Heterochromatin protein 1 is recruited to various types of DNA damage


Published in:
Journal of Cell Biology

DOI:
10.1083/jcb.200810035

Citation for published version (APA):
Luijsterburg, M. S., Dinant, C., Lans, H., Stap, J., Wiernasz, E. S., Lagerwerf, S., ... van Driel, R. (2009). Heterochromatin protein 1 is recruited to various types of DNA damage. Journal of Cell Biology, 185(4), 577-586. DOI: 10.1083/jcb.200810035

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Heterochromatin protein 1 (HP1) family members are chromatin-associated proteins involved in transcription, replication, and chromatin organization. We show that HP1 isoforms HP1-α, HP1-β, and HP1-γ are recruited to ultraviolet (UV)-induced DNA damage and double-strand breaks (DSBs) in human cells. This response to DNA damage requires the chromo shadow domain of HP1 and is independent of H3K9 trimethylation and proteins that detect UV damage and DSBs. Loss of HP1 results in high sensitivity to UV light and ionizing radiation in the nematode Caenorhabditis elegans, indicating that HP1 proteins are essential components of DNA damage response (DDR) systems. Analysis of single and double HP1 mutants in nematodes suggests that HP1 homologues have both unique and overlapping functions in the DDR. Our results show that HP1 proteins are important for DNA repair and may function to reorganize chromatin in response to damage.

Introduction

DNA can be damaged by various agents, including ionizing radiation (IR) and UV radiation. Cells respond to genotoxic stress by activating DNA damage response (DDR) systems, including DNA repair, cell cycle arrest, senescence, and apoptosis (Bartek and Lukas, 2007). DNA repair pathways each deal with specific types of lesions (Hoeijmakers, 2001). Nucleotide excision repair (NER) removes bulky adducts and UV-induced photoproducts such as cyclobutane pyrimidine dimers and 6-4 photoproducts from the genome, whereas DNA double-strand breaks (DSBs) are removed by homologous recombination or nonhomologous end joining (NHEJ; Hoeijmakers, 2001).

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Abbreviations used in this paper: CD, chromodomain; CSB, Cockayne syndrome protein B; CSD, chromo shadow domain; DDR, DNA damage response; DSB, double-strand break; FLP, fluorescence loss in photobleaching; FP, fluorescent protein; GGR, global genome NER; HP1, heterochromatin protein 1; IR, ionizing radiation; mRFP, monomeric RFP; NER, nucleotide excision repair; NHEJ, nonhomologous end joining; PCNA, proliferating cell nuclear antigen; SCFP, super cyan FP; TCR, transcription-coupled NER; XP, xeroderma pigmentosum.

The heterochromatin protein 1 (HP1) isoforms HP1-α, HP1-β, and HP1-γ are versatile epigenetic regulators with functions in chromatin organization, transcription regulation, and DNA replication (Maison and Almouzni, 2004). Recent studies suggested that HP1 may respond to chromosomal breaks in heterochromatin (Ayoub et al., 2008; Goodarzi et al., 2008). HP1 proteins bind to histone H3 that is methylated at lysine 9 (H3K9me) via their N-terminal chromodomain (CSD), including epigenetic regulators and chromatin remodelling complexes (Nielsen et al., 2002; Fuks et al., 2003; Eskeland et al., 2007). Additionally, HP1 interacts with a large number of nuclear proteins via its C-terminal chromo shadow domain (CSD), including epigenetic regulators and chromatin remodelling complexes (Nielsen et al., 2002; Fuks et al., 2003; Eskeland et al., 2007).

In this study, we show that all three HP1 proteins are recruited to UV-induced lesions and DSBs in living human cells.
This recruitment of HP1 depends on the CSD but not on H3K9 methylation or on the repair systems that remove these lesion types. Loss of HP1 proteins renders the nematode Caenorhabditis elegans highly sensitive to UV and ionizing irradiation. Our data suggest that HP1 homologues have both distinct and overlapping essential functions in the DDR.

