Published in final edited form as:

Exp Cell Res. 2008 July 1; 314(11-12): 2224–2230.

PRC1 associates with the hsp70i promoter and interacts with HSF2 during mitosis

Lynea A. Murphy^a, Donald C. Wilkerson^a, Yiling Hong^b, and Kevin D. Sarge^{a*}

^aDepartment of Molecular and Cellular Biochemistry, Chandler Medical Center, University of Kentucky, Lexington, KY, 40536.

Abstract

Mitosis is a series of events leading to division of a cell by the process known as cytokinesis. Protein regulating cytokinesis 1 (PRC1) is a CDK substrate that associates with the mitotic spindle and functions in microtubule bundling. Previous studies revealed that loss of PRC1 is associated with chromosomal mis-segregation and atypical chromosome alignment. HSF2 is a DNA binding protein that we previously showed bookmarks the hsp70i gene during mitosis, an epigenetic mechanism which allows the hsp70i gene to re-establish transcriptional competence early in G1. Another study demonstrated that HSF2^{-/-} mouse embryonic fibroblasts (MEFs) exhibit increased numbers of multinucleated cells vs. wild-type MEFs. This suggests that HSF2 is important for proper cytokinesis, but the mechanism was unknown. Here we report the existence of a direct interaction between HSF2 and PRC1. HSF2 and PRC1 associate during mitosis and co-localize during this phase of the cell cycle. PRC1 does not interact with the related protein HSF1, indicating the specificity of the HSF2-PRC1 interaction. Intriguingly, PRC1 is associated with the hsp70i promoter during mitosis. These results provide a potential mechanistic basis for the defective cytokinesis phenotype exhibited by HSF2^{-/-} cells, as well as suggest a potential role for PRC1 in HSF2-mediated gene bookmarking.

Keywords

HSF2; PRC1; hsp70i gene; bookmarking; cytokinesis

Introduction

Mitosis is comprised of an intricately regulated series of events ultimately leading to division of a cell into two daughter cells in a process known as cytokinesis. In the first part of mitosis, chromosomes are condensed and segregated to facilitate correct alignment later during cytokinesis. Protein regulating cytokinesis 1 (PRC1) is a 71 kDa CDK substrate protein that associates with the mitotic spindle and has been found to play a crucial role in the completion of cytokinesis [1,2]. PRC1 bundles microtubules (MT) and is thought to be critical for spindle formation [2]. Previous studies demonstrated that siRNA-mediated knockdown of PRC1 causes aberrant chromosomal alignment and segregation in mitotic cells as well as an increase in binucleated cells [2,3]. Other studies identified binding partners of PRC1 which regulate or mediate PRC1 function during mitosis. For example, the interaction between the

*To whom correspondence should be addressed. Email: kdsarge@uky.edu.

^bCurrent address: University of Dayton, Department of Biology, Dayton, OH, 45469.

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chromokinesin Kif4 and PRC1, regulated by the Cdk phosphorylation state of PRC1, occurs at the metaphase-to-anaphase transition and is responsible for translocating PRC1 along the spindle, allowing PRC1 to bundle interdigitating microtubules (MTs) and form the spindle midzone [4,5]. PRC1 also interacts with the motor proteins MKLP-1 and CENP-E in late mitosis [4]. In more recent studies, PRC1 was found to interact with mitotic-specific kinases. For example, the MAPKK-like kinase, TOPK, associates with PRC1 and enhances cdk1/Cyclin B1 phosphorylation of PRC1 [6]. Another study suggested that PRC1 acts as a docking site for Polo-like kinase 1 (Plk1) and is necessary to localize Plk1 to the central spindle where it participates in regulation of cytokinesis [7].

