The role of chromatin proteins in DNA damage recognition and repair. Histochem Cell Biol

ARTICLE *in* HISTOCHEMIE · FEBRUARY 2006

Impact Factor: 3.05 · DOI: 10.1007/s00418-005-0053-5 · Source: PubMed

3 AUTHORS, INCLUDING:

Piotr Widlak
Maria Sklodowska Curie Memorial Cancer C…
119 PUBLICATIONS **1,996** CITATIONS
SEE PROFILE

Monika Pietrowska
Cancer Center and Institute of Oncology
58 PUBLICATIONS **351** CITATIONS
SEE PROFILE

Histochem Cell Biol (2006) 125: 119–126
DOI 10.1007/s00418-005-0053-5

REVIEWS

Piotr Widlak · Monika Pietrowska · Joanna Lanuszewska

The role of chromatin proteins in DNA damage recognition and repair

Published online: 15 September 2005

Abstract The structure of chromatin is the major factor determining the rate and efficiency of DNA repair. Chromatin remodeling events such as rearrangement of nucleosomes and higher order chromatin structures are indispensable features of repair processes. During the last decade numerous chromatin proteins have been identified that preferentially bind to different types of DNA damage. The HMGB proteins, which preferentially interact with DNA intrastrand crosslinks induced by cisplatin, are the archetypal example of such proteins. Several hypothetical models have been proposed describing the role of such damage-binding chromatin proteins. The damage shielding model postulates that binding of chromatin proteins to damaged DNA might disturb damage recognition by repair factors and impair its removal. Alternatively, the damage-recognition/signaling model proposes that the binding of specific chromatin proteins to damaged DNA could serve as a hallmark to be recognized by repair proteins. Additionally, the binding of specific chromatin proteins to damaged DNA could induce chromatin remodeling at the damage site and indirectly affect its repair. This paper aims to critically review current experimental data in relation to such possible roles of chromatin proteins.

Keywords Damage recognition · DNA repair · Chromatin · Damaged DNA-binding proteins

P. Widlak (✉) · M. Pietrowska · J. Lanuszewska
Department of Experimental and Clinical Radiobiology,
Maria Sklodowska-Curie Cancer Center and Institute of Oncology,
Wybrzeze AK 15, 44-100 Gliwice,
Poland
E-mail: widlak@io.gliwice.pl
Tel.: +48-32-2789672
Fax: +48-32-2313512

Introduction

The genomes of all organisms are prone to physical and chemical assaults coming from either endogenous sources or the environment. Some genotoxic agents can induce modification (or loss) of bases; among them are alkylating agents or UV radiation. Others, like ionizing radiation and reactive oxygen species, lead to the formation of breaks in the phosphodiester backbone of DNA as well as oxidative modifications of bases. All of these lesions are particularly dangerous for proliferating cells and DNA damage must be efficiently recognized and removed before DNA replication and chromosome segregation. Organisms have evolved different mechanisms to repair damaged DNA. Modified bases are usually repaired in excision repair pathways. Among them are: (a) Nucleotide Excision Repair (NER) that removes bulky modifications of bases induced by UV or aromatic compounds; (b) Base Excision Repair (BER) that removes alkylated or oxidatively modified bases; and (c) Mismatch Repair (MMR) that replaces misincorporated nucleotides with the correct ones. Broken DNA ends and interstrand cross-links are repaired in recombination-based mechanisms. In addition, DNA damage checkpoints delay cell cycle progression, providing more time for repair of lesions before DNA replication and chromosome segregation. All repair pathways start from recognition of DNA damage, which depends on specific sensor proteins that recognize either modified bases or altered DNA helical structure. For example, XPC-HR23B, XPA-RPA and XPE protein complexes recognize a broad spectrum of DNA lesions and seem to be general recognition factors indispensable in NER (though the first one is not essential for repair of the transcribed strand in the transcription-coupled subpathway of NER) (reviewed in Sancar et al. 2004). It is obvious, yet frequently not deeply considered, that the process of DNA repair must be connected with vast structural changes in chromatin—the true physiological substrate for all DNA transactions in the cell nucleus.

The genetic material of eukaryotic cells is packed into a nucleoprotein complex termed chromatin. The fundamental structural unit of chromatin is the nucleosome, which contains 146 base pairs of DNA wrapped around an octamer of core histones (two copies each of H2A, H2B, H3 and H4) forming the core particle. In addition, the nucleosome consists of a linker region of variable length (usually less than 50 base pairs). Linker DNA interacts with the linker histone H1 and/or specific non-histone chromatin proteins, primarily certain HMG proteins. The polynucleosomal chain is further looped and folded into various higher order structures. The organization of chromatin domains (or loops) seems to be maintained by anchorage of specific DNA sequences to a nucleoskeleton protein network. Such packing of DNA provides a compaction and organization for the replication, recombination and repair processes and is the major epigenetic factor determining the expression of genetic information. Nucleosomes positioned in the regulatory regions of genes often hinder the accessibility of binding sites for transcription factors. In addition, formation of specific chromatin structures leads to transcriptional repression of extended chromatin regions. Among the different mechanisms that act on chromatin to regulate transcription are non-covalent ATP-dependent remodeling of nucleosome structure and covalent post-translational modifications of histones. The most common modifications of core histones are acetylation/deacetylation, methylation, phosphorylation, ubiquitination and polyADP-ribosylation, which determine the so-called ''histone code''. The structural transitions triggered by histone modifications and/or chromatin remodeling ''machines'' facilitate the binding of transcription factors to gene regulatory regions, which allows assembling of the RNA polymerase complex to activate transcription (reviewed in Narlikar et al. 2002; Felsenfeld and Groudine 2003; Iizuka and Smith 2003; Peterson and Laniel 2004).