**Results and discussion**

**HP1 proteins are recruited to UV lesions by the CSD**

To study whether HP1 proteins respond to UV-induced DNA damage, we locally damaged nuclei of cultured cells with UV-C light either using a UV-C laser (266 nm; Dinant et al., 2007) or by irradiation with a UV-C lamp (254 nm) through a polycarbonate mask (Moné et al., 2001). These methods recruit NER proteins but not the DSB repair proteins NBS1 (Fig. S1 A), Rad50, Rad54, and Ku80 (Houtschmuller et al., 1999; Dinant et al., 2007). At UV-irradiated sites, we observed recruitment of all three HP1 isoforms expressed as fluorescent protein (FP)-tagged fusion proteins (monomeric RFP [mRFP]–HP1–α, super cyan FP [SCFP]–HP1–β, and EGFP–HP1–γ) in human cells (Fig. 1, A–C) and mouse cells (not depicted). The FPs (CFP, YFP, and GFP) alone do not accumulate at locally damaged sites, indicating that accumulation of HP1 is dependent on the CSD (Fig. S1, F–I). Fluorescent immunolabeling with HP1–β–specific antibodies showed that GFP–HP1–β is expressed at ~20% of the level of endogenous HP1–β (Fig. S1, D and E). Importantly, endogenous HP1–α, HP1–β, and HP1–γ accumulate at local UV damage in primary human fibroblasts (Fig. 1, D–F). Comparing the same cells before and after local UV irradiation (Fig. S1, B and C) showed that SCFP–HP1–β accumulated at damaged sites with a t1/2 of 180 s after local UV irradiation (Fig. 1 G), which is comparable to the binding kinetics of XPA (xeroderma pigmentosum [XP] group A) at DNA repair sites (Fig. S1 J). To further investigate the binding of HP1 at lesions, we performed photobleaching experiments on mouse cells that express EGFP–HP1–β and that were globally UV-C irradiated with 25 J/m². These experiments confirmed that a small but significant fraction of EGFP–HP1–β is immobilized in UV-irradiated cells on a time scale of several minutes but not in nondamaged control cells (Fig. 1, H and I). Global UV irradiation did not result in visible changes in the nuclear distribution of HP1 (Fig. S2 A) nor did it result in immobilization of GFP-NLS (Fig. S1, K and L).

HP1 proteins contain three distinct domains: the N-terminal CD, the C-terminal CSD, and the hinge region that separates the CD from the CSD. Deletion mutants of HP1–β lacking the CD, CSD, or hinge (Fig. 1 J) were tagged with EGFP and tested for recruitment to UV-irradiated regions. Interestingly, UV lesions triggered binding of EGFP–HP1–β (ΔCD) and EGFP–HP1–β (Δhinge), but EGFP–HP1–β (ΔCSD) failed to accumulate (Fig. 1, K–M). To test whether the CSD (amino acids 98–185 of HP1–β) is not only necessary but also sufficient for recruitment, we fused this domain to EYFP and found that EYFP–CSD is indeed recruited to sites of local UV irradiation (Fig. 1 N). Our results show that HP1 recruitment to damaged sites is independent of the CD, suggesting that recruitment of HP1 is independent of H3K9 trimethylation (H3K9me3). To verify that HP1 recruitment does not require H3K9me3, we examined accumulation of HP1 in MEFs deficient for Suv39h1 and Suv39h2 (Peters et al., 2001). After UV irradiation, SCFP–HP1–β accumulated at sites of damage, confirming that binding of HP1 requires neither the CD nor H3K9me3 (Fig. 1 O). In agreement, we did not detect recruitment of EYFP-tagged H3K9 methyltransferase Suv39h1 (not depicted) nor did we detect increased H3K9me3 at sites of UV lesions (Fig. S2 B). A recent study showed that HP1 is phosphorylated at residue T51 (in the CD) in response to chromosomal breaks, which was suggested to initiate the DDR (Ayoub et al., 2008). We show that HP1–β lacking the T51 phosphorylation site (YFP–HP1–β (ΔT51)) is recruited to sites of UV-induced lesions (Fig. 1 P). Together, our results indicate that HP1 proteins are recruited to UV-induced DNA damage through their CSD independently of the CD and of trimethylation at H3K9.