During mitosis, chromosomes are condensed and segregated to facilitate correct alignment later during cytokinesis. Previous studies investigated an epigenetic phenomenon known as gene bookmarking, in which specific gene promoters remain relatively uncompacted in comparison to most genomic DNA [8–11]. A study in our laboratory defined the mechanism by which heat shock transcription factor 2 (HSF2) mediates gene bookmarking at the hsp70i promoter, one of the promoters known to be bookmarked. Specifically, HSF2 binds to the heat shock elements (HSEs) in hsp70i and other heat shock gene promoters during mitosis and recruits the phosphatase PP2A, while simultaneously interacting with the CAP-G subunit of condensin to facilitate dephosphorylation/inactivation of adjacent condensin complexes, thereby reducing compaction of this specific region of chromosomal DNA in contrast to the majority of genomic DNA which remains compacted [12]. The reduced compaction at the hsp70i promoter allows rapid reassembly to a transcriptionally-competent state in early G1, which ensures the ability of the cell to induce this crucial proteoprotective heat shock protein if stress conditions occur [13].

In this study we have identified PRC1 as an interacting partner of HSF2. This interaction is specific for HSF2 as PRC1 does not interact with HSF1, a related HSF family member which functions to upregulate hsp70i gene expression in response to cellular stressors. Immunofluorescence analysis demonstrates that HSF2 and PRC1 co-localize during mitosis, and chromatin immunoprecipitation data reveals that PRC1 is present at the hsp70i promoter. Given the role of HSF2 in hsp70i bookmarking, these results suggest a potential role for PRC1 in the mechanism of HSF2-mediated gene bookmarking.

Materials and Methods

Plasmids/Antibodies

pOTB7 Plasmid containing full length PRC1 cDNA clone (MGC3669) was purchased from Invitrogen. The plasmid pEGFP-PRC1 was cloned using primers to add XhoI and EcoRI sites to the 5' and 3' ends of PRC1. Following digestion with XhoI and EcoRI, the insert was cloned into the XhoI and EcoRI sites of pEGFP-C2 (Clontech). Affinity purified antibodies synthesized against the peptides CSKASKSDATSGILNSTNIQS or CYLCELAPAPLDSDMPLLDs which correspond to the C-terminal residues 601 to 620 of PRC1 [1] 498 to 517 of mouse HSF2 (which is identical to the C-terminal sequence of human HSF2), respectively, are from Bethyl Laboratories, Inc.

Enrichment of mitotic cell populations/Transient transfection of HeLa cells

HeLa ATCC and Jurkat cells were treated with nocodazole (Sigma-Aldrich) at 250ng/mL for 18 hours or with 10nM Taxol (T7402 Sigma-Aldrich) for 24 hours [12,14,15]. For transfections, cells were transfected with pEGFP HSF2 or pEGFP-PRC1 using Effectene (Qiagen) or jetPEI (Bridge Bioscience) according to the manufacturer's instructions. HeLa cells were grown in DMEM, 10% fetal bovine serum (FBS), 50 µg/ml gentamicin at 37°C with

5% CO₂. Jurkat cells were cultured in RPMI 1640, 10% FBS, and 50µg/ml gentamicin at 37°C with 5% CO₂.

Yeast two-hybrid analysis and β -galactosidase assay

An HSF2 or HSF1 “bait” construct consisting of full length HSF2 or HSF1 inserted in-frame into the vector pGBD-C1 was transformed into yeast strain pJ69-4A. The resulting strain was then transformed with a mouse whole embryo cDNA library [16]. To confirm the interaction, the pGBD-HSF2 and pVP16 plasmid containing the partial mPRC1 cDNA, referred to as mPRC1 (118–233), were transformed back into yeast and the ability of HSF2 or HSF1 and the mPRC1 clone to interact was determined by growth on selective media lacking adenine or histidine. For assay of interaction strength using β -galactosidase activity, yeast extracts were incubated with Z Buffer (60mM Na₂HPO₄ and 40mM NaH₂PO₄ (pH 7.0), 10mM KCl, 1mM MgSO₄, 50mM β -mercaptoethanol). After addition of 4mg/ml o-nitrophenyl- β -D-galactoside (ONPG) substrate, samples were incubated at 30°C for 30 minutes and then the OD₄₂₀ was measured.