At the moment, knowledge about the nature of chromatin structural changes related to repair processes is much less complete. However, it is generally accepted that in principle, similar mechanisms of chromatin remodeling may be involved in both transcription and repair (reviewed in Meijer and Smerdon 1999). It is well recognized that chromatin structure determines the rate and efficiency of removal of DNA damage. Active (or potentially active) genes, which are organized in transcriptionally competent chromatin structures, are repaired faster than heterochromatin. Consequently, chromatin structural changes are required to increase the accessibility of damage to repair proteins. The activity of histone acetyltransferases and chromatin remodeling ''machines'' seems to be essential for nucleosome rearrangement during repair. In fact, several repair proteins (Rad16, CSB/Rad26, Rad5 and Rad54) belong to the SWI2/SNF2 family of helicases, which are typical components of chromatin remodeling ''machines'' (Fyodorov and Kadonaga 2001). Similarly, it has been reported that CBP/p300 histone acetyltransferase interacts with the p48 subunit of the XPE damage recognition factor (Datta et al. 2001). Once repair is completed, reconstitution of nucleosomes is required to recover the original primary chromatin structure. Such chromatin assembly is frequently coupled with repair DNA synthesis and depends on specific or general chromatin assembly factors (e.g. CAF1 and RCAF (Gaillard et al. 1996; Tyler et al. 1999). Disassembly/assembly of nucleosomes during DNA replication is apparently another factor that facilitates recognition and removal of DNA damage. The role of chromatin structure for DNA repair processes and mechanisms responsible for its rearrangement during repair have been addressed in several excellent review articles (Meijer and Smerdon 1999; Moggs and Almouzni 1999; Thoma 1999; Peterson and Cote 2004; Fousteri et al. 2005; Gong et al. 2005; Reed 2005; Reeves and Adair 2005; Thoma 2005).

Moreover, there is another aspect of the inter-relationship between chromatin and DNA repair—chromatin proteins that bind to DNA damage. Cellular proteins that preferentially bind damage-containing DNA are collectively called DDB (damaged-DNA-binding) proteins. In addition to damage sensors specific for DNA repair or involved in DNA damage checkpoints, many other proteins with distinct molecular functions show higher affinity for damaged DNA than to undamaged DNA of the same sequence. There are several chromatin proteins and transcription factors among such ''non-repair'' DDB proteins. It has been postulated that binding of chromatin proteins to damaged DNA might disturb damage recognition by repair factors and impair its removal. Alternatively, binding of specific chromatin proteins to damaged DNA could serve as a hallmark to be recognized by repair proteins. Additionally, binding of chromatin proteins to damaged DNA could induce chromatin remodeling at the damage site and indirectly affect its repair. The aim of this paper is to critically review current data regarding such possible roles of chromatin proteins.

DNA damage induced by cisplatin and its recognition by HMG-box containing proteins

Cisplatin (*cis*-diamminedichloroplatinum, *cis*-DDP) is one of the most effective chemotherapeutic anti neoplastic agents, whose action is believed to result from its interactions with DNA. The N7 atoms of guanine and adenine are the main binding sites for platinum complexes in DNA. The DNA adducts induced by cisplatin include monoadducts, interstrand and intrastrand DNA cross-links and DNA–protein cross-links (reviewed in Siddik 2003). Quantitative studies showed that 1,2-intrastrand d(GpG) and d(ApG) cross-links account for ~65 and 25%, respectively, of all the cisplatin adducts formed in vitro. Such 1,2-intrastrand cross-links produce severe local distortion in DNA

structure, bending the helix by 34° towards its major groove and unwinding it by 13° (Bellon et al. 1991).

The High-mobility-group (HMG) proteins are the largest group of non-histone chromosomal proteins found in all cells of higher eukaryotes. They are operationally defined as nuclear proteins extracted with 0.35 M NaCl that are soluble in 5% trichloroacetic (or perchloric) acid; they have relatively low molecular weights (below 30 kDa) and a high content of charged aminoacids. The HMG proteins probably serve as architectural elements rather facilitating, than performing specific functions in chromatin. The HMG proteins are grouped into three families, currently termed HMGB, HMGN and HMGA (previously termed HMG-1/−2, HMG-14/−17 and HMG-I/Y, respectively) (Bustin 2001a). Members of the HMGB family are the most abundant (~1 molecule per 10–15 nucleosomes) and highly conserved (two major proteins of this family, HMGB1 and HMGB2, share >80% identity). These proteins serve as structural components of chromatin and as ancillary transcription factors. The HMGB1 and HMGB2 have a tripartite structure. They possess an N-terminal A domain and a central B domain, which are called "HMG-boxes". These boxes are largely homologous and consist of ~80 highly basic residues forming three α-helices, which constitute non-specific DNA-binding regions. An additional C-terminal segment contains ~30 highly acidic residues and is involved in interactions with other proteins. The HMG-box is found in several other proteins, and HMG-box-containing proteins can be divided into two subgroups. One group includes HMGB1/2 as well as UBF and mtTFA (its nuclear testis-specific splicing variant is termed tsHMG), which contain two or more HMG-boxes and bind to chromatin with low sequence-specificity. The other subgroup, including SRY, SSRP and certain yeast non-histone chromatin proteins, consists of known or putative transcription factors that contain a single HMG-box embedded in a larger protein (reviewed in Bustin and Reeves 1996; Thomas and Travers 2001). All HMG-box-containing proteins interact with DNA primarily through contacts with the minor groove, similar to TBP binding to the TATA element, whose mode is responsible for low sequence-specificity of DNA binding. Binding of HMG-boxes to DNA induces characteristic bending, unwinding, looping or supercoiling. In addition, HMG-box-containing proteins (as well as isolated HMG-boxes) preferentially bind to DNA that is intrinsically bent and underwound or organized as cruciforms or four-way junctions. This feature of HMG-boxes explains their ability to bind to the 1,2-intrastrand DNA cross-links induced by cisplatin (reviewed in Landsman and Bustin 1993; Thomas and Travers 2001). The preferential binding of HMGB proteins to cisplatin-induced DNA cross-links was originally reported in the early 1990s by the groups of Lippard (Pil and Lippard 1992) and Billings (Hughes et al. 1992). Structural details of interactions between HMG-box and cisplatin-damaged DNA are well known; the crystal structure of the complex between domain A of HMGB1 and 1,2-intrastrand cisplatin adduct was solved by Lippard and co-authors (Ohndorf et al. 1999). Currently, numerous HMG-box-containing proteins have been shown to bind cisplatin-damaged DNA; some of them are listed in Table 1.

