The impact of heterochromatin on DSB repair

Aaron A. Goodarzi, Angela T. Noon and Penny A. Jeggo1
Genome Damage and Stability Centre, University of Sussex, Brighton BN1 9RQ, U.K.

Abstract
DNA NHEJ (non-homologous end-joining) is the major DNA DSB (double-strand break) repair pathway in mammalian cells. Although NHEJ-defective cell lines show marked DSB-repair defects, cells defective in ATM (ataxia telangiectasia mutated) repair most DSBs normally. Thus NHEJ functions independently of ATM signalling. However, ~15% of radiation-induced DSBs are repaired with slow kinetics and require ATM and the nuclease Artemis. DSBs persisting in the presence of an ATM inhibitor, ATMi, localize to heterochromatin, suggesting that ATM is required for repairing DSBs arising within or close to heterochromatin. Consistent with this, we show that siRNA (small interfering RNA) of key heterochromatic proteins, including KAP-1 (KRAB (Krüppel-associated box) domain-associated protein 1), HP1 (heterochromatin protein 1) and HDAC (histone deacetylase) 1/2, relieves the requirement for ATM for DSB repair. Furthermore, ATMi addition to cell lines with genetic alterations that have an impact on heterochromatin, including SuV39H1/2 (suppressor of variegation 3–9 homologue 1/2)-knockout, ICFa (immunodeficiency, centromeric region instability, facial anomalies syndrome type a) and Hutchinson–Guilford progeria cell lines, fails to have an impact on DSB repair. KAP-1 is a highly dose-dependent, transient and ATM-specific substrate, and mutation of the ATM phosphorylation site on KAP-1 influences DSB repair. Collectively, the findings show that ATM functions to overcome the barrier to DSB repair posed by heterochromatin. However, even in the presence of ATM, γ-H2AX (phosphorylated histone H2AX) foci form on the periphery rather than within heterochromatic centres. Finally, we show that KAP-1’s association with heterochromatin is diminished as cells progress through mitosis. We propose that KAP-1 is a critical heterochromatinic factor that undergoes specific modifications to promote DSB repair and mitotic progression in a manner that allows localized and transient chromatin relaxation, but precludes significant dismantling of the heterochromeric superstructure.

Introduction
ATM (ataxia telangiectasia mutated) is a PIKK (phosphoinositide 3-kinase-like kinase) that lies at the heart of a signal transduction response to DNA DSBs (double-strand breaks) [1]. The very existence of a highly complex DSB signalling response attests to the significance of DSBs as lesions promoting genomic instability and/or cell death. The dramatic clinical features caused by loss of ATM in A-T (ataxia telangiectasia) patients serves further to demonstrate the importance of an appropriate response to DSB formation [2]. ATM signalling activated by DSB formation promotes cell-cycle-checkpoint arrest, apoptosis and also influences DSB repair. Whereas apoptosis functions to remove damaged cells from the cycling population, cell-cycle-checkpoint arrest can both enhance the opportunity for repair and serve as an alternative to apoptosis to limit the proliferative capacity of damaged cells. DNA NHEJ (non-homologous end-joining) functions as the major DSB-rejoining pathway in mammalian cells [3]. A-T cell lines rejoin the majority of DSBs with normal kinetics, demonstrating that most NHEJ occurs independently of ATM signalling [4,5]. However, the rejoining of ~15% of IR (ionizing radiation)-induced DSBs require ATM and additional proteins that function in the ATM signal transduction process, including γ-H2AX (phosphorylated histone H2AX) and 53BP1 (p53-binding protein 1) [5]. Previous studies have demonstrated that, although the majority of DSBs are repaired with fast kinetics in mammalian cells, a subset of breaks is rejoined with slower kinetics [6]. Strikingly, ATM is required for the slow component of DSB repair, which represents a ~15% subset of X- or γ-ray-induced DSBs [5]. ATM-dependent DSB repair also requires the nuclease Artemis [5]. Artemis nuclease activity has the capacity to cleave hairpin-ended DSBs and to remove 3’- or 5’-single-stranded overhangs following remodelling of the DNA ends by the DNA-PK (DNA-dependent protein kinase) [7,8]. Furthermore, Artemis is an ATM-dependent substrate after radiation [5,9–11]. For this and other reasons, it was suggested that ATM may function to promote Artemis-dependent end processing before rejoining by NHEJ [5]. However, in a more recent study, a closer look at the ATM-dependency for DSB repair following exposure to a range of agents producing DNA ends of differing complexity suggested that there was only a weak correlation between end...
DSBs that persist following IR treatment in the absence of ATM kinase activity are localized around the periphery of chromocentres

