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REVIEW

CREBBP and p300 lysine acetyl transferases in the DNA damage response

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Abstract

The CREB-binding protein (CREBBP, or in short CBP) and p300 are lysine (K) acetyl transferases (KAT) belonging to the KAT3 family of proteins known to modify histones, as well as non-histone proteins, thereby regulating chromatin accessibility and transcription. Previous studies have indicated a tumor suppressor function for these enzymes. Recently, they have been found to acetylate key factors involved in DNA replication, and in different DNA repair processes, such as base excision repair, nucleotide excision repair, and non-homologous end joining. The growing list of CBP/p300 substrates now includes factors involved in DNA damage signaling, and in other pathways of the DNA damage response (DDR). This review will focus on the role of CBP and p300 in the acetylation of DDR proteins, and will discuss how this post-translational modification influences their functions at different levels, including catalytic activity, DNA binding, nuclear localization, and protein turnover. In addition, we will exemplify how these functions may be necessary to efficiently coordinate the spatio-temporal response to DNA damage. CBP and p300 may contribute to genome stability by fine-tuning the functions of DNA damage signaling and DNA repair factors, thereby expanding their role as tumor suppressors.

Keywords DNA repair · DNA replication · DNA repair enzymes · Protein acetylation · Post-translational modification

Introduction

The CREB-binding protein (CREBBP or CBP) and its paralogue p300 belong to the type 3 family of lysine acetyl transferases (KAT3) present not only in all mammals, but also found in many multicellular organisms such as flies, worms and plants [1, 2]. These enzymes are involved in the regulation of important physiological processes such as proliferation, differentiation and apoptosis, thanks to their ability to interact and regulate more than 400 factors [3, 4].

Both CBP and p300 (KAT3a and 3b, respectively) are transcriptional co-activators binding to transcription factors and bridging them to large protein complexes in the transcriptional machinery [5, 6]. However, the function of CBP and p300 in gene transcription is not restricted to their scaffold properties, but also involves the KAT activity required for acetylation of transcription factors and histones, to allow chromatin accessibility [7, 8].

This unique KAT3 family shows a characteristic structure composed of four transactivation domains (TADS): (1) cysteine–histidine-rich region 1 (CH1), which includes the transcriptional adapter zing finger 1 (TAZ1); (2) the CREB-interacting kinase-inducible (KIX) domain; (3) the cysteine–histidine 3 region (CH3), also including the TAZ2 domain and (4) the nuclear receptor co-activator binding domain (NCBD). In addition, a catalytic domain (HAT) responsible for lysine acetylation is adjacent to the bromodomain (BrD), which recognizes acetylated substrates [9, 10]. The CH2 region contains a plant homeodomain (PHD) and a RING domain, which are thought to cooperate with HAT function for chromatin modification [11]. The principal domains of CBP and p300 are shown in Fig. 1.

CBP and p300 are fundamental for embryonic development, as demonstrated by the lethality of *CREBBP*$^{-/-}$ and *EP300*$^{-/-}$ mice, as well as the heterozygous double-mutant knockout mice [12, 13]. *CREBBP* and *EP300* genes are often mutated in several types of solid tumors including colorectal, breast, ovarian and hepatocellular carcinomas, as well as in hematological malignancies [14–16]. Heterozygous

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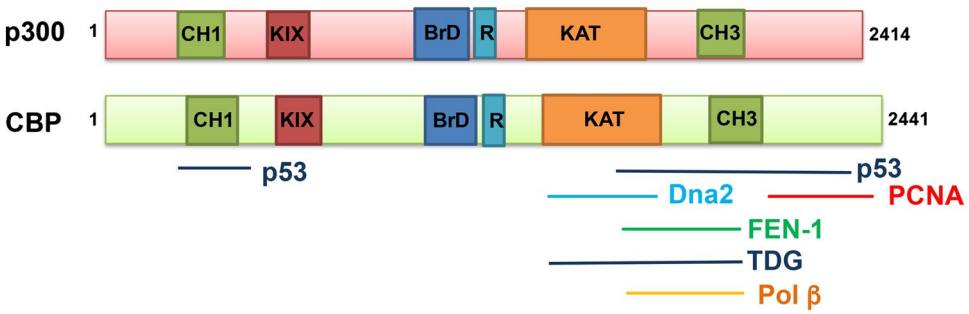