The damage-shielding model

The HMG-box-containing proteins have high affinity to bind to DNA damaged by cisplatin, especially to the most toxic 1,2-intrastrand cross-links, but not to DNA damaged by its much less cytotoxic *trans* isomer (*trans*-DDP). This suggests that such proteins could be involved in the sensitivity of cells to cisplatin due to their interfering with recognition and repair of cisplatin-induced lesions. Two possible models have been initially proposed. The HMG-box proteins bound to damaged DNA might serve as a recognition element for recruiting other repair proteins. The alternative model, called the damage-shielding model, assumes that HMG-box proteins bind tightly to damaged DNA and prevent access of the repair proteins to the sites of damage (Chu 1994). Several lines of evidence suggest that in fact HMG-box proteins do shield cisplatin–DNA adducts from repair and increase the sensitivity of cells to cisplatin. First, it has been shown that interruption of the gene encoding the *Saccharomyces cerevisiae* HMG-box protein Ixr1 resulted in desensitization of the yeast cells to cisplatin (Brown et al. 1993; McA'Nulty et al. 1996). Inversely, overexpression of HMG-box proteins increases sensitivity of human cells to cisplatin, which was shown for HeLa cells ectopically expressing tsHMG (Zamble et al. 2002) or MCF-7 cells with up-regulated HMGB1 (He et al. 2000). In addition, the results of several in vitro experiments have shown that HMG-box proteins do reduce the repair efficiency of cisplatin-damaged DNA. Such experiments revealed that a variety of HMG-box proteins, including HMGB1 and tsHMG, block the removal of cisplatin 1,2-intrastrand d(GpG) adducts when added in a NER assay (Huang et al. 1994). Moreover, the efficiency of in vitro NER in the presence of HMGB1 depends on the affinity of HMGB1 to bind to particular analogs of cisplatin (Malina et al. 2002). Taking into account that some HMG-box proteins are transcription factors, one can assume that their binding to damaged DNA might affect the transcription processes as well. The transcription factor hijacking model proposes that cisplatin adducts sequester transcription factors away from appropriate promoters, resulting in the disruption of the transcription of target genes. The human ribosomal RNA transcription factor UBF binds to a 1,2-d(GpG) cisplatin adduct with the highest affinity known for a platinum adduct binding protein (K_d = 60 pM), apparently because of the presence of multiple HMG-boxes. Interestingly, it has been found that cisplatin causes redistribution of UBF in the nucleolus and inhibits the synthesis of rRNA in vivo (Zhai et al. 1998).

There are two aspects that make the damage-shielding model more general. First, adducts induced by cisplatin

Table 1 HMG-box-containing-proteins and other non-repair proteins that preferentially bind to cisplatin-DNA 1,2-intrastrand crosslinks

Protein	Number of HMG-boxes	Approximate mol weight (kDa)	Species	Reference
HMGB1	2	28	Mammals	Pil and Lippard (1992)
HMGB2	2	27		Hughes et al. (1992)
Testis-specific HMG (ts-HMG)	2	28	Mammals	Ohndorf et al. (1997)
Mitochondrial transcription factor A (mtTFA)				Yoshida et al. (2002)
Upstream-binding factor 1 (UBF1)	5	97	Mammals	Treiber et al. (1994)
Structure-specific recognition protein 1 (SSRP1)	1	81	Mammals	Bruhn et al. (1992)
Ixr1			*S. cerevisiae*	Brown et al. (1993)
Sex-determining region Y (SRY)	1	34	Mammals	Trimmer et al. (1998)
Non-histone chromatin protein 6A (Nhp6A)	1	11	*S. cerevisiae*	Wong et al. (2002)
Cytosine-mismatch binding 1 (Cmb1)	1	22	*S. pombe*	Kunz et al. (2003)
Histone H1	–	23	Mammals	Yaneva et al. (1997)
TATA-binding protein (TBP)	–	38	Mammals	Vichi et al. (1997)
Y-Box Binding Protein 1 (YB-1)	–	43	Mammals	Ise et al. (1999)

Table 2 Other types of DNA damage that are preferentially bound by HMG-box-containing proteins

Protein	Damaged DNA	Reference
HMGB1, HMGB2	Chromium damaged	Wang et al. (1997)
HMGB1, HMGB2	UV damaged	Pasheva et al. (1998)
HMGB1, HMGB2	AAAF damaged	Lanuszewska and Widlak (2000)
HMGB1, HMGB2	BPDE damaged	Lanuszewska and Widlak (2000)
mtTFA	8-oxo-dG	Yoshida et al. (2002)
mtTFA	AAAF damaged	MP and PW (unpublished)
Cmb1	O^6 -methyl-dG	Kunz et al. (2003)

are not the only DNA lesions recognized by HMG-box-containing proteins. In fact, several other types of DNA damage are recognized and bound by HMGB proteins (listed in Table 2). These include bulky DNA lesions caused either by other DNA-crosslinking agents (chromium, UVC-irradiation) or aromatic compounds that induce monofunctional adducts (examples shown in Fig. 1). More surprisingly, some HMG-box proteins show enhanced affinities to bind to lesions that barely distort the DNA helix: 8-oxo- and ^{6} *O*-methyl-guanines.

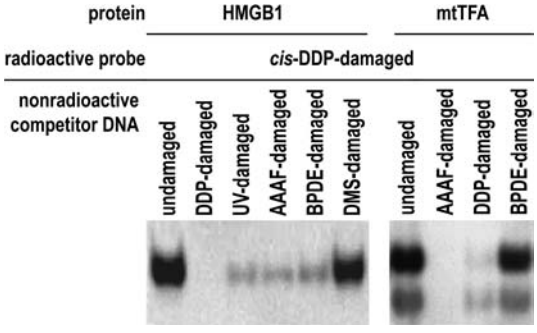

Fig. 1 HMGB proteins bind preferentially to different types of bulky DNA damage. Complexes between radioactive DNA damaged by cisplatin (*cis-DDP*) and human recombinant HMGB1 or mtTFA were analyzed using the gel band-shift assay. Complexes were formed in the presence of 50-fold excess of homologous non-radioactive competitor either undamaged or damaged with different genotoxic factors: cis-DDP, UVC radiation, *N*-acetoxy-acetylaminofluorene (*AAAF*), benzo(*a*)pyrene diol epoxide (*BPDE*) or dimethyl sulfate (*DMS*). Removal of the protein from radioactive complexes was in direct proportion to its affinity to the non-radioactive competitor