(A) DMSO or 10 μM KU55933 ATMi was added to confluent NIH 3T3 cells. After 30 min, cells were irradiated with 2 Gy of IR and harvested 0.5 or 24 h after IR treatment, as indicated. Cells were fixed and immunostained for γ-H2AX (red) and DAPI (green). Images shown are representative of the total cell population. At 0.5 h after IR treatment, ~20% of DSBs overlap or touch chromocentres. In contrast, by 24 h, ~70% of DSBs are in contact with the periphery of chromocentres. (B) NIH 3T3 cells were treated with or without ATMi as in (A) and irradiated with 2 Gy of IR. Cells were fixed at the times indicated and immunostained as in (A). Total γ-H2AX foci and γ-H2AX foci overlapping with the periphery of heterochromatic regions (assessed by DAPI staining) were enumerated. Euchromatic numbers were estimated by subtracting the heterochromatin number of foci from the total number. Results are means ± S.D. for three independent experiments.

Knockdown or loss of heterochromatic building factors relieves the requirement for ATM for DSB repair

The findings above predicted that reducing the heterochromatin density content might diminish the requirement for ATM for DSB repair. A range of DNA and histone modifications, as well as the recruitment of specific heterochromatic building factors, function co-ordinately to create heterochromatic DNA [16,17]. DNA methylation by DNA methyltransferases is the most significant DNA modification, whereas histone H3 acetylation promotes a more open chromatin conformation and histone H3 methylation at Lys9 (H3K9me) confers a more closed conformation. The HDACs (histone deacetylases) HDAC1, HDAC2 and HDAC3 are important factors, function co-ordinately to create heterochromatic DNA [16,17]. DNA methylation by DNA methyltransferases is the most significant DNA modification, whereas histone H3 acetylation promotes a more open chromatin conformation and histone H3 methylation at Lys9 (H3K9me) confers a more closed conformation. The HDACs (histone deacetylases) HDAC1, HDAC2 and HDAC3 are important for DSB repair.
Table 1 | DSB repair has a diminished dependence on ATM when heterochromatin is impaired

The results show the number of γ-H2AX foci present 24 h after 3 Gy of IR in the absence (first column) or presence (second column) of ATMi. ATMi was added 30 min prior to irradiation. Data are only shown for the 24 h time point. Full analysis is given in [12]. In some instances, there was a high background number of γ-H2AX foci. We assessed whether the number of foci was elevated relative to this background. Thus, for example, following HDAC1/2 siRNA, there were elevated γ-H2AX foci in the absence of ATMi, but this was not increased further by ATMi addition, and the foci remaining were fewer than observed in cells treated with scrambled siRNA and ATMi. Thus we consider that HDAC1/2 siRNA at least partially alleviates the requirement for ATM for DSB repair.