Fig. 1 Schematic representation of p300 and CBP proteins. The cysteine/histidine (CH) rich regions 1 and 3 are shown, while the CH2 region (not indicated) contains both the bromodomain (BrD) and RING (R) domains. Also shown is the region containing the lysine acetyl transferase (KAT) catalytic activity. Numbers indicate the length of each protein. Colored bars shown below represent the regions involved in the interaction with the indicated DDR factors. For comparison, the regions responsible for p53 binding are also shown

germ line mutations are the cause of the Rubinstein–Taybi syndrome, which is characterized by developmental anomalies and predisposition to cancer [17]. Somatic mutations contribute to loss of heterozygosity, thereby affecting CBP and p300 cell functions and promoting cancer development or progression [18]. However, some mutations may provide gain-of-function properties contributing to cancer [19]. CBP and p300 are regarded as tumor suppressor genes since they acetylate p53, the guardian of genome stability [20–22]. In addition, they may contribute to DNA repair through histone acetylation, thereby activating transcription and facilitating the recruitment of DNA repair factors to site of damage [23, 24]. Acetylation of histones and transcription factors (e.g., p53) in the DNA damage response (DDR) has been extensively studied and reviewed [20–22, 25, 26], and they will not be further discussed here. CBP and/or p300 have been shown to acetylate specific DNA replication and repair factors [27]. For some of them (described below) the regions responsible for interaction with CBP and/or p300 are also shown in Fig. 1. The list of CBP/p300 substrates now includes factors participating in base excision repair (BER), nucleotide excision repair (NER), non-homologous end joining (NHEJ), as well as double-strand break repair (DSBR) (Fig. 2).

In this review, we will discuss the role of CBP- and p300-mediated acetylation of proteins participating in the cell response to DNA damage, with a particular focus on DNA repair factors.

The DNA damage response

DDR is a complex network of cellular processes including DNA damage recognition, signaling, DNA repair, cell cycle checkpoint activation, as well as DNA replication-associated specific pathways that are activated in response to genotoxic stress to safeguard genome integrity [28]. Post-translational

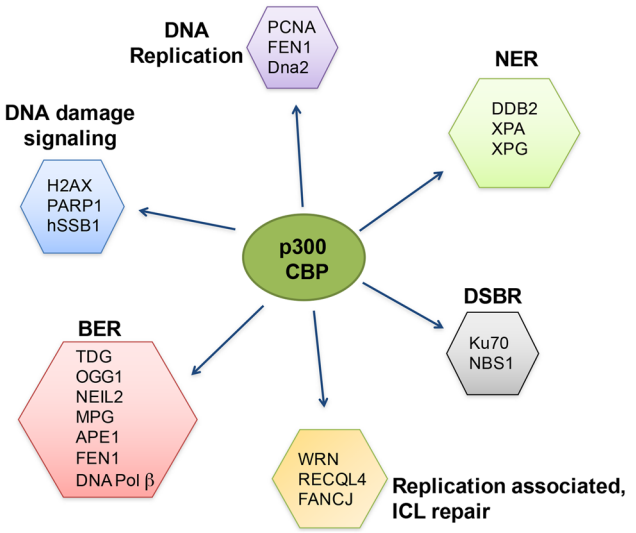

Fig. 2 Schematic representation of protein substrates of p300 and CBP participating in different aspects of the DNA damage response. Each block represents a group of proteins involved in the same process (e.g., DNA replication/repair, DNA damage signaling, NER, BER, etc.)

modifications are part of the signaling mechanisms, and acetylation contributes to dynamically control protein function [29–31], thus indicating that acetylation of DNA repair factors by CBP and/or p300 may play an active role in DDR.

Acetylation of DNA damage sensing/signaling factors

PARP-1

Poly(ADP-ribose) polymerase 1 (PARP-1) is one of the most characterized factors involved in the cellular response to DNA damage, acting specifically as a sensor of DNA

breaks. It belongs to the PARP superfamily utilizing NAD^+ to produce ADP-ribose polymers, and it is required in several pathways, from DNA replication and repair to cell death response, transcription, mitochondrial activity regulation and chromatin remodeling [32–34].