Second, HMG-box proteins are not the only chromatin proteins and/or transcription factors that have enhanced affinity to damaged DNA. In fact, histone H1, another abundant chromatin protein, has also been found to preferentially bind to cisplatin-modified DNA (Yaneva et al. 1997). Both HMGB and histone H1 bind to internucleosomal linker DNA in chromatin and may compete for the same binding sites. However, structural aspects of interactions between histone H1 and cisplatin-damaged DNA remain unclear at the moment. In addition to HMG-box proteins some other transcription factors, including the TATA-box binding protein TBP and Y-box binding proteins, have also been shown to preferentially bind cisplatin damaged DNA (Table 1).

Initially, the damage-shielding model assumed that HMG-box proteins (and, according to current knowledge, other chromatin proteins and transcription factors) shield damaged sites in chromatin and prevent recognition of adducts by repair factors, blocking their repair (Fig. 2a). Consequently, the persistence of unrepaired damage would induce apoptosis and lead to cell death (reviewed in Rich et al. 2000). However, the relationship between the binding of HMG-box proteins to damaged DNA and the induction of apoptosis could be much more complicated. It has been shown that the mechanism of cisplatin-induced apoptosis requires the p53 protein, at least in testicular cancer cells that favorably respond to cisplatin therapy (Zamble et al. 1998). Interestingly, HMGB1 interacts with p53 and functions as its co-activator, enhancing sequence-specific binding of p53. In addition, interactions between the C-terminal domain of p53 and domain A of HMGB1

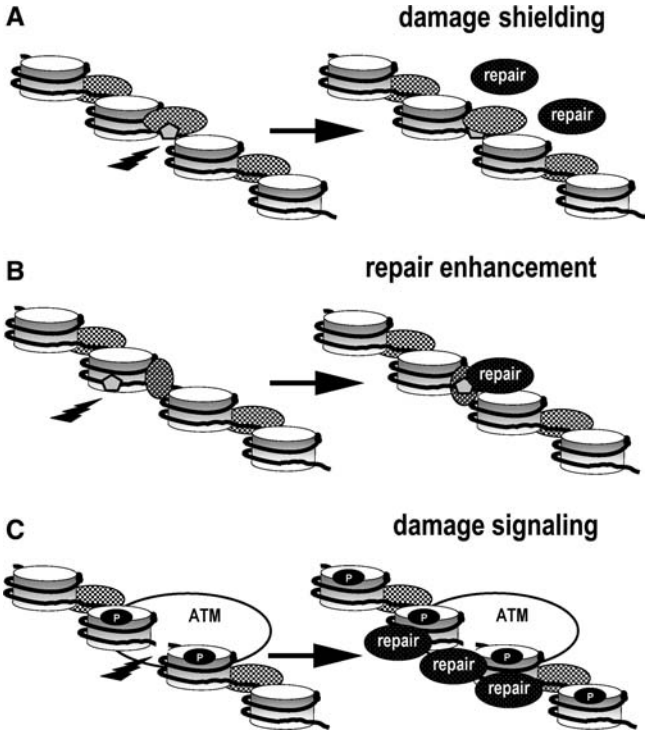

Fig. 2 Possible roles for damage-binding chromatin proteins. **a** Binding of a chromatin protein (exemplified by HMGB) to the damage (presented as a *pentagon*) shields the damage from repair. **b** rearrangement of nucleosomes induced by specific chromatin proteins makes the damage more accessible to repair factor and enhances its repair. **c** The presence of specific chromatin proteins (exemplified by phosphorylation of H2AX induced by DNA double-strand breaks) serves as a signal recognized by repair factors

increase the affinity of HMGB1 to bind to DNA damaged by cisplatin (Imamura et al. 2001). Similarly, p53 interacts with mtTFA and enhances its affinity to bind to cisplatin-damaged DNA, but reduces its affinity to bind to oxidatively damaged DNA (Yoshida et al. 2003). It is known that some apoptotic stimuli, including DNA damage and oxidative stress, trigger mitochondrial localization of p53 (Marchenko et al. 2000). All these findings suggest an intriguing possibility that binding of HMG-box proteins to damaged DNA, either in the nucleus or in mitochondria, might affect cell death due to their direct interactions with p53, a major cellular activator of apoptosis. However, the details of such a mechanism remain to be elucidated.

The repair of damaged DNA could be facilitated in the presence of specific chromatin proteins

It has been unequivocally demonstrated that the structure of chromatin is the major factor determining the rate and efficiency of DNA repair processes. The HMG proteins are generally believed to "fluidize" chromatin, making it accessible for transcription regulators (reviewed in Agresti and Bianchi 2003). Thus, it might be expected that binding of HMG proteins to damaged DNA would facilitate local repair due to promoting chromatin remodeling at sites of damage. One could propose that binding of HMG proteins to the damaged sites may promote nucleosome unfolding or nucleosome movement away from them so as to locate the damage to the much more favorably repaired internucleosomal linker (Fig. 2b).

In fact, there is clear evidence that HMGN proteins are required for efficient repair of UV-induced DNA damage in vivo. The HMGN proteins (also termed HMG-14/−17) have a high content of lysine residues, which is somewhat similar to linker histones. They can specifically interact with nucleosomal core particles and destabilize higher order structures of the chromatin fiber, being involved in activation of transcription and replication (reviewed in Bustin 2001b). In addition, chromatin unfolding induced by HMGN enhances repair by the NER system. It has been shown that HMGN1 enhances repair of UV-induced damage in chromatin, and cells lacking the *Hmgn1* gene are hypersensitive to UV (Birger et al. 2003). However, there is no evidence that HMGN proteins specifically interact with damaged DNA.

