromatin, known as heterochromatic genes, such as *light* and *rolled*, seem to require HP1 to maintain their active transcription (47,48). The level of transcription of heterochromatic genes was dramatically reduced in a mutated HP1 background (47–49). It was therefore proposed that HP1 may function as a positive regulator of transcription of these genes (50,51), although the exact regulation mechanism remains unclear. A study of heat-shock genes found that HP1 is associated with RNA transcripts in the coding region, and is also a positive regulator of their transcription (52,53). The chromatin association of HP1 at the promoter region of active euchromatic genes demonstrated from this work and others, and its independence from histone H3K9 methylation (21), all suggest that mechanism whereby HP1 modulates transcription of euchromatic genes is potentially distinct from its role in heterochromatin formation.

Collectively, the results of this study demonstrate that HP1 plays an essential role in cell-cycle progression, and support the view that HP1, in addition to its role in heterochromatin, can act as a positive transcriptional regulator of euchromatic genes.

SUPPLEMENTARY MATERIAL

Supplementary Material is available at NAR Online.

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