PARP-1 was first identified as a target of p300 activity in a study of PARP-1 regulation of NF-κB-dependent transcription after inflammatory stimuli [35]. Both p300 and CBP acetylated PARP-1 in vivo and in vitro, on lysine residues K498, K505, K508, K521 and K524, as detected by autoradiography and mass spectrometry (MS) analysis. The acetylation of these residues was required for stabilizing the interaction of PARP-1 with p50 for the transcriptional activation of NF-κB [35].

Sumoylation of PARP-1 completely abrogated lysine acetylation, thereby controlling the transcription co-activating function of PARP-1 target genes [36]. Among acetylated lysines, K498, K521, and K524 are positioned in the PARP-1 auto-modification domain and may function as acceptor site for auto-polyADP-ribosylation (PAR). Therefore, the same residues may compete for acetylation vs ADP-ribosylation [37]. Only more recently a relationship between PARP-1 acetylation and DNA repair has been shown: an increased modification induced by histone deacetylase (HDAC) inhibitors resulted in a reduced efficiency in NHEJ which was attributed to an anomalous persistent binding of PARP-1 to DNA breaks [38]. Reversal of this effect was obtained by inhibiting p300/CBP with the small molecule C646, thus suggesting that PARP-1 acetylation stabilized the interaction with DNA, with a consequent inhibition of DNA repair activity [38]. Given that PAR synthesis must terminate to allow PARP-1 release from DNA damage sites and DNA repair to proceed [32], by competing with the same residues, acetylation might be the signal to reset PARP-1 to a transcription mode. However, further studies are necessary to clarify this role.

H2AX

Phosphorylation on serine 139 (γ-H2AX) of histone H2AX promotes the recruitment and retention of proteins associated with DDR signaling [28]. This is one of the first steps necessary for DNA repair of DNA lesions, such as those induced by ionizing radiation (IR). Acetylation of histone H2AX by CBP and p300 has been reported to occur constitutively on lysine 36 [39]. Expression of the mutant forms K36A, or K36R in $H2AX^{-/-}$ MEFs did not complement their radio-sensitivity, although serine 139 was efficiently phosphorylated upon DNA damage. These results suggested that K36 acetylation is required for cell survival, although no increase in acK36 was observed after DNA damage. When both K36 and S139 were mutated, the double-mutant cells showed higher sensitivity to IR than cells carrying the single mutation, indicating that the two modifications affect different pathways [39]. In conclusion, the constitutive acetylation of H2AX on K36 is required for cell survival, independently of the canonical pathway of DDR signaling.

NBS1

Nibrin (NBS1)—the product of *NBN* gene involved in Nijmegen Breakage syndrome (NBS)—is part of the MRN complex, formed by MRE11–RAD50–NBS1, playing an essential role in the recognition and signaling of DNA damage (DNA breaks) and in the checkpoint activation [28]. DNA damage induced the association of NBS1 with p300, and the ATM-mediated phosphorylation of p300 at serine 106 (S106) was shown to regulate the stability of NBS1 and its recruitment to DNA damage sites [40]. The interaction of S106-phosphorylated p300 with NBS1 was required for acetylation of latter factor, as detected with anti-acetyl-lysine antibody. A dominant negative and catalytically inactive mutant form of p300 did not interact with, nor stabilized NBS1 after DNA damage [40]. These results indicated that the participation of NBS1 in DDR occurs in an acetylation-dependent manner.