**HP1 recruitment to UV lesions is independent of NER**

Cells from placental mammals are fully dependent on NER for the removal of UV-induced DNA injuries, involving transcription-coupled NER (TCR) and global genome NER (GGR; Hoeijmakers, 2001). UV lesions trigger several chromatin-related events, such as recruitment of CAF-1, incorporation of histone H3.1 (Polo et al., 2006), and ubiquitylation of H2A (Bergink et al., 2006). These events strictly depend on the binding and activity of NER proteins that are involved in recognition and subsequent processing of DNA lesions (Bergink et al., 2006; Polo et al., 2006). To investigate whether HP1 binding is a late or early event in NER, we tested accumulation of HP1–β in repair-deficient XP-A cells that have compromised GGR and TCR. SCFP–HP1–β accumulated in XPA mutant cells after UV irradiation (Fig. 2 A), suggesting that HP1–β binding does not occur after DNA repair is finished. We then considered the possibility that HP1 binding
is caused by replication stress, we determined the cell cycle stage by expressing mCherry–proliferating cell nuclear antigen (PCNA) together with SCFP–HP1-β and DDB2-mVenus in repair-deficient XP-A cells and wild-type MRC5 cells. Recruitment of HP1-β was observed in wild-type (not depicted) and NER-deficient cells (Fig. S2, C and D) in S phase as well as in non–S phase cells, as shown by the distribution of PCNA. Clear accumulation of endogenous HP1-β was observed in G0 cells (Ki67-negative cells) at sites of UV irradiation (Fig. S2 E), showing that HP1 accumulation is not the result of stalled replication.

Together, these results show that HP1 binding to UV-damaged areas occurs in both cycling and quiescent cells and is independent of the activity of preincision GGR proteins, the TCR factor CSB, and ATR kinase.

UV-induced HP1 accumulation persists in the absence of functional NER

To determine whether loss of HP1 accumulation depends on the presence of UV-induced DNA lesions, we measured HP1 accumulation in repair-deficient XP-A cells and wild-type MRC5 cells. Recruitment of HP1-β was observed in wild-type (not depicted) and NER-deficient cells (Fig. S2, C and D) in S phase as well as in non–S phase cells, as shown by the distribution of PCNA. Clear accumulation of endogenous HP1-β was observed in G0 cells (Ki67-negative cells) at sites of UV irradiation (Fig. S2 E), showing that HP1 accumulation is not the result of stalled replication.

Together, these results show that HP1 binding to UV-damaged areas occurs in both cycling and quiescent cells and is independent of the activity of preincision GGR proteins, the TCR factor CSB, and ATR kinase.

is an early step after damage detection and tested accumulation in DDB2-deficient and XPC-deficient cells. Accumulation of SCFP–HP1-β was observed in both cell types (Fig. 2, B–D). Because binding of repair proteins involved in GGR is dependent on XPC (Volker et al., 2001), these results suggest that HP1 recruitment to damaged sites is independent of the activity of GGR proteins. Damage detection in TCR requires stalled RNA polymerase II, and subsequent recruitment of NER factors depends on the Cockayne syndrome protein B (CSB) protein (Fousteri et al., 2006). Recruitment of HP1 was also observed in CSB-deficient cells, indicating that HP1 binding is not dependent on TCR (Fig. 2 E).

In addition to DNA repair, cells respond to damaged DNA by activating ATM (ataxia telangiectasia mutated) or ATR (ATM and Rad3-related) kinase signaling pathways, resulting in activation of cell cycle checkpoints and phosphorylation of a variety of proteins involved in the DDR (Bartek and Lukas, 2007). To determine whether ATR is required for the recruitment of HP1 to UV lesions, we examined the accumulation of HP1-α, HP1-β, and HP1-γ in Seckel cells, which have severely reduced ATR expression (O’Driscoll et al., 2003). Accumulation of the HP1 isoforms after local UV irradiation was not affected in these cells (Fig. 2 F and not depicted), demonstrating that HP1 recruitment to sites of DNA damage does not require DNA damage-induced signaling mediated by the ATR kinase.