In vitro binding assay

GST-HSF2, GST-HSF1, and GST expressed in E. coli were bound to glutathione-agarose beads and then incubated with ³⁵S-labeled in vitro translated full length in 0.5 ml of 20 mM Tris-HCl (pH 7.5), 50 mM NaCl, 0.1% Triton X-100, 1 mM DTT, 1mM PMSF, and 1X protease inhibitor (Roche) for 16 hours at 4°C. The beads containing bound proteins were then washed four times at 4°C with 10 mM Tris-HCl (pH 7.0), 1% Triton X-100, 150 mM NaCl, 2 mM DTT, 1 mM PMSF. The beads were then resuspended in 20 µl SDS-PAGE loading buffer, boiled for 5 minutes and then subjected to SDS-PAGE on a 10% gel. The gels were then dried and exposed to X-ray film to detect the ³⁵S-labeled in vitro translated PRC1 proteins. The amounts of GST-HSF2, GST-HSF1, and GST proteins bound to the beads were determined by SDS-PAGE followed by Western blot using goat polyclonal anti-GST antibody (Amersham). For experiments using HeLa ATCC, mitotic cells were lysed in 10mM Tris-HCl (pH 7.0), 1% Triton X-100, 150mM NaCl, 2mM DTT, 1mM PMSF and incubated and washed as described above. The amount of bound PRC1 was determined by SDS-PAGE followed by Western blot using goat polyclonal anti-PRC1 antibody (Bethyl).

Immunoprecipitation analysis

Nocodazole or taxol treated HeLa ATCC cells were lysed in 25 mM HEPES, 100 mM NaCl, 5 mM EDTA, 0.5% Triton X-100, 20 mM γ -glycerophosphate, 20 mM paranitrophenylphosphate, 100 µM sodium orthovanadate, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 1x Complete mini-protease inhibitor cocktail [Roche Diagnostics]) supplemented with 20 mM N-ethylmaleimide and 5 µM MG132, followed by centrifugation for 25 min at 15,000 × g at 4°C [17]. Lysates were precleared with 4µg rabbit or goat IgG and 40µl of a 50% slurry of Protein G Sepharose in supplemented TEG buffer (20 mM Tris-HCl [pH 7.5], 1 mM EDTA, 10% glycerol) containing 150 mM NaCl and 0.1% Triton X-100 for 1 hour at 4°C. Samples were incubated with 3µL anti-HSF2 rabbit polyclonal or 4µg anti-PRC1 goat antibody for 30 minutes at room temperature followed by incubation with 40µl of Protein G Sepharose overnight at 4°C. Beads were washed with supplemented TEG buffer and resuspended in 20µL SDS loading buffer. Immunoprecipitated proteins were analyzed by SDS-PAGE and Western blot according to established lab protocols using anti-PRC1 antibody (Bethyl) or GFP mouse monoclonal antibody (Clontech) [18].

Immunofluorescence analysis

HeLa ATCC cells were seeded onto coverslips that were acid-washed and flamed, and then coated with laminin (5µg/mL) (Sigma-Aldrich). Cells were washed once in ice-cold 1×PBS,

followed by fixation in 4% paraformaldehyde and permeabilization in 0.5% Triton X-100/0.5% saponin. Cells were blocked in 5% bovine serum album followed by incubation with anti-HSF2 goat polyclonal antibody or anti- α -tubulin mouse monoclonal antibody clone B-5-1-2 (T5168 from Sigma-Aldrich) for 1 hour at 37°C. After multiple washes with PBS, the coverslips were incubated as above with either Horse anti-mouse Texas Red (Vector Laboratories), Alexa Fluor® 488 donkey anti-goat IgG or Alexa Fluor® 594 donkey anti-goat IgG (Invitrogen). After a final wash with PBS, coverslips were mounted on a slide with Vectashield mounting medium plus 1.5µg/mL DAPI (4', 6 diamidino-2-phenylindole) (Vector Laboratories). Immunostaining was visualized using a Nikon fluorescent microscope with a 100x oil immersion objective and a Nikon Spotcam digital-imaging camera.