hSSB1

The human single-stranded DNA binding protein 1 (hSSB1) plays a crucial role in the DNA damage response, although it has higher similarity to the bacterial protein rather than to human RPA [41]. After DNA damage, hSSB1 relocates to sites containing the lesions thus facilitating ATM kinase activity and checkpoint activation, thanks also to the binding to p300, thereby promoting acetylation of p53 [42]. Interestingly, acetylation of hSSB1 itself by p300 was detected at K94 by immunoprecipitation and by MS, and found to increase after DNA damage [43]. This modification stabilized the protein since a mutant form (K94R) was degraded more rapidly than the wild-type (WT) protein. Stabilization of hSSB1 was obtained by antagonizing with ubiquitination, as also indicated by an increase in ubiquitinated forms of hSSB1 after p300/CBP inhibition with C646. In contrast, hSSB1 acetylation did not affect its recruitment to DNA damage sites, since the K94R mutant was similarly accumulated. Stabilization of the protein was also indicated by a positive correlation between p300 and hSSB1 in tumor samples [43]. The biological function of hSSB1 acetylation in DDR was further demonstrated, after knockdown of hSSB1, since WT protein, but not the K94R mutant rescued cell sensitivity to radiotherapy and chemotherapy [43].

The binding between p300 and hSSB1 was also shown to regulate the acetylation of p53 at lysine 382, which is a crucial event for the p53-mediated expression of p21 in checkpoint activation [42].

Acetylation of DNA replication/repair factors

Several proteins participating in DNA replication are also involved in DNA repair, because these factors perform functions in common with both processes, such as DNA synthesis. The acetylation of these factors has been investigated in both processes and the influence of this modification on protein function appears to be similar.

Proliferating cell nuclear antigen

The proliferating cell nuclear antigen (PCNA) is a homotrimeric protein acting as a ring platform required for tethering DNA replication and repair factors to DNA [44]. The first evidence of an interaction with the C-terminal region of p300 suggested that PCNA could be acetylated during DNA repair [45]. The post-translational modification was subsequently investigated with an anti-acetyl-lysine antibody following peptide separation by 2D electrophoresis [46]. The functional role of acetylation was investigated by immunoprecipitation and the results suggested that the modification could increase the PCNA interaction with DNA polymerases (pol) δ and β. In addition, the acetylated form of PCNA supported more efficiently a DNA synthesis reaction in vitro [46]. Another study by mutational analysis showed that after DNA damage, a unique residue of PCNA (K14) could modulate the interaction with MTH2 protein, a *MutT*-homolog involved in maintenance of DNA replication fidelity [47]. A large-scale MS study provided the first evidence of PCNA acetylation by detecting modification on K77, K80, and K248 [29]. In a more recent study new acetylation sites were identified by MS to occur, in vitro and in vivo, not only at K77 and K80, but also at K13 and K14 [48]. The same study showed that PCNA was modified not only by p300, but also by CBP, through binding to its C-terminal domain. Interestingly, all the residues modified by p300 and CBP are located in the internal rim of the ring contacting the negative charges of DNA phosphates [49]. Mutation of these residues (K > R) increased the stability of the protein after DNA damage by inhibiting PCNA ubiquitination and consequent proteasomal degradation. Furthermore, these mutations impaired DNA replication and repair, inhibiting DNA synthesis when the protein was already loaded on DNA. In contrast, K > A mutations inhibited DNA replication because mutant PCNA could not be loaded onto DNA [48], in agreement with previous in vitro findings [49]. All together, these results indicate that PCNA acetylation, although not required for loading onto DNA, significantly supports both DNA replication and repair syntheses, probably by enhancing the processivity of DNA polymerases. In addition, acetylation of PCNA is the signal connecting its release from DNA repair sites for proteasomal degradation [48].

Flap endonuclease 1

Human flap endonuclease 1 (FEN1) is an endonuclease, which interacts with PCNA, and it is involved in the lagging strand DNA replication, in BER and also in NER [50]. In particular, FEN1 participates in the removal of RNA/DNA primers in the Okazaki fragment maturation, or in the cleavage of flaps generated by the DNA repair machinery [50]. FEN1 was shown to interact with p300, while CBP was not investigated. Acetylation was found by MS to occur in four lysine residues (K354, K375, K377 and K389) located at the C terminus of the protein, near the PCNA binding box [51]. Acetylation was stimulated by UV irradiation in human epithelial kidney 293 cells, and inhibited the nuclease activity, while not affecting the interaction with PCNA. The lysine modifications influenced both endo- and exonuclease activities of FEN1 and were important for DNA binding, since acetylation reduced the FEN1 affinity for DNA [51]. It was suggested that inhibition of FEN1 activity by acetylation after UV damage may trigger the error-free repair system by homologous recombination (HR). Consistent with this regulatory role, haploid organisms lack the C-terminal portion of the protein [51]. However, FEN1 mutant proteins in the C-terminal lysines (K > A) were as active as the WT enzyme on a double-flap substrate that was shown to be the preferred substrate in vitro, suggesting that a similar intermediate might be the in vivo substrate for FEN1 [52]. It was thus concluded that further studies are required to provide a clear mechanistic role of FEN1 acetylation at the cellular level. The possibility that the inhibition of FEN1 activity by acetylation could occur on specific pools of the enzyme involved in different cellular processes [51] remains to be investigated.