During S phase, stalling of replication forks at UV-induced lesions can result in DSBs. To exclude that HP1 accumulation is caused by replication stress, we determined the cell cycle stage by expressing mCherry–proliferating cell nuclear antigen (PCNA) together with SCFP–HP1-β and DDB2-mVenus in repair-deficient XP-A cells and wild-type MRC5 cells. Recruitment of HP1-β was observed in wild-type (not depicted) and NER-deficient cells (Fig. S2, C and D) in S phase as well as in non–S phase cells, as shown by the distribution of PCNA. Clear accumulation of endogenous HP1-β was observed in G0 cells (Ki67-negative cells) at sites of UV irradiation (Fig. S2 E), showing that HP1 accumulation is not the result of stalled replication.

Together, these results show that HP1 binding to UV-damaged areas occurs in both cycling and quiescent cells and is independent of the activity of preincision GGR proteins, the TCR factor CSB, and ATR kinase.

UV-induced HP1 accumulation persists in the absence of functional NER

To determine whether loss of HP1 accumulation depends on the presence of UV-induced DNA lesions, we measured HP1 accumulation in repair-deficient cells. Binding of HP1-β was observed up to ~4 h after local UV irradiation in XP-A cells (Fig. 3 A). Conversely, in XPA-deficient cells that were transiently transfected with mVenus-XPA to restore the repair capacity, bound HP1-β levels gradually decreased and HP1 accumulation had almost disappeared 4 h after UV irradiation (Fig. 3 B), which is consistent with the rate of DNA repair (van Hoffen et al., 1995). Accordingly, accumulation of YFP-tagged CSD became...
Repair-deficient XP-A cells were transfected with DDB2-mVenus and SCFP-HP1-β. Cells were irradiated at 100 J/m², and accumulation of HP1-β was monitored for 4 h after UV irradiation. Repair-deficient XP-A cells were transfected with mVenus-XPA (to complement the repair-deficient phenotype), DDB2-mCherry, and SCFP-HP1-β. Cells were irradiated at 100 J/m², and accumulation of HP1-β was monitored for 4 h after UV irradiation. Wild-type (MRC5) cells were transfected with DDB2-mCherry and YFP-CD, locally irradiated (100 J/m²), and accumulation of the CSD was monitored for 4 h after UV irradiation. The accumulation of DDB2-mVenus or DDB2-mCherry indicates the site of local damage.

Long-term accumulation of HP1-β in repair-proficient and repair-deficient cells. (A) Repair-deficient XP-A cells were transfected with DDB2-mVenus and SCFP-HP1-β. Cells were irradiated at 100 J/m², and accumulation of HP1-β was monitored for 4 h after UV irradiation. (B) Repair-deficient XP-A cells were transfected with mVenus-XPA (to complement the repair-deficient phenotype), DDB2-mCherry, and SCFP-HP1-β. Cells were irradiated at 100 J/m², and accumulation of HP1-β was monitored for 4 h after UV irradiation. (C) Wild-type (MRC5) cells were transfected with DDB2-mCherry and YFP-CD, locally irradiated (100 J/m²), and accumulation of the CSD was monitored for 4 h after UV irradiation. Expression of DDB2-mVenus or DDB2-mCherry indicates the site of local damage.

HP1 is recruited to DSBs

To study the response of HP1 to chromosomal breaks, we performed photobleaching experiments on human cells that express EGFP–HP1-β and that were globally irradiated with x-rays (5 and 10 Gy). Photobleaching experiments indicated that a small fraction of HP1 became immobile in response to IR (Fig. S3 A), suggesting increased binding of HP1 to chromatin in response to chromosomal breaks. IR did not result in visible changes in the nuclear distribution of HP1 proteins (Fig. S3 B). To confirm immobilization of HP1 at sites of double-strand DNA breaks, we tested HP1 recruitment to locally inflicted DSBs. Cells were irradiated with a dose of ~20 Gy of soft x-rays (extended UV 20 nm) through a nickel filter containing 5-μm pores, resulting in local accumulation of γH2AX. At locally damaged sites, we observed binding of GFP–HP1-β (Fig. 4 A), which is consistent with our fluorescence loss in photobleaching (FLIP) data (Fig. S3 A). To confirm these results, we irradiated cells with α-particles from a radioactive Americium (Am-241) source (Aten et al., 2004; Stap et al., 2008). Irradiation of human U2OS and mouse NIH/3T3 cells with α-particles resulted in linear tracks of γH2AX. Binding of EGFP–HP1-β in mouse cells (Fig. 4 B) and endogenous HP1-β in human U2OS cells (Fig. S3 C) colocalized with the linear γH2AX pattern. In addition, accumulation of GFP–HP1-α and GFP–HP1-γ (Fig. S3, D and E) was observed in MRC5 cells, showing that all HP1 isoforms are recruited to DSBs.