The data regarding involvement of possible HMGB-induced chromatin remodeling in different aspects of DNA repair are less clear at the moment. It has been shown that HMGB1 enhances MMR in extracts from human cells and directly interacts with the MutSα protein (Yuan et al. 2004). Similarly, HMGB1/2 enhances in vitro ligation of double-strand breaks by DNA ligase IV, being possibly involved in non-homologous end-joining repair (Nagaki et al. 1998). Interestingly, in contrast to other data mentioned above that support the damage-shielding model, it has been reported that HMGB1 is overexpressed in human epidermoid cancer cells that are resistant to cisplatin (Nagatani et al. 2001). Surprisingly, mouse embryonic fibroblasts from *Hmgb1*$^{-/-}$ animals do not differ in cisplatin sensitivity from wild-type counterparts (Wei et al. 2003). These data suggest that the influence of HMG-box proteins upon repair of cisplatin adducts and other bulky DNA lesions largely depends on a cell type context, and models that depict the role of HMGB proteins in DNA repair and cellular responses to stress may need to be re-evaluated. In fact, it has to be noted that in vitro experiments that validated an inhibitory role of HMGB proteins in repair of cisplatin-damaged DNA had been performed on non-chromatin substrates (Huang et al. 1994) and might not reflect the actual situation in vivo.

The damage-signaling model a lesson from histone H2AX

The model of "damage recognition" was among several models proposed after the discovery of preferential binding of HMGB proteins to cisplatin-damaged DNA (Pil and Lippard 1992). The model assumed that the

binding of HMG-box proteins to damaged DNA would serve as a recognition element recruiting other repair proteins. However, experimental evidence is not available for such a role. The hypothetical model of chromatin proteins serving as recognition elements for recruiting repair proteins to sites of DNA damage has received experimental support after the discovery of the specific phosphorylation of histone H2AX. There are three isoforms of histone H2A present in mammalian cells and among these, histone H2AX makes up 5–25% of the total H2A pool. Bonner and colleagues discovered that serine 139 in the unique carboxy-terminus of H2AX becomes phosphorylated following exposure of cells to agents that cause DNA double-strand breaks. The degree of such phosphorylation is directly proportional to the amount of DNA breaks that are introduced and reaches a maximum after 10–30 min (Rogakou et al. 1998). In fact, the phosphorylation of histone H2AX at serine 139 (so-called γ-H2AX) is a general cellular response to the formation of DNA double-strand breaks induced not only by DNA damaging factors but also by apoptosis and recombination processes (Rogakou et al. 2000; Chen et al. 2000). Phosphorylation of serine 139 of H2AX in response to double-strand breaks induced by ionizing radiation is catalyzed by ATM and DNA-PK serine/threonine kinases (Burma et al. 2001; Stiff et al. 2004). Surprisingly, 100–1000 of phosphorylated H2AX molecules are formed per DNA break, implying that a single double-strand break leads to the modification of a whole chromatin domain around the damaged site (Rogakou et al. 1999). Gamma H2AX forms microscopically visible foci (Rogakou et al. 1999), which are excellent and very sensitive markers of the induction of double-strand breaks. The induction and persistence of γ-H2AX foci correlates well with the radiosensitivity of cells; γ-H2AX foci persist longer in radiosensitive cells while radioresistant cells have a rapid loss of such foci (Taneja et al. 2004). Cells and animals lacking γ-H2AX are radiosensitive and show a characteristic phenotype, which involves chromosomal instability, growth retardation, immune deficiency and male infertility (Celeste et al. 2002; Bassing et al. 2002).

Most interestingly, γ-H2AX contributes to the assembly of specific DNA repair complexes on damaged DNA. Several essential factors involved in homologous recombination repair (Brca1 and Rad51) or non-homologous end joining (the complex of Rad50, Nbs1 and Mre11 (reviewed in Hefferin and Tomkinson 2005) of double-strand breaks form foci that co-localize with γ-H2AX foci. The formation of γ-H2AX foci is the earliest response to ionizing radiation (within a few minutes after irradiation), and Brca1 is recruited to such sites before Rad51 and the Mre11/Rad50/Nbs1 (MRN) complex (Paull et al. 2000). Gamma H2AX is essential for recruitment of Brca1 and MRN complex, but not for Rad51, to such radiation-induced foci (Celeste et al. 2002). In addition, proteins involved in DNA damage-induced cell cycle checkpoints also interact with γ-H2AX: 53BP1 (Ward et al. 2003) and Mdc1/NFBD1 (Lukas et al. 2004). Gamma H2AX binds with Nbs1 through the BRCT (BRCA1 C-terminal) domain of Nbs1, which is also present in other proteins interacting with γ-H2AX (Brca1, 53BP1 and Mdc1/NFBD1) (Kobayashi et al. 2002). Interestingly, it has been reported that ATM, BRCA, MRN complex and several other repair proteins may form large complexes termed BASC (BRCA-associated Surveillance Complex) (Wang et al. 2000). The physical interaction between γ-2AX and proteins containing the BRCT domain is indispensable for their massive recruitment to sites of DNA double-strand breaks. However, the role of γ-H2AX in the initial recognition of DNA breaks remains unclear. In fact, more recent study showed that the initial recruitment of repair factors (including Brca1 and MRN complex) to double-strand breaks did not require γ-H2AX. Instead, γ-H2AX was required for further concentration of repair factors in the vicinity of DNA lesion (Celeste et al. 2003). The microscopically visible foci of γ-H2AX and interacting repair factors contain thousands of molecules, thus the functional significance of their formation remain elusive. Alternatively, one may assume that a primary role of γ-H2AX is to modify the higher order chromatin structure at a site of damage. Initially, it had been proposed that phosphorylation of H2AX relaxes the structure of chromatin, facilitating the repair of DNA breaks (Rogakou et al. 1998, 1999). In fact, the carboxy-terminal tail of histone H2A interacts with linker DNA and histone H1, both being involved in regulation of the structure of the nucleosomal fiber (Lindsey et al. 1991). Recently, more "sophisticated" pathways of involvement of γ-H2AX in the regulation of chromatin structure have been identified. Gamma H2AX has been shown to functionally interact with the INO80 ATP-dependent chromatin-remodeling complex (Morrison et al. 2004; van Attikum et al. 2004). Additionally, histone deacetylase (HDAC) 4 is recruited to radiation-induced foci and interacts with 53BP1 (Kao et al. 2003).