Chromatin Immunoprecipitation (ChIP)

Jurkat cells were treated with nocodazole (250ng/mL for 18 hours) or Taxol (10nM for 24 hours) as indicated and subjected to ChIP analysis according to established protocols in our laboratory [12] with the following modifications. Pre-cleared chromatin was incubated with 13µg of goat PRC1 antibodies or control goat IgG, and rotated at 4°C for 16 hours. DNA was purified using a QIAquick PCR Purification Kit (Qiagen Inc.) and eluted in 50µl of 10mM Tris (pH 8.5). Immunoprecipitated DNA and input samples obtained prior to immunoprecipitation were analyzed by traditional PCR and by quantitative real time PCR (QPCR). QPCR was performed with a Stratagene Mx 4000 system using Brilliant SYBR Green QPCR master mix (Stratagene). Samples were checked for specific amplification using dissociation curves analysis software. PCR products were also assayed on polyacrylamide gels with ethidium bromide staining to ensure they were of the expected size. The Ct values were normalized to input DNA (DNA before immunoprecipitation step) and IgG controls using the formula $2^{[(Ct \text{ IgG}-Ct \text{ Input})-(Ct \text{ Ab}-Ct \text{ Input})]}$ (where Ab=PRC1 antibodies or IgG). Data is represented as fold differences relative to the IgG antibody, which was set to 1. The data shown represents at least three independent ChIP assays. Error bars represent standard error of the means. Traditional PCR was performed using a Stratagene Robocycler. The linear range of amplification was identified by assaying a variety of cycle numbers. PCR products were resolved by polyacrylamide gel electrophoresis and DNA visualized with ethidium bromide staining. The following primers (shown 5' to 3') from Integrated DNA Technologies (Coralville, IA) were used for all PCR reactions: hhsp70i: (+) CAACACCCTTCCACCGCCACTC, (–) CCAGCCTTCCTTGACCAATCAG.

Results

Interaction between HSF2 and PRC1 is specific

In order to obtain a better understanding of the regulation and functions of HSF2 in cells, a yeast two-hybrid screen was performed using the HSF2 protein as the bait to identify protein partners of this HSF. One of the interacting clones identified by this screen represents a sequence in the amino terminal half of mouse PRC1, comprising amino acids 118–233, which are homologous to amino acids 117–246 of human PRC1 (Fig. 1A–1C). As a control to test the specificity of this interaction, a yeast two-hybrid assay was performed using HSF1 as bait. Analysis of yeast two-hybrid selective growth and β -galactosidase activity data reveals that the interaction between PRC1 is specific for HSF2 as HSF1 does not appear to interact (Fig. 1A and 1B).

Recombinant HSF2 associates with in vitro translated PRC1 and endogenous PRC1 from mitotic cell lysates

To independently confirm this interaction between HSF2 and PRC1, and to test whether the interaction is direct, in vitro binding experiments were performed using ^{35}S -labeled in vitro translated human PRC1 that was expressed from a full-length cDNA clone (MGC3669).

The ^{35}S -labeled PRC1 was incubated with GST-HSF2, or GST bound to glutathione agarose beads, washed, and then subjected to SDS-PAGE and autoradiography to determine the amount of ^{35}S -labeled PRC1 that was bound. The results of this experiment, shown in Figure 2A, indicate that the in vitro translated PRC1 interacts with the GST-HSF2 but not GST alone, thereby supporting the yeast two-hybrid finding of a specific interaction between HSF2 and PRC1 and also suggesting that the interaction is direct.

Next, to test whether recombinant HSF2 could associate with endogenous mitotic PRC1, we incubated GST-HSF2, GST-HSF1 or GST bound to glutathione agarose beads with lysates of mitotic HeLa cells. Following washing, the amount of PRC1 from these extracts that remained bound was determined by SDS-PAGE and Western blot using antibodies against PRC1. The results indicate that endogenous PRC1 present in mitotic cell extracts is able to interact specifically with purified recombinant HSF2 (Figure 2B).

