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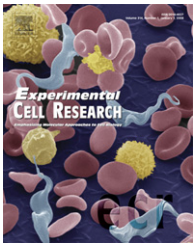

Research Article

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

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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.

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 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

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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 immunopreciptation 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 *Xho*I and *Eco*RI sites to the 5' and 3' ends of PRC1. Following digestion with *Xho*I and *Eco*RI, the insert was cloned into the *Xho*I and *Eco*RI 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 250 ng/ml for 18 h or with 10 nM Taxol (T7402 Sigma-Aldrich) for 24 h [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% CO_2. Jurkat cells were cultured in RPMI 1640, 10% FBS, and 50 μg/ml gentamicin at 37 °C with 5% CO_2.

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 (60 mM Na_2HPO_4 and 40 mM NaH_2PO_4 (pH 7.0), 10 mM KCl, 1 mM $MgSO_4$, 50 mM β-mercaptoethanol). After addition of 4 mg/ml o-nitrophenyl-β-D-galactoside (ONPG) substrate, samples were incubated at 30 °C for 30 min and then the OD_{420} 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 ^{35}S-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, 1 mM PMSF, and 1X protease inhibitor (Roche) for 16 h 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 min and then subjected to SDS-PAGE on a 10% gel. The gels were then dried and exposed to X-ray film to detect the ^{35}S-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 10 mM Tris–HCl (pH 7.0), 1% Triton X-100, 150 mM NaCl, 2 mM DTT, 1 mM 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 ß-glycerophosphate, 20 mM paranitrophenylphosphate, 100 μM sodium orthovanadate, 0.5 mM phenylmethylsulfonyl fluoride, 1 mM dithiothreitol, 1× 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