Mammalian cells use homologous recombination or NHEJ to remove DSBs from the genome (Wyman and Kanaar, 2006). The latter pathway is initiated by the KU70/80 dimer, which was shown to interact with HP1-α (Song et al., 2001). To test whether HP1 accumulation at DSBs depends on NHEJ, we irradiated wild-type and KU80-deficient CHO cells with α-particles. Recruitment of endogenous HP1-β was observed in both cell types at all γH2A.X tracks, showing that HP1 association is independent of NHEJ (Fig. 4 C). These results show that HP1 proteins are recruited to DSBs, which is in contrast to the mobilization of HP1 after DNA damage, as reported recently (Ayoub et al., 2008). In that study, microscopic analysis showed spreading of GFP–HP1-β into a larger area at sites of laser-assisted DNA damage inflicted at heterochromatic sites (Ayoub et al., 2008), which was interpreted as dissociation of HP1-β from heterochromatin. An alternative explanation could be that HP1 does not spread into neighboring chromatin but that the apparent spreading of HP1 actually reflects accumulation of HP1 at sites of DNA damage. To explore this, we have used the same procedure that Ayoub et al. (2008) used to damage DNA locally. Cells were sensitized with Hoechst, and a narrow strip spanning the nucleus was irradiated using a 405-nm laser, resulting in clear accumulation of NBS1-mCherry. At sites marked by NBS1 accumulation, we observed accumulation of GFP–HP1-β (Fig. 4, D and E). The CD and T51 residue (a protein kinase target in HP1) of HP1-β are dispensable for recruitment to these damaged sites (Fig. 4, F and G). Similar to what is observed for UV-induced lesions, we find that the CSD alone is sufficient for binding and that GFP–HP1-β (ΔCSD) does not bind to the damaged DNA sites (Fig. 4, H and I). We could not detect loss or dispersal of HP1 in the damage region before accumulation of HP1. Moreover, monitoring the same cells before and after damage induction showed that HP1 accumulated at higher levels in the locally damaged area compared with the predamage distribution of HP1 (Fig. 4, D and E), showing that the accumulation of HP1-β reflects de novo binding of HP1 molecules at damaged sites. We subsequently measured the binding kinetics of GFP–HP1-β in cells sensitized with BrdU in which a strip spanning the nucleus was irradiated using a 337-nm laser (Fig. 4 J). HP1-β rapidly accumulated at damaged sites with a t½ of 85 s. The CSD-dependent accumulation of HP1 is markedly different from the recently reported reappearance of HP1 at heterochromatic sites after a transient (5 min) dispersal of HP1 (Ayoub et al., 2008). The latter study showed phosphorylation of HP1 at T51 (T51P) in response to localized laser-assisted DNA damage, IR, and etoposide treatment. Incubation of the CD of HP1-β with CK2 resulted in a weakened interaction with the H3K9me peptide in vitro (Ayoub et al., 2008). However, it remains unclear whether T51-phosphorylated HP1-β has a lowered affinity for chromatin in vivo, as binding of HP1-β to chromatin is influenced by its dimerization with HP1-α, interactions with the H3 histone fold, and an RNA component.
Loss of HP1 renders *C. elegans* highly sensitive to UV irradiation and chromosomal breaks