HSF2 and PRC1 interact during mitosis

PRC1 is a CDK substrate protein known to associate with the mitotic spindle during mitosis [1,2]. Since PRC1 is known to be expressed predominantly during the S and G2/M phase of the cell cycle, and to function during mitosis [1], and in light of the mitotic function of HSF2 in bookmarking the hsp70i promoter, we hypothesized that the interaction between endogenous HSF2 and PRC1 may occur during this stage of the cell cycle. To test this hypothesis, immunoprecipitation analysis was performed using mitotic-enriched populations of HeLa cells. Cells were enriched for mitotic populations by treatment with nocodazole, which depolymerizes microtubules or with taxol, which stabilizes microtubules, and then extracts of these cells were subjected to immunoprecipitation using HSF2 antibodies followed by anti-PRC1 western blot (Figures 3A and 3C). As an additional approach, cells were transfected with a construct expressing GFP-HSF2, enriched for mitotic cells by nocodazole or taxol treatment, subjected to immunoprecipitation using anti-PRC1 antibodies, followed by western blot using anti-GFP antibodies to detect the transfected GFP-HSF2 protein (Figures 3B and 3D). The results of this experiment, shown in Figure 3, suggest that complexes between PRC1 and both endogenous as well as transfected tagged HSF2 are indeed present in extracts of mitotic cells. In addition, HSF2-PRC1 interaction appears to be independent of microtubule dynamics, as they are observed in extracts of cells treated with nocodazole as well as those of taxol treated cells.

HSF2 and PRC1 co-localize during mitosis

To determine when and where the interaction could be occurring between HSF2 and PRC1 in mitosis with respect to subcellular localization, immunofluorescence analysis was performed. Asynchronous cells were transfected with pEGFP-PRC1 followed by immunofluorescence analysis for endogenous HSF2, with DAPI (4',6 diamidino-2-phenylindole) staining used to identify cells in different stages of mitosis. Previous studies showed that PRC1 localizes predominantly to the mitotic spindle but is inhibited from binding microtubules prior to metaphase in a phosphorylation-dependent manner [2,5]. α -tubulin was co-stained with PRC1 and HSF2 separately to identify the location of the mitotic spindle in relation to these two proteins. The results of this experiment indicated that HSF2 and PRC1 appear to co-localize at the periphery of the mitotic spindle at the prometaphase (Prometa) and metaphase (Meta) stages of mitosis but not during anaphase (Ana) or telophase (Telo) (Figure 4C). HSF2 and α -tubulin do not appear to co-localize during mitosis (Figure 4B) which corresponds to our earlier immunoprecipitation data which indicates that the HSF2/PRC1 interaction occurs independently of microtubule dynamics during mitosis.

PRC1 associates with the hsp70i promoter during mitosis

HSF2 has previously been shown to bind to the hsp70i promoter during mitosis to mediate bookmarking of the hsp70i gene promoter [12]. Since our results showed that HSF2 and PRC1 interact during mitosis, we hypothesized that PRC1 could also be present at the hsp70i promoter. To test this hypothesis, we treated Jurkat cells with nocodazole (Figure 5A) or taxol (Figure 5B). Chromatin Immunoprecipitation assay was performed on treated cells using antibodies against PRC1. Precipitated DNA from three independent experiments was analyzed by quantitative PCR using primers specific for the human Hsp70i promoter region. We found that in Jurkat cells treated with nocodazole, PRC1 bound to the Hsp70i promoter approximately 2.2-fold better than the control IgG (Figure 5A, upper panel). Furthermore, in Jurkat cells treated with taxol, PRC1 bound to the Hsp70i promoter approximately 6.2-fold better than the control IgG (Figure 5B, upper panel). DNA samples from the ChIP assay were also amplified by PCR and analyzed by polyacrylamide gel electrophoresis and ethidium bromide staining. The results of this analysis also indicate association of PRC1 with the hsp70i promoter region, and confirm that the PCR products were of the expected size (Figure 5A and 5B, lower panels).