<table>
<thead>
<tr>
<th>Treatment or cell line</th>
<th>DMSO-treated cells</th>
<th>ATMi-treated cells</th>
</tr>
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<tbody>
<tr>
<td>Scrambled siRNA</td>
<td>2.1 ± 0.4</td>
<td>10.7 ± 0.8</td>
</tr>
<tr>
<td>KAP-1 siRNA</td>
<td>3.2 ± 1.5</td>
<td>3.6 ± 1.0</td>
</tr>
<tr>
<td>HP1αβγ γ siRNA</td>
<td>2.8 ± 1.5</td>
<td>4.4 ± 0.4</td>
</tr>
<tr>
<td>HDAC1/2 siRNA</td>
<td>5.2 ± 1.1</td>
<td>5.8 ± 1.3</td>
</tr>
<tr>
<td>SUV39H1/2 +/- MEFs</td>
<td>0.8 ± 0.4</td>
<td>8.6 ± 0.4</td>
</tr>
<tr>
<td>SUV39H1/2 +/- MEFs</td>
<td>1.9 ± 0.4</td>
<td>5.0 ± 0.4</td>
</tr>
<tr>
<td>Normal fibroblasts</td>
<td>0.9 ± 0.2</td>
<td>9.3 ± 0.6</td>
</tr>
<tr>
<td>HGPS fibroblasts</td>
<td>1.1 ± 0.3</td>
<td>5.0 ± 0.6</td>
</tr>
<tr>
<td>ICFa fibroblasts</td>
<td>5.2 ± 2.6</td>
<td>7.5 ± 2.6</td>
</tr>
</tbody>
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in removing heterochromatin-inhibiting histone H3 acetylation, whereas Suv39H1/2 (suppressor of variegation 3–9 homologue 1/2) are important SET (suppressor of variation 3–9, enhancer of zeste, trithorax)-domain histone methyltransferases. Additionally, HP1 and the co-repressor, KAP-1, are critical heterochromatin building proteins [18]. Heterochromatin building occurs in an interwoven manner with loss of any component adversely affecting compaction similar to the demise of a carefully stacked pack of cards following the removal of any single card. We therefore examined the impact of a range of critical heterochromatic components on the ATM-dependency for DSB repair. Strikingly, we observed that knockdown or loss of any one of several heterochromatin factors relieved or reduced the DSB-repair defect imparted by the addition of ATMi [12] (summarized in Table 1). Thus, following KAP-1 siRNA (small interfering RNA), DSBs were repaired at a similar rate to that observed in control cells with or without addition of ATMi. Similar results were obtained following HDAC1/2 siRNA or HP1 siRNA. Furthermore, mouse embryonic fibroblasts knocked out for the Suv39H1/2 histone methyltransferases, which show reduced HP1 localization within pericentric heterochromatin, also showed a diminished DSB-repair defect following ATMi addition, reflecting a reduced dependency on ATM [19]. We also examined cells from ICFa (immunodeficiency, centromeric region instability, facial anomalies syndrome type a) and HGPS (Hutchinson–Gilford progeria syndrome), both of which show progressive heterochromatin disorganization, and, in both cases, observed a diminished DSB repair defect 24 h after ATMi addition compared with normal human cell lines. Collectively, these results suggest that the manipulation of essential heterochromatic building factors, although not affecting the normal rate of DSB repair as such, has an impact on the requirement for ATM for DSB repair, consolidating the notion that ATM is specifically needed for repair of DSBs located within or close to heterochromatic DNA.

KAP-1 is a highly DSB dose-dependent and specific ATM substrate

KAP-1 harbours the motif LSSQE, which encompasses the consensus ATM-phosphorylation motif (S/T/Q). We raised antibodies that recognize KAP-1 phosphorylated at the predicted ATM-phosphorylation site (Ser824). Western blotting using these anti-pSer824-KAP-1 (pKAP-1) antibodies confirmed that, although there was no detectable signal in undamaged cells, they recognize an IR-inducible substrate that has the mobility of KAP-1 (Figure 2). Moreover, KAP-1 phosphorylation was almost entirely ATM-dependent, with no detectable signal observed in an ATM−/− lymphoblastoid cell line (LBL) and no significant reduction being found in an ATR (ATM-and Rad3-related)-deficient LBL (ATR-SS cells) following treatment with IR or etoposide, an agent that induced DSBs following topoisomerase II inhibition (Figure 2). Furthermore, we did not detect any evidence of pKAP-1 following exposure to hydroxyurea or UV agents which do not directly induce DSBs. These findings strongly suggest that KAP-1 is an unusually specific ATM substrate that is only induced by direct DSB induction. Additionally, pKAP-1 was observed at early times post-IR (10–30 min, depending on dose), its magnitude was highly dose-dependent and was relatively transient with its rate of decay being dose-dependent, largely disappearing by 6 h post-physiological exposure levels.