Because loss of all HP1 isoforms in mammalian cells is lethal (Filesi et al., 2002; Schotta et al., 2004), we used the nematode *C. elegans* to test whether HP1 is functionally required for the DDR, as conditional HP1-deficient nematodes are available (Coustham et al., 2006). Nematodes are a very suitable model (Nielsen et al., 2001; Maison et al., 2002; Dialynas et al., 2007; Mateos-Langerak et al., 2007). Our results indicate that neither the CD nor the T51 residue are required for the binding of HP1 at sites of DNA damage. It cannot be excluded that phosphorylation of HP1 bound at damaged sites plays a role in phospho-dependent interactions with DDR proteins. Interestingly, HP1 is also recruited to oxidative DNA lesions (unpublished data), indicating that HP1 responds to a variety of DNA lesions in mammalian cells.

**Figure 4. Recruitment of HP1-β to DSBs.** (A) Mouse cells expressing EGFP–HP1-β (green) were locally irradiated with soft x-rays through a nickel mask with pores of 5 µm and subsequently labeled for γH2AX (red). (B) Mouse cells expressing EGFP–HP1-β (green) were irradiated with α-particles and subsequently labeled for γH2AX (red). (C) Hamster cells deficient in Ku80 were irradiated with α-particles and subsequently labeled for endogenous HP1-β (green) and γH2AX (red). Cells are shown 30 min after irradiation. (D–I) Wild-type U2OS cells expressing various HP1-β fusion proteins were sensitized with 10 µg/ml Hoechst 33342 for 5 min and locally irradiated (five iterations) in a strip spanning the nucleus using a 405-nm laser at 70% output. GFP–HP1-β before and after laser-assisted damage (the damaged area is indicated by arrows; 5 min; D and E), GFP–HP1-βΔCD (F), YFP–HP1-βT51A (G), GFP–HP1-βΔCSD (H), and YFP-CSD (I). Accumulation of NBS1-mCherry indicates the site of laser-induced DNA damage. (J) GFP–HP1-β accumulation in BrdU-sensitized U2OS cells during the first 800 s after irradiation using a 337-nm laser. (K) Quantification of GFP–HP1-β accumulation as described in J. Error bars indicate SD.
A system to study the DDR because their response to DNA damage is similar to that of mammals (O’Neil and Rose, 2006; van Haften et al., 2006). Two HP1 homologues (HPL-1 and HPL-2) are present in C. elegans. To study sensitivity to DNA damage, we used animals lacking HPL-1 (hpl-1Δ) and carrying a temperature-sensitive allele of HPL-2 (hpl-2ts), which is expressed at 20°C but not at 25°C (Coustham et al., 2006). Eggs of single- and double-mutant animals were exposed to UV-B radiation (80 J/m²) and transferred to 25°C. Wild-type and NER-deficient xpa-1 null eggs (Stergiou et al., 2007) were assayed in parallel (Fig. 5). Exposure to UV-B was used because it penetrates nematodes much better than UV-C light (unpublished data). Irradiation with UV-B caused an immediate growth arrest in hpl-2ts/hpl-1Δ double-mutant worms, similar to NER-deficient xpa-1 mutant worms. In contrast, single HP1-like protein mutants exhibit comparable UV sensitivity as wild-type worms (Fig. 5, A–C). It should be noted that hpl-2ts worms displayed considerably slower growth after UV irradiation, resulting in a smaller size compared with irradiated hpl-1Δ worms (Fig. 5 B). An increased UV-sensitive phenotype was also observed in hpl-2ts/hpl-1Δ mutant eggs, the survival of xpa mutant eggs was also quantified. Representative assays performed in duplicate or in quintuplicate are shown. For each assay, at least 120 animals were scored. Error bars indicate SD.
gested by Kruhlak et al. (2006) and Solimando et al. (2009),
ings, we favor a scenario in which HP1 proteins play a role in
kinds of HP1 (Quivy et al., 2008). In analogy to these find
duced (Goodarzi et al., 2008). However, our results also indicate
that loss of HPL-2 results in strong IR sensitivity, suggesting an
essential function in the DDR after chromosomal breaks. In con-
clusion, it appears that HP1 proteins have partly redundant roles
in response to UV damage, whereas they seem to have unique
functions in response to IR. This reveals an essential role for the
HP1 proteins in response to UV-induced DNA damage and chro-
mosomal breaks, possibly through different mechanisms.