Conclusions

Preferential binding of the major non-histone chromatin proteins HMGB to DNA damaged by cisplatin had been originally reported in 1992 (Pil and Lippard 1992; Hughes et al. 1992). Since that time, numerous "non-repair" chromatin proteins and transcription factors have been identified that preferentially bind to different types of DNA damage. Several models have been proposed to describe the possible relationships between DNA repair mechanisms and the chromatin proteins (or transcription factors) that recognize DNA damage. However, none of these models are supported by unequivocal experimental evidence and the role of damage-interacting chromatin proteins in DNA repair remains elusive. Undoubtedly, several questions need to be addressed in further experiments, which include the extent of occupancy of damage sites by non-repair

proteins and the effects of damage-interacting chromatin proteins on repair of chromatin substrates.

Acknowledgements The authors wish to thank Michel Bustin for a human recombinant HMGB1, Kimitoshi Kohno for a human recombinant mtTFA and William T. Garrard for help in preparation of the manuscript. This work was supported by the Polish Ministry of Science and Informatics, Grant KBN 4T11F01824.

References

Agresti A, Bianchi ME (2003) HMGB proteins and gene expression. Curr Opin Genet Dev 13:170–178

van Attikum H, Fritsch O, Hohn B, Gasser SM (2004) Recruitment of the INO80 complex by H2A phosphorylation links ATP-dependent chromatin remodeling with DNA double-strand break repair. Cell 119:777–788

Bassing CH, Chua KF, Sekiguchi J, Suh H, Whitlow SR, Fleming JC, Monroe BC, Ciccone DN, Yan C, Vlasakova K, Livingston DM, Ferguson DO, Scully R, Alt FW (2002) Increased ionizing radiation sensitivity and genomic instability in the absence of histone H2AX. Proc Natl Acad Sci U S A 99:8173–8178

Bellon SF, Coleman JH, Lippard SJ (1991) DNA unwinding produced by site-specific intrastrand cross-links of the antitumor drug cis-diamminedichloroplatinum(II). Biochemistry 30:8026–8035

Birger Y, West KL, Postnikov YV, Lim Y-H, Furusawa T, Wagner JP, Laufer CS, Kraemer KH, Bustin M (2003) Chromosomal protein HMGN1 enhances the rate of DNA repair in chromatin. EMBO J 22:1665–1675

Brown SJ, Kellett PJ, Lippard SJ (1993) Ixr1, a yeast protein that binds to platinated DNA and confers sensitivity to cisplatin. Science 261:603–605

Bruhn SL, Pil PM, Essigmann JM, Housman DE, Lippard SJ (1992) Isolation and characterization of human cDNA clones encoding a high mobility group box protein that recognizes structural distortions to DNA caused by binding of the anticancer agent cisplatin. Proc Natl Acad Sci USA 89:2307–2311

Burma S, Chen BP, Murphy M, Kurimasa A, Chen DJ (2001) ATM phosphorylates histone H2AX in response to DNA double-strand breaks. J Biol Chem 276:42462–42467

Bustin M (2001a) Revised nomenclature for high mobility group (HMG) chromosomal proteins. Trends Biochem Sci 26:152–153

Bustin M (2001b) Chromatin unfolding and activation by HMGN* chromosomal proteins. Trends Biochem Sci 26:431–437

Bustin M, Reeves R (1996) High-mobility-group chromosomal proteins: architectural components that facilitate chromatin function. Prog Nucleic Acid Res Mol Biol 54:35–100

Celeste A, Petersen S, Romanienko PJ, Fernandez-Capetillo O, Chen HT, Sedelnikova OA, Reina-San-Martin B, Coppola V, Meffre E, Difilippantonio MJ, Redon C, Pilch DR, Olaru A, Eckhaus M, Camerini-Otero RD, Tessarollo L, Livak F, Manova K, Bonner WM, Nussenzweig MC, Nussenzweig A (2002) Genomic instability in mice lacking histone H2AX. Science 296:922–927

Celeste A, Fernandez-Capetillo O, Kruhlak MJ, Pilch DR, Staudt DW, Lee A, Bonner RF, Bonner WM, Nussenzweig A (2003) Histone H2AX phosphorylation is dispensable for the initial recognition of DNA breaks. Nat Cell Biol 5:675–679

Chen HT, Bhandoola A, Difilippantonio MJ, Zhu J, Brown MJ, Tai X, Rogakou EP, Brotz TM, Bonner WM, Ried T, Nussenzweig A (2000) Response to RAG-mediated VDJ cleavage by NBS1 and gamma-H2AX. Science 290:1962–1965

Chu G (1994) Cellular responses to cisplatin. The roles of DNA-binding proteins and DNA repair. J Biol Chem 269:787–790

Datta A, Bagchi S, Nag A, Shiyanov P, Adami GR, Yoon T, Raychaudhuri P (2001) The p48 subunit of the damaged-DNA binding protein DDB associates with the CBP/p300 family of histone acetyltransferase. Mutat Res 486:89–97

Felsenfeld G, Groudine M (2003) Controlling the double helix. Nature 421:448–453

Fousteri M, van Hoffen A, Vargova H, Mullenders LHF (2005) Repair of DNA lesions in chromosomal DNA. Impact of chromatin structure and Cockayne syndrome proteins. DNA Repair 4:919–925

Fyodorov DV, Kadonaga JT (2001) The many faces of chromatin remodeling: switching beyond transcription. Cell 106:523–525

Gaillard PH, Martini EM, Kaufman PD, Stillman B, Moustacchi E, Almouzni G (1996) Chromatin assembly coupled to DNA repair: a new role for chromatin assembly factor I. Cell 86:887–896

Gong F, Kwon Y-H, Smerdon MJ (2005) Nucleotide excision repair in chromatin and the right of entry. DNA Repair 4:884–896

He Q, Liang CH, Lippard SJ (2000) Steroid hormones induce HMG1 overexpression and sensitize breast cancer cells to cisplatin and carboplatin. Proc Natl Acad Sci USA 97:5768–5772