Importantly, ectopic expression of S824A KAP-1 in cells that were knocked down for endogenous KAP-1 showed a similar DSB-repair defect to that observed in A-T cell lines, whereas expression of S824D KAP-1 conferred repair kinetics that were similar to wild-type and unaffected by ATMi addition [12]. Collectively, these findings argue that Ser824 is a critical phosphorylation site on KAP-1, which is regulated by ATM-dependent phosphorylation and is required for ATM-dependent DSB repair.

Heterochromatin is a barrier to DSB repair and signalling which ATM partially relieves

The findings above, taken together with additional published observations [12], strongly suggest that ATM is specifically required to repair DSBs that arise within or proximal to heterochromatin, which is achieved by phosphorylation of KAP-1, a co-repressor that interacts with HP1. The finding that ATM is specifically required for the slow component of DSB repair and that DSBs associated with heterochromatin are repaired with slower kinetics compared with DSBs located within euchromatin [12], suggest that heterochromatin poses a barrier to the DSB repair that is relieved, at least in part, by...
Figure 2 | KAP-1 is an efficient and specific ATM phosphorylation substrate

(A) GM02188 [WT (wild-type)], GM03189D (A-T) and DK0064 [hypomorphic ATR-SS (ATRhyp)] were irradiated with 0, 2, 5 or 10 Gy of IR and harvested 30 min later, irradiated with 0, 10, 20 or 40 J/m² UVC (UV) radiation and harvested 120 min later, treated with 1, 2 or 5 mM hydroxyurea (HU) and harvested 120 min later or treated with 5, 10 or 20 μM etoposide (ETP) and harvested 120 min later. KAP-1 was immunoblotted as in Figure 1. Then, 40 μg of whole-cell extract (prepared as described previously [12]), was immunoblotted for either pSer824 KAP-1 (pKAP1) or total KAP-1. (B) GM02188 (WT) cells were exposed to the indicated doses of IR and examined from 0.1 to 8 h post-IR for levels of phosphorylated KAP-1 (pKAP1) and total KAP-1 as described in (A).

ATM. We aimed to evaluate how KAP-1 phosphorylation impacts upon heterochromatin. In Figure 1, we show that, in the presence of ATMi, the persisting γ-H2AX foci at 24 h after 3 Gy of IR are located on the periphery of the densely DAPI-staining chromocentres. It is striking that, although the γ-H2AX foci partially encroach into the densely DAPI-staining regions, complete overlap is rarely observed. These findings raise the possibility that ATM-dependent KAP-1 phosphorylation might overcome the barrier to γ-H2AX foci expansion posed by heterochromatin. We therefore examined the relationship between γ-H2AX foci formation and chromocentre location in wild-type cells, where ATM is functional. We observed that the γ-H2AX foci persisting at 0.5 h after 1 Gy of IR were, like those remaining in the presence of ATMi, predominantly located around the periphery of the chromocentres (Figure 3). Using high-resolution imaging, we delineated the chromocentre regions (shown in blue), the γ-H2AX foci (green) and the regions of overlap (red). The regions of overlap are predominantly on the edge of the chromocentres, consistent with the notion that heterochromatin poses a barrier to signal expansion. Strikingly, there was only limited encroachment of γ-H2AX formation into the chromocentric mass (Figure 3), similar to our previous observations in cells treated with ATMi [12]. These findings therefore argue that, although ATM phosphorylation events may serve to relax the heterochromatin to an extent that allows DSB repair, they do not result in overt dismantling of the heterochromatic superstructure and do not fully relieve the barrier to foci expansion posed by heterochromatin. In this context, it is also important to note that the densely DAPI-staining chromocentres in KAP-1 siRNA-treated NIH 3T3 cells are clearly detectable albeit with moderately diminished levels of H3K9me3 (see supplementary material in [12]).