DNA 2 endonuclease/helicase

The DNA 2 endonuclease/helicase (Dna2) protein is not only endowed with both 5′–3′ and 3′–5′ endonuclease activities, but also shows ATPase and 5′–3′ helicase activities [50]. Together with FEN1, Dna2 participates in the Okazaki fragment processing during DNA replication, and in the long-patch BER. At difference from FEN1, Dna2 removes longer flap structures (> 20 nucleotides) that may have escaped FEN1 activity [50]. Dna2 interacted with and was acetylated by p300 in vitro and in vivo, with a consequent stimulation of both endonuclease and helicase activities [53]. Acetylation significantly increased the binding efficiency of Dna2 to DNA substrate, as shown by gel shift assay [53]. An increase in Dna2 acetylation was observed in UV-treated

cells, suggesting that DNA damage induced the activity of p300 and/or reduced that of enzymes de-acetylating Dna2 [53]. This condition may result in Dna2 stimulation and concomitant FEN1 inhibition, thereby enhancing the processing of longer flaps. In DNA replication, this pathway may be more effective for the removal of incorrect base possibly introduced by the error-prone DNA Pol α during the priming synthesis. This mechanism may be also applied during DNA repair (see below), thus implying that global acetylation may underlie a protecting role for p300 in regulating DNA metabolism [53].

Acetylation of BER factors

As compared with other DNA repair systems, the BER process includes a significant number of factors that have been identified as substrates for p300/CBP activity. However, it is still unclear whether the modification of each factor acts synergistically with all the others.

Thymine DNA glycosylase

Thymine DNA glycosylase (TDG) is an enzyme which acts preferentially on G/T and G/U mismatches and, together with MBD4, is primarily involved in DNA demethylation to maintain genetic and epigenetic integrity of CpG sites [54, 55]. Both CBP and p300 were capable to acetylate TDG in vitro and in vivo. Acetylation was detected in the N-terminal region of the protein by [^{14}C] acetylCoA labeling and located at residues K70, K94, K95 and K98 [56].

The interaction between CBP/p300 and TDG influenced gene transcription and concomitant effect on DNA repair, since the complex retained both the TDG ability to cleave G/T and G/U mispaired bases, and the histone acetylation by CBP. In addition, TDG stimulated CBP-dependent transcription, even in a catalytically deficient mutant, indicating that the DNA repair and transcription functions of TDG are independent [56]. Remarkably, acetylation of TDG resulted in a reduced ability to bind apurinic/apyrimidinic (AP) endonuclease (APE), suggesting that TDG modification may promote a switch from DNA repair to the transcription process [56]. Interestingly, acetylation required uncoupling from DNA, since when TDG was already bound to DNA, modification by CBP/p300 was prevented. Conversely, TDG acetylation abrogated the processing of G/T mispair, and this effect was mutually excluded by TDG phosphorylation, thus highlighting the tight regulation of TDG activity [57].

8-Oxoguanine DNA glycosylase 1

The human 8-oxoguanine DNA glycosylase 1 (OGG1) is the most important enzyme responsible for the repair of oxidative DNA base damage, such as 8-oxoguanine (8-oxoG) and also various types of oxidized bases [58]. Studies in vitro and in vivo have shown that p300 (possibly also CBP) acetylates OGG1 on K338 and K341 (K335 to a low level). K to R mutation of these sites resulted in lower OGG1 activity in vitro, while the presence of APE1 increased the activity by reducing OGG1 affinity for the AP site produced by the reaction [59]. OGG1 was found to interact with histone deacetylase 1 (HDAC1); accordingly, the HDAC inhibitor trichostatin A (TSA), but not the SIRT1 inhibitor nicotinamide, increased the levels of acetylated OGG1, suggesting that class 1 HDAC enzymes are involved in OGG1 deacetylation [59].