Hefferin ML, Tomkinson AE (2005) Mechanism of DNA double-strand break repair by non-homologous end joining. DNA Repair 4:639–648

Huang JC, Zamble DB, Reardon JT, Lippard SJ, Sancar A (1994) HMG-domain proteins specifically inhibit the repair of the major DNA adduct of the anticancer drug cisplatin by human excision nuclease. Proc Natl Acad Sci USA 91:10394–10398

Hughes EN, Engelsberg BN, Billings PC (1992) Purification of nuclear proteins that bind to cisplatin-damaged DNA; identity with high mobility group proteins 1 and 2. J Biol Chem 267:13520–13527

Iizuka M, Smith MM (2003) Functional consequences of histone modifications. Curr Opin Gen Dev 13:154–160

Imamura T, Izumi H, Nagadani G, Ise T, Nomoto M, Iwamoto Y, Kohno K (2001) Interaction with p53 enhances binding of cisplatin-modified DNA by high mobility group 1 protein. J Biol Chem 276:7534–7540

Ise T, Nagatani G, Imamura T, Kato K, Takano H, Nomoto M, Izumi H, Ohmori H, Okamoto T, Ohga T, Uchiumi T, Kuwano M, Kohno K (1999) Transcription factor Y-box binding protein 1 binds preferentially to cisplatin-modified DNA and interacts with proliferating cell nuclear antigen. Cancer Res 59:342–346

Kao GD, McKenna WG, Guenther MG, Muschel RJ, Lazar MA, Yen TJ (2003) Histone deacetylase 4 interacts with 53BP1 to mediate the DNA damage response. J Cell Biol 160:1017–1027

Kobayashi J, Tauchi H, Sakamoto S, Nakamura A, Morishima K, Matsuura S, Kobayashi T, Tamai K, Tanimoto K, Komatsu K (2002) NBS1 localizes to gamma-H2AX foci through interaction with the FHA/BRCT domain. Curr Biol 12:1846–1851

Kunz C, Zurbriggen K, Fleck O (2003) Mutagenesis of the HMGB (high-mobility group B) protein Cmb1 (cytosine-mismatch binding 1) of *Schizosaccharomyces pombe*: effects on recognition of DNA mismatches and damage. Biochem J 372:651–660

Landsman D, Bustin M (1993) A signature for the HMG-1 box DNA-binding proteins. Bioessays 15:539–546

Lanuszewska J, Widlak P (2000) High mobility group 1 and 2 proteins bind preferentially to DNA that contains bulky adducts induced by benzo(a)pyrene diol epoxide and *N*-acetoxy-acetylaminofluorene. Cancer Lett 158:17–25

Lindsey GG, Orgeig S, Thompson P, Davies N, Maeder DL (1991) Extended C-terminal tail of wheat histone H2A interacts with DNA of the "linker" region. J Mol Biol 218:805–813

Lukas C, Melander F, Stucki M, Falck J, Bekker-Jensen S, Goldberg M, Lerenthal Y, Jackson SP, Bartek J, Lukas J (2004) Mdc1 couples DNA double-strand break recognition by Nbs1 with its H2AX-dependent chromatin retention. EMBO J 23:2674–2683

Malina J, Kasparkova J, Natile G, Brabec V (2002) Recognition of major DNA adducts of enantiomeric cisplatin analogs by HMG box proteins and nucleotide excision repair of these adducts. Chem Biol 9:629–638

Marchenko ND, Zaika A, Moll UM (2000) Death signal-induced localization of p53 protein to mitochondria. A potential role in apoptotic signaling. J Biol Chem 275:16202–16212

McA'Nulty MM, Whitehead JP, Lippard SJ (1996) Binding of Ixr1, a yeast HMG-domain protein, to cisplatin-DNA adducts in vitro and in vivo. Biochemistry 35:6089–6099

Meijer M, Smerdon MJ (1999) Accessing DNA damage in chromatin: insights from transcription. BioEssays 21:596–603

Moggs JG, Almouzni G (1999) Chromatin rearrangements during nucleotide excision repair. Biochimie 81:45–52

Morrison AJ, Highland J, Krogan NJ, Arbel-Eden A, Greenblatt JF, Haber JE, Shen X (2004) INO80 and gamma-H2AX interaction links ATP-dependent chromatin remodeling to DNA damage repair. Cell 119:767–775

Nagaki S, Yamamoto M, Yumoto Y, Shirakawa H, Yoshida M, Teraoka H (1998) Non-histone chromosomal proteins HMG1 and 2 enhance ligation reaction of DNA double-strand breaks. Biochem Biophys Res Commun 246:137–141

Nagatani G, Nomoto M, Takano H, Ise T, Kato K, Imamura T, Izumi H, Makishima K, Kohno K (2001) Transcriptional activation of the human HMG1 gene in cisplatin-resistant human cancer cells. Cancer Res 61:1592–1597

Narlikar GJ, Fan H-Y, Kingston RE (2002) Cooperation between complexes that regulate chromatin structure and transcription. Cell 108:475–487

Ohndorf UM, Whitehead JP, Raju NL, Lippard SJ (1997) Binding of tsHMG, a mouse testis-specific HMG-domain protein, to cisplatin-DNA adducts. Biochemistry 36:14807–14815

Ohndorf UM, Rould MA, He Q, Pabo CO, Lippard SJ (1999) Basis for recognition of cisplatin-modified DNA by high-mobility-group proteins. Nature 399:708–712

Pasheva EA, Pashev IG, Favre A (1998) Preferential binding of high mobility group 1 protein to UV-damaged DNA. Role of the COOH-terminal domain. J Biol Chem 273:24730–24736

Paull TT, Rogakou EP, Yamazaki V, Kirchgessner CU, Gellert M, Bonner WM (2000) A critical role for histone H2AX in recruitment of repair factors to nuclear foci after DNA damage. Curr Biol 10:886–895

Peterson CL, Cote J (2004) Cellular machineries for chromosomal DNA repair. Genes Dev 18:602–616

Peterson CL, Laniel M-A (2004) Histones and histone modifications. Curr Biol 14:R546–R551