The distribution of KAP-1 on chromatin is reorganized during mitosis

The results described above and published findings suggest that ATM's impact on heterochromatin occurs via phosphorylation of KAP-1 on Ser824, which modifies KAP-1 binding to heterochromatin and triggers heterochromatin relaxation [12,14]. The interaction of HP1 with chromatin during interphase is promoted by H3K9me3. Notably, although H3K9me3 does not alter during mitosis, the binding of HP1 to chromatin has been reported to diminish [20]. Rather than affecting the histone-methylation code, evidence suggests that mitotic cells regulate HP1 chromatin binding via histone H3 Ser10 phosphorylation by Aurora B kinase [21]. Driven in part by our goal to examine ATM's role in DSB repair in G2-phase, we examined the localization of KAP-1 during the cell cycle. Immunofluorescence analysis of G1-phase cells using α-KAP-1 antibodies showed a pronounced enrichment of KAP-1 at the densely DAPI-staining chromocentres, consistent with the substantial evidence that these represent regions of heterochromatin, to which KAP-1 is preferentially localized (Figure 4A). In G1-phase, these densely staining chromocentres were also enriched for H3K9me3, but were depleted of the euchromatic marker, H3K9ac (histone H3...
acetylated at Lys\(^9\)) (Figures 4B and 4C). In contrast, in G\(_2\)-phase, the DAPI regions were more diffuse compared with G\(_1\)-phase and contained KAP-1, H3K9me3 and H3K9ac, suggesting that these regions no longer uniquely represent heterochromatic chromocentres (Figures 4B and 4C). We monitored KAP1 localization through G\(_2\)/M-phase using phospho-H3 staining to delineate cell-cycle positions. Cells with minimal (but detectable) phospho-H3 signal, determined to be at the very beginning of G\(_2\)-phase, have similar KAP-1-enriched chromocentres to G\(_1\)/S-phase cells. As the levels of phospho-H3 increase and cells progress through G\(_2\)-phase towards the mitotic boundary, some (but not all) KAP-1 is redistributed from densely DAPI-stained regions to give pan-nuclear staining, indicating the active reorganization of heterochromatin in preparation for mitotic chromosome hypercondensation (Figure 4D). It was notable that the pan-nuclear KAP-1 localization observed during mid/late-G\(_2\)-phase was unaffected by detergent extraction (Figure 4E), suggesting that KAP-1 remains bound to chromatin, but that the densely DAPI-staining regions may less stringently represent ‘pure’ heterochromatic chromocentres. In further contrast, as cells progressed through mitosis, the localization...
Figure 4. KAP-1 is removed from chromatin during progression through mitosis

(A) Confluent G0/G1-phase NIH 3T3 cells were fixed and immunostained with KAP-1 (green), H3K9me3 (TriMeK9 H3, red) and DAPI (blue). (B) Exponential-phase NIH 3T3 cells were fixed and immunostained with H3K9ac (AcetylK9 H3, green), H3K9me3 (TriMeK9 H3, red) and DAPI (blue). (C) Exponential-phase NIH 3T3 cells were fixed and immunostained with H3K9ac (AcetylK9 H3, green), KAP-1 (red) and DAPI (blue). (D) Exponential-phase NIH 3T3 cells were fixed and immunostained with KAP-1 (green), histone H3 pSer10 (Phospho H3, red) and DAPI (blue). Progression through G2/M-phase was assessed by phospho-H3 staining and DAPI morphology. (E) Exponential-phase NIH 3T3 cells were first extracted with 0.1% (v/v) Triton X-100 in PBS for 30 s before being fixed and immunostained as in (D). NB: the specific morphologies of KAP-1, H3K9me3 and H3K9ac during G2-phase shown in (B) and (C) were confirmed by co-staining each with a G2/M-phase marker such as pSer10 of histone H3 or cyclin B1.