HP1 and the DDR
What is the molecular role of HP1 in the DDR? Our data suggest
that HP1 recruitment does not require DNA repair activity be-
cause binding of HP1 at sites of UV lesions and DSBs is indepen-
dent of any of the known damage recognition proteins (Fig. 2 and
Fig. 4 C). In TCR, stalled RNA polymerase II initiates NER, which
could trigger binding of HP1 proteins (Mateescu et al., 2008).
However, HP1 proteins also accumulate at damaged sites in cells
in which transcription is blocked with α-amanitin (un-
published data), suggesting that HP1 proteins are recruited through
a damage detection system that is different from that for TCR and
GGR. HP1 binding depends on its CSD but not the CD or H3K9
trimethylation. It is possible that DNA damage-induced changes in
local chromatin structure are recognized by HP1 proteins. In
agreement with this, we show that HP1 accumulation persists in
repair-deficient cells in which lesions are not removed (Fig. 3 A).
ACF1 interacts with the CSD of HP1 and accumulates at UV les-
sions (Fig. S3, F and G; Eskeland et al., 2007), suggesting that this
remodelling factor may cooperate with HP1 to modify chro-
matin structure in damaged areas. However, HP1 recruitment was
still observed in cells depleted for ACF1 (unpublished data). Con-
sistent with an essential role for HP1 in facilitating DNA repair,
we found that HP1-deficient nematodes are extremely sensitive to
UV-induced DNA damage. HP1 isoforms each distinctly contrib-
ute to the sensitivity to UV- and IR-induced DNA damage, sug-
gesting divergent functions for HP1 family members to different
types of DNA damage. In support of this idea, neuronal cells de-
derived from HP1-Δ-deficient mice but not HP1-α-deficient ani-
mals display genomic instability (Aucott et al., 2008). Recently,
it was shown that replication of pericentromeric heterochromatin
in mouse cells depends on binding of the p150 subunit of CAF-1
to the CSD of HP1 (Quivy et al., 2008). In analogy to these find-
ings, we favor a scenario in which HP1 proteins play a role in
reorganizing higher order chromatin structure, as recently sug-
gested by Kruhlak et al. (2006) and Solimando et al. (2009),
which is essential for DNA repair. In conclusion, our experi-
ments reveal an intriguing link between HP1 proteins and DNA
repair systems.

Materials and methods

Cell lines
Cell lines used in this study were HeLa, U2OS, CHOK1, NIH/3T3,
NIH/3T3 EGFP–HP1-Δ (Meteos-Langerok et al., 2007), VH110 hTERT fibro-
blasts, ATIR-deficient GM13636-hTERT Seckel cells (Bergink et al., 2006),
Suv3-9h double-knockout MEFs (provided by T. Jenuwien, Research Insti-
tute of Molecular Pathology, Vienna, Austria; Peters et al., 2001; Schotta
et al., 2004), and KU80-deficient XR-V15B CHO cells (Mari et al., 2006).
The NER-deficient SV40-immortalized cell lines were XP-FA (XP-C), XPDS
(XP-A), XP12KO (XP-A), XP23PV (XP-E), MEFs XPC−/−, and GSTAN (CS-B).
All cell lines were cultured as described previously (Luijsterburg et al.,
2007). For immunolocalization experiments, hTERT human fibroblasts
were grown to confluency for 10 d. Subsequently, cells were synchronized
in GO by keeping them for a minimum of 5 d in medium supplemented
with 0.2% FCS.