Pil PM, Lippard SJ (1992) Specific binding of chromosomal protein HMG1 to DNA damaged by the anticancer drug cisplatin. Science 256:234–237

Reed SH (2005) Nucleotide excision repair in chromatin: the shape of things to come. DNA Repair 4:909–918

Reeves R, Adair JE (2005) Role of high mobility group (HMG) chromatin proteins in DNA repair. DNA Repair 4:926–938

Rich T, Allen RL, Wyllie AH (2000) Defying death after DNA damage. Nature 407:777–783

Rogakou EP, Pilch DR, Orr AH, Ivanova VS, Bonner WM (1998) DNA double-stranded breaks induce histone H2AX phosphorylation on serine 139. J Biol Chem 273:5858–5868

Rogakou EP, Boon C, Redon C, Bonner WM (1999) Megabase chromatin domains involved in DNA double-strand breaks in vivo. J Cell Biol 146:905–916

Rogakou EP, Nieves-Neira W, Boon C, Pommier Y, Bonner WM (2000) Initiation of DNA fragmentation during apoptosis induces phosphorylation of H2AX histone at serine 139. J Biol Chem 275:9390–9395

Sancar A, Lindsey-Boltz LA, Unsal-Kacmaz K, Linn S (2004) Molecular mechanisms of mammalian DNA repair and the DNA damage checkpoints. Annu Rev Biochem 73:39–85

Siddik ZH (2003) Cisplatin: mode of cytotoxic action and molecular basis of resistance. Oncogene 22:7265–7279

Stiff T, O'Driscoll M, Rief N, Iwabuchi K, Lobrich M, Jeggo PA (2004) ATM and DNA-PK function redundantly to phosphorylate H2AX after exposure to ionizing radiation. Cancer Res 64:2390–2396

Taneja N, Davis M, Choy JS, Beckett MA, Singh R, Kron SJ, Weichselbaum RR (2004) Histone H2AX phosphorylation as a predictor of radiosensitivity and target for radiotherapy. J Biol Chem 279:2273–2280

Thoma F (1999) Light and dark in chromatin repair: repair of UV-induced DNA lesions by photolyase and nucleotide excision repair. EMBO J 18:6585–6598

Thoma F (2005) Repair of UV lesions in nucleosomes—intrinsic properties and remodeling. DNA Repair 4:855–869

Thomas JO, Travers AA (2001) HMG1 and 2, and related 'architectural' DNA-binding proteins. Trends Biochem Sci 26:167–174

Treiber DK, Zhai X, Jantzen HM, Essigmann JM (1994) Cisplatin-DNA adducts are molecular decoys for the ribosomal RNA transcription factor hUBF (human upstream binding factor). Proc Natl Acad Sci USA 91:5672–5676

Trimmer EE, Zamble DB, Lippard SJ, Essigmann JM (1998) Human testis-determining factor SRY binds to the major DNA adduct of cisplatin and a putative target sequence with comparable affinities. Biochemistry 37:352–362

Tyler JK, Adams CR, Chen SR, Kobayashi R, Kamakaka RT, Kadonaga JT (1999) The RCAF complex mediates chromatin assembly during DNA replication and repair. Nature 402:555–560

Vichi P, Coin F, Renaud JP, Vermeulen W, Hoeijmakers JH, Moras D, Egly JM (1997) Cisplatin- and UV-damaged DNA lure the basal transcription factor TFIID/TBP. EMBO J 16:7444–7456

Wang JF, Bashir M, Engelsberg BN, Witmer C, Rozmiarek H, Billings PC (1997) High mobility group proteins 1 and 2 recognize chromium-damaged DNA. Carcinogenesis 18:371–375

Wang Y, Cortez D, Yazdi P, Neff N, Elledge SJ, Qin J (2000) BASC, a super complex of BRCA1-associated proteins involved in the recognition and repair of aberrant DNA structures. Genes Dev 14:927–939

Ward IM, Minn K, Jorda KG, Chen J (2003) Accumulation of checkpoint protein 53BP1 at DNA breaks involves its binding to phosphorylated histone H2AX. J Biol Chem 278:19579–19582

Wei M, Burenkova O, Lippard SJ (2003) Cisplatin sensitivity in $Hmgb1^{-/-}$ and $Hmgb1^{+/+}$ mouse cells. J Biol Chem 278:1769–1773

Wong B, Masse JE, Yen YM, Giannikopoulos P, Feigon J, Johnson RC (2002) Binding to cisplatin-modified DNA by the *Saccharomyces cerevisiae* HMGB protein Nhp6A. Biochemistry 41:5404–5414

Yaneva J, Leuba SH, van Holde K, Zlatanova J (1997) The major chromatin protein histone H1 binds preferentially to cis-platinum-damaged DNA. Proc Natl Acad Sci USA 94:13448–13451

Yoshida Y, Izumi H, Ise T, Uramoto H, Torigoe T, Ishiguchi H, Murakami T, Tanabe M, Nakayama Y, Itoh H, Kasai H, Kohno K (2002) Human mitochondrial transcription factor A binds preferentially to oxidatively damaged DNA. Biochem Biophys Res Commun 295:945–951

Yoshida Y, Izumi H, Torigoe T, Ishiguchi H, Itoh H, Kang D, Kohno K (2003) p53 physically interacts with mitochondrial transcription factor A and differentially regulates binding to damaged DNA. Cancer Res 63:3729–3734

Yuan F, Gu L, Guo S, Wang C, Li G-M (2004) Evidence for involvement of HMGB1 protein in human DNA mismatch repair. J Biol Chem 279:20935–20940

Zamble DB, Jacks T, Lippard SJ (1998) p53-dependent and -independent responses to cisplatin in mouse testicular teratocarcinoma cells. Proc Natl Acad Sci USA 95:6163–6168

Zamble DB, Mikata Y, Eng CH, Sandman KE, Lippard SJ (2002) Testis-specific HMG-domain protein alters the responses of cells to cisplatin. J Inorg Biochem 91:451–462

Zhai X, Beckmann H, Jantzen HM, Essigmann JM (1998) Cisplatin-DNA adducts inhibit ribosomal RNA synthesis by hijacking the transcription factor human upstream binding factor. Biochemistry 37:16307–16315