A T cell lines display dramatic radiosensitivity, which, more than 20 years ago, was proposed to be due to a defect in the repair of chromosomal DSBs [22]. Despite this early observation, consolidation of this notion was slow in surfacing, primarily because the majority of DSBs are repaired normally in A-T cells when assessed by physical methods such as PFGE (pulsed-field gel electrophoresis) [4]. Although DSB-repair defects were detected by PFGE, the modest impact and high doses necessitated by the technique limited the significance of the findings [4]. Later, A-T cells were demonstrated to display cell-cycle-checkpoint defects, and the biological impact of ATM loss was attributed to defective checkpoint function of KAP-1 was observed to be completely distinct from that of the DAPI staining, indicating the release of KAP-1 from chromatin during progression through mitosis (Figure 4). Thus, although KAP-1 partly co-localized with DAPI staining in very late G2-phase, in prophase, metaphase and anaphase, there was almost no KAP-1 co-localizing with DAPI staining. Strikingly, in late telophase, KAP-1 rapidly relocalized to chromatin. These findings closely parallel studies of HP1 localization during progression through mitosis [21]. Collectively, these findings suggest that, in G2-phase, heterochromatic DNA becomes more diffusely organized than in G1-phase and during transition through mitosis both KAP-1 and HP1 are released from the chromatin.