Discussion

Previous studies in our laboratory determined that HSF2 mediates the bookmarking of the hsp70i gene during mitosis. In this epigenetic mechanism, HSF2 bound to the hsp70i promoter recruits PP2A and inactivates the CAP-G subunit of condensin resulting in a region of DNA that remains "open" and allows for a rapid transition to a transcriptionally active state in early G1 of the cell cycle [12]. With the characterization of this phenomenon involving HSF2, the question remained as to whether other functions and interactions involving HSF2 in mitosis have yet to be discovered. Consistent with such potential additional mitotic functions of HSF2, a previous study demonstrated that HSF2^{-/-} mouse embryonic fibroblasts (MEFs) exhibited increased numbers of binucleate cells [19], suggesting that HSF2 function could play a role in the mechanism or regulation of cytokinesis.

This study reports the interaction between HSF2 and PRC1. The results indicate that HSF2 and PRC1 interact during mitosis and that this interaction is specific for HSF2, which is consistent with the hypothesis which suggests that, in addition to bookmarking, HSF2 could have other functions during mitosis. Data obtained from immunoprecipitations using cell lysates treated with nocodazole, a microtubule depolymerizing drug, or taxol, a microtubule stabilizing drug indicate that HSF2 and PRC1 interact independently of microtubule dynamics during mitosis. Previous data indicated that PRC1 does not bind to microtubules during metaphase due to cell cycle-dependent phosphorylation [1,2]. Consistent with our hypothesis that the HSF2/PRC1 interaction is not dependent on microtubule dynamics, our immunofluorescence analysis of untreated cells indicates that HSF2 does not co-localize with α -tubulin during mitosis and that HSF2 and PRC1 localize together at prometaphase and metaphase but not in the later stages of mitosis when PRC1 is localized at the spindle midzone [1,2,4]. As mentioned above, previous studies in our laboratory indicated that HSF2 is present at the hsp70i promoter during mitosis to mediate bookmarking of the hsp70i gene [12]. Interestingly, the results of Chromatin Immunoprecipitation analysis in this current study indicate that PRC1 is also present at the hsp70i promoter during mitosis, suggesting a possible new function for PRC1 in the regulation/mechanism of hsp70i gene bookmarking.

Supporting the hypothesis of a functional complex involving HSF2 and PRC1 is the finding that CAP-G, the subunit of the condensin complex that HSF2 binds to during bookmarking of the hsp70i promoter [12], also interacts with Kif4 [20], a kinesin-4 family member that has been shown to interact with and translocate PRC1 along the mitotic spindle [4,5,20]. The interaction between Kif4 and PRC1 is regulated by phosphorylation of PRC1 [4,5]. Early in mitosis both PRC1 and Kif4 are present at the mitotic spindle, when PRC1 is phosphorylated,

but these two proteins do not interact at that time [5]. Dephosphorylation of PRC1 allows it to interact with Kif4, and as a result Kif4 is then able to translocate PRC1 onto MTs where PRC1 bundles the MTs to facilitate formation of the midzone, a necessary component for cytokinesis [5]. Interestingly, a previous study showed that Kif4 interacts with PRC1 at the amino terminus of the protein [4], the same region of PRC1 that our studies indicate is involved in interactions with HSF2. Thus, one possibility in elucidating the biological role that this interaction plays is that HSF2 could be responsible for negatively regulating PRC1 early in mitosis by binding to the same region in PRC1 with which Kif4 interacts, thereby mediating the lack of PRC1-Kif4 interaction at that stage.

Recent studies have also reported that PRC1 acts as a docking site for Plk1 in a phosphorylation-dependent manner [7,21]. Given that HSF2 and PRC1 interact in the early stages of mitosis, an intriguing question for future studies would be to determine if HSF2 acts in a similar manner to bring PRC1 into proximity with an as yet undetermined binding partner.