DNA constructs
HP1-α and HP1-β cDNA were ligated in frame with mRFP and SCP3a.
EGFP–HP1-Δ and EGFP–HP1-γ were provided by P. Hemmerich (Fritz
Lipmann Institute, Jena, Germany; Schmiedeberg et al., 2004). All constructs
were transiently transfected in various cell lines using Lipofectamine
2000 (Invitrogen). EGFP–HP1-Δ was stably expressed in mouse NIH/3T3
cells (Meteos-Langerok et al., 2007), and EGFP–HP1-α and EGFP–HP1-γ
were stably expressed in MRC5-SV cells. HP1-β (ΔCD) was tagged with
EGFP (Meteos-Langerok et al., 2007). EGFP–HP1-β (ΔCSD) and EGFP–HP1-β
(ΔShingle) were provided by T. Mistelli (National Cancer Institute, Bethesda,
MD; Cheulin et al., 2003), and EYFP-tagged CSD was provided by Y. Hiraoka
(Osaka University, Osaka, Japan). HP1-βT51A was created by overlap PCR
and fused to EYFP. XPC and DBD2 were fused to mVen. In addition, DBD2
was fused to mCherry (Luijsterburg et al., 2007). ACF1-EGFP was provided
by P.D. Varga-Weisz (Babraham Institute, Cambridge, England, UK). EYFP-
Su3-9H1 was provided by R.W. Dirks (Leiden University Medical Center,
Leiden, Netherlands). NBS1-mCherry was provided by J. Lukas (Institute of
Cancer Biology and Centre for Genotoxic Stress Research, Copenhagen,
Denmark). The cDNAs for SCP3a and mVen were provided by J. Goedhart
(University of Amsterdam, Amsterdam, Netherlands), and mCherry and
mRFP cDNA were provided by R.Y. Tsien (University of California, San
Diego, La Jolla, CA).

UV-C irradiation
UV lamp–induced damage was inflicted using a UV source containing
four UV lamps (TUV 9W PL-S; Philips) as described previously (Moné et al.,
2004; Luijsterburg et al., 2007) or a custom UV cross-linker (CL-
1000; UVP) containing two UV-C lamps. UV laser–induced damage was
inflicted by using a 2-mW pulsed (7.8 kHz) diode-pumped solid-state laser
emitting at 266 nm (Rapp OptoElectronic GmbH) as described previously
(Dinan et al., 2007).

Irradiation with x rays
We used an x-ray generator (150 kV; 15 mA; dose rate, 2.18 Gy/min;
HI 60; Pantak) to irradiate cells globally as previously described (Syuljasen
et al., 2004).

Irradiation with soft x rays
Cells were plated in custom-made culture dishes containing an ultrathin
Mylar bottom (Aten et al., 2004; Stap et al., 2008). The dishes were placed
on a soft x-ray source. We used a modified EG2 electron bombard-
ment evaporation source (VG Scienta), which was fitted with a carbon
anode to generate 277-eV photons. For detailed information about this type
of source, see Agarwal and Sparrow (1981). The source was operated at
3-kV electron energy and 8-mA electron current. The unit was mounted in
a vacuum chamber (P < 10−3 Torr) equipped with a Mylar film window
(2-µm thick), which was supported by a stainless steel grid (1-mm-maze
size) to withstand atmospheric pressure. Cells were irradiated through a
metal filter (Stork Veko BV) with pores of 5 µm to inflict local damage. Cells
were irradiated for 6 min, corresponding with a dose of ∼20 Gy.

α-Particle irradiation
Cells were cultured in carbon-coated Mylar dishes. Cells were irradiated
using 241Am (Am-241) source with an activity of 140 kBq at on
angle of 30° with the horizontal plane to obtain long linear arrays of DSBs. The radioactive source just contacts the Mylar as described previously (Stap et al., 2008). Cells were irradiated at room temperature for 0.5 min and subsequently fixed using paraformaldehyde (final concentration 2%).

**Laser irradiation after sensitization with Hoechst**

U2OS cells expressing EGFP-HF1-β were sensitized with 10 μg/ml Hoechst 33342 (Invitrogen) for 5 min and locally irradiated (five iterations) in a strip spanning the nucleus using a 405-nm laser at 70% output. NBS1-mCherry was used as a marker for laser-induced damage.

**UV-A (337 nm) irradiation after sensitization with BrdU**

U2OS cells expressing EGFP-HF1-β were incubated with 10 μM BrdU one day before irradiation. Cells were irradiated in a strip spanning the nucleus with a 30-Hz 337-nm laser (PALM Laser Technologies) at ~61% laser output. For more details, see Lukas et al. (2003).