Discussion

A-T cell lines display dramatic radiosensitivity, which, more than 20 years ago, was proposed to be due to a defect in the repair of chromosomal DSBs [22]. Despite this early observation, consolidation of this notion was slow in surfacing, primarily because the majority of DSBs are repaired normally in A-T cells when assessed by physical methods such as PFGE (pulsed-field gel electrophoresis) [4]. Although DSB-repair defects were detected by PFGE, the modest impact and high doses necessitated by the technique limited the significance of the findings [4]. Later, A-T cells were demonstrated to display cell-cycle-checkpoint defects, and the biological impact of ATM loss was attributed to defective checkpoint function.
order chromatin structure is not dismantled following IR-
KAP-1 phosphorylation is unclear and, clearly, the higher-
heterochromatic structure. However, the precise impact of
ings suggest that ATM-dependent KAP-1 phosphorylation
in an ATM-dependent manner. Taken together, these find-
enriched for heterochromatic proteins diminished after IR
previously that the presence of KAP-1 in a chromatin fraction
enhanced accessibility respectively. Additionally, we showed
they showed that KAP-1 siRNA also increased accessibility
nuclease to DNA in an ATM-dependent manner. Critically,
phosphorylation being rapidly lost as DSB repair proceeds.
Finally, KAP-1 is a highly sensitive ATM substrate with its
superstructure including KAP-1, HDAC1/2, Suv39H1/2 and
loss of multiple factors that contribute to the heterochromatic
in the repair of HC-DSBs. We also show that knockdown or
specifically defective in the slow component of DSB repair, i.e.
that form in A-T cells are smaller than those in control cells,
we observed a similar magnitude of γ-H2AX encroachment
into the chromocentres in either the presence or absence
of ATM kinase activity. Furthermore, the γ-H2AX foci
that remain at later times in a control or an A-T cell line
are preferentially located at the periphery of chromocentres
and show a similar magnitude of overlap with them. This
is consistent with our finding that DSBs associated with
chromocentres (HC-DSBs) are repaired with slower kinetics
than DSBs located within euchromatic DNA and that A-T is
specifically defective in the slow component of DSB repair, i.e.
in the repair of HC-DSBs. We also show that knockdown or
loss of multiple factors that contribute to the heterochromatic
superstructure including KAP-1, HDAC1/2, Suv39H1/2 and
HP1 can relieve the requirement for ATM for DSB repair.
Finally, KAP-1 is a highly sensitive ATM substrate with its
phosphorylation being rapidly lost as DSB repair proceeds.
We also showed previously that KAP-1 phosphorylation at Ser289
is required for its role in ATM-dependent DSB repair [12]. Collectively, these findings strongly suggest that,
following the introduction of DSBs, ATM phosphorylates
KAP-1, which promotes the repair of DSBs located within
or close to heterochromatic DNA. This raises the question
of how KAP-1 phosphorylation has an impact on the hetero-
chromatic superstructure. Ziv et al. [14] showed previously
that exposure to neocarzinostatin, a radiomimetic DNA-
damaging agent, increased the accessibility of micrococcocal
nuclease to DNA in an ATM-dependent manner. Critically,
they showed that KAP-1 siRNA also increased accessibility
and that expression of S824A or S824D KAP-1 prevented or
enhanced accessibility respectively. Additionally, we showed
previously that the presence of KAP-1 in a chromatin fraction
enriched for heterochromatic proteins diminished after IR
in an ATM-dependent manner. Taken together, these find-
ings suggest that ATM-dependent KAP-1 phosphorylation
modifies its chromatin binding, which, in turn, influences heterochromatic structure. However, the precise impact of
KAP-1 phosphorylation is unclear and, clearly, the higher-
order chromatin structure is not dismantled following IR-
induced KAP-1 phosphorylation. We therefore suggest that
KAP-1 phosphorylation confers sufficient localized hetero-
chromatic relaxation to allow DSB repair without necessi-
tating significant disassembly of the higher-order chromatin
structure. Possibly consistent with this, we observed that
the duration and extent of KAP-1 phosphorylation is short
and highly dose-dependent. We thus propose that the DSB
response restricts the duration, location and extent of KAP-1
phosphorylation to limit the overall impact on higher-order
structure, but promotes localized DSB repair. In contrast, we
show that KAP-1’s distribution and chromatin association
is altered dramatically as cells progress through mitosis,
similar to the release observed previously for HP1. Global
chromatin alterations are required to mediate the dramatic
DNA condensation needed for cell division, which coincides
with a significant decrease in many nuclear processes (e.g.
transcription). In this context, it is perhaps not surprising that
the mechanisms of chromatin alteration following DSB form-
ation and mitotic entry are distinct. Finally, we demonstrate
that KAP-1 is phosphorylated predominantly by ATM with
minimal phosphorylation contributed by ATR following,
for example, replication-fork stalling or UV irradiation.
It is likely that, since higher-order chromatin structure is
dismantled during replication, KAP-1 phosphorylation
might be less important for DSB repair during S-phase or
at collapsed replication forks, thus eliminating the need for
ATR to phosphoryse this substrate. Given the fact that the
majority of DSB-response substrates are phosphorylated re-
dundantly by ATM and ATR, the unusual specificity of KAP-
1 phosphorylation is noteworthy and potentially important.
In summary, KAP-1 is a strong and functionally critical
ATM substrate, whose phosphorylation is highly dose-
dependent and transient. Collectively, the emerging data
suggest that KAP-1 is a ‘keystone’ heterochromatic building
factor that can become modified after DSB formation as well
as during the cell cycle to allow critical, but highly defined,
changes to the higher-order structure necessary for DSB
repair and potentially for events during mitosis.

Funding
Work in the P.A.J. laboratory is supported by the Medical Research
Council, the International Association for Cancer Research, the
Wellcome Trust and European Union grant [grant number
FIHG-CT-200200207] (DNA Repair).

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to ionizing radiation is associated with a repair deficiency of DNA

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Received 8 December 2008
doi:10.1042/BST0370569