Acknowledgements

This research was supported by NIH grants GM61053 and GM64606 to K.D.S., Training Grant T32 ES-07266 (Department of Toxicology, P.I.: Dr. Mary Vore) support for L.A.M., and NIH postdoctoral support (HD50043) to D.C.W.

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Fig. 1.

Yeast two-hybrid analysis of HSF2 interaction with PRC1. (A) Yeast strain pJ694A transformed with pGBD-HSF2 or pGBD-HSF1 along with either pVP16 PRC1(118–233) or pVP16 alone (or pGBD alone with pVP16 PRC1(118–233)) were streaked on plates lacking tryptophan and leucine (–TL), tryptophan, leucine, and histidine (–HTL), or tryptophan, leucine, and alanine (–ATL). (B) β -galactosidase activity was measured in extracts of yeast strain pJ694A transfected with pVP16 PRC1(118–233) along with either pGBD-HSF2, pGBD-HSF1, or empty pGBD-C1 vector. (C) Schematic showing the location of the HSF2-interacting region of PRC1 identified by the yeast two-hybrid analysis, which comprises amino acids 118–233 of mouse PRC1.

Fig. 2.

Interaction with PRC1 is specific for HSF2. (A) ³⁵S-labeled in vitro translated full length human PRC1 was incubated with GST-HSF2 or GST that were bound to glutathione-agarose beads. After washing, the amount of bound ³⁵S-labeled full length human PRC1 was determined by SDS-PAGE and autoradiography. Amounts of GST-HSF2 and GST bound to beads were determined by GST Western blot. (B) Lysates of nocodazole-blocked HeLa cells were incubated with GST-HSF2, GST-HSF1 or GST bound to glutathione agarose. After washing, the amount of bound PRC1 was determined by SDS-PAGE followed by Western blot using goat polyclonal anti-PRC1 antibodies (Bethyl).

Fig. 3. HSF2 interacts with PRC1 during mitosis. Extracts of nocodazole (nocod.) (250 ng/ml for 18 hours) or taxol treated (10 nM for 24 hours) HeLa cells were subjected to immunoprecipitation analysis using anti-HSF2 (A and C) or PRC1 antibodies (B and D) or non-specific IgG, followed by Western blot analysis of the immunoprecipitates using anti-PRC1 or anti-GFP antibodies.

Fig. 4.

HSF2 and PRC1 co-localize during prometaphase and metaphase of mitosis. (A and C) HeLa cells were transfected with pEGFP-PRC1. After transfection, cells were subjected to immunofluorescence analysis using either anti-HSF2 (Bethyl) or anti α -tubulin antibodies (Sigma-Aldrich) followed by incubation with Texas Red anti-mouse IgG (Vector Laboratories), Alexa Fluor® 488 donkey anti-goat IgG or Alexa Fluor® 594 donkey anti-goat IgG (Invitrogen). Cells were visualized using a 100 $\times$ oil immersion objective on a Nikon fluorescent microscope. (A) GFP-PRC1 was co-stained with α -tubulin as a positive control for the location of the mitotic spindle. (B) Untransfected HeLa cells were subjected to immunofluorescence analysis as above. HSF2 and α -tubulin do not appear to co-localize during

mitosis. (C) GFP-PRC1 and HSF2 appear to co-localize during prometaphase and metaphase but not in the later stages of mitosis.

Fig. 5. PRC1 is associated with the Hsp70i promoter during mitosis. Jurkat cells treated with nocodazole (A) or taxol (B) were subjected to ChIP analysis using antibodies against PRC1 or IgG (negative control). Precipitated DNA, amplified using primers specific to the Hsp70i promoter, was quantitated by real time PCR (A and B, upper panels). The data represent the results of three independent ChIP assays, and error bars represent the standard error of the means. Precipitated DNA was also amplified with the same Hsp70i primers on a Robocycler PCR instrument. Amplified products were resolved by polyacrylamide gel electrophoresis and bands detected by ethidium bromide staining (A and B, lower panels).