In vivo stimulation of OGG1 activity by acetylation was further demonstrated in human skeletal muscle, in which an inverse correlation between 8-oxoG levels and acetylated OGG1 was found [60]. In addition, the levels of acetylated OGG1 were correlated to the amount of oxidative stress induced, and to the balanced expression of p300/CBP and the deacetylase SIRT1, indicating that deacetylation of OGG1 may involve distinct proteins in different cells and tissues [59, 60]. In fact, regulation of OGG1 levels in lens epithelial cells was markedly influenced by RNA interference of p300 and SIRT1, although the effect of depleting other acetylase/deacetylase proteins was not investigated [61]. OGG1 is also localized in mitochondria where the enzyme protects these organelles from oxidative injury [62]. In glioblastoma cell lines, SIRT3 deacetylase was found to interact with the mitochondrial form of OGG1 and to reduce its acetylation levels, with a concomitant negative influence on mitochondrial DNA repair of oxidative damage. This effect was attributed to an influence on protein stability, since a higher degradation of OGG1 occurred upon SIRT3 silencing, indicating that OGG1 deacetylation protected cells from mitochondrial DNA damage induced by oxidative stress [62]. Therefore, acetylation of OGG1 seems to influence DNA repair efficiency both by enhancing turnover of the catalytic reaction, and by stabilizing protein levels.

Nei-like 2 DNA glycosylase

This DNA glycosylase is one of the two human orthologs of bacterial enzymes (Fpg and Nei), named NEIL1 and NEIL2, endowed with lyase activity in addition to the glycosylase function. NEIL2 is primarily responsible for removing oxidative lesions on cytosine and other pyrimidine lesions, such as 5,6-dihydrouracil and 5-hydroxy uracil. In contrast with the expression level of NEIL1 that is increased in S phase, NEIL2 is expressed throughout all the cell cycle phases [58]. Similar to other DNA glycosylases, NEIL2 was found to interact with p300 and to be acetylated in vitro and in vivo at two major lysine residues, K49 and K153, and to a minor extent at lysine residues K149 and K150 [63]. Remarkably,

K49 is located in the active site of the protein necessary for the glycosylase activity and acetylation of this residue, but not of K153, resulted in the inhibition of both glycosylase and AP lyase activities. The mutation of this residue to arginine (K49R) to maintain the positive charge induced inactivation of NEIL2, highlighting the importance of modification of this residue. In contrast, K153 acetylation was not relevant to the enzymatic activity, suggesting a distinct function for the modification at this site, e.g., for interaction with other BER factors, such as DNA ligase IIIα and DNA pol β. The evidence that acetylation inhibited the DNA glycosylase activity against oxidative damage suggested that inactivation might occur after completion of the BER process. Alternatively, the enzyme inactivation under physiological conditions is counteracted by deacetylation to induce the DNA repair function [63]. However, the deacetylase activity required for NEIL2 reactivation has not yet been identified.

3-Methyladenine DNA glycosylase

This enzyme, known as alkyladenine or methylpurine DNA glycosylase (AAG/MPG), is another member of DNA glycosylases catalyzing the excision of alkylated bases from DNA in BER [58]. MPG acetylation by p300 was reported after in vitro experiments with purified recombinant proteins and [^{3}H]acetylCoA labeling [64]. The presence of the estrogen receptor (ER) α increased MPG labeling, suggesting that acetylation was stimulated after receptor binding. In addition, the modification increased the MPG catalytic activity toward DNA substrate containing a modified base (hypoxanthine). In turn, MPG decreased the p300-mediated acetylation of ER α. These results suggested that MPG was recruited to ER elements to maintain genome integrity in transcribed genes. However, the occurrence of MPG acetylation after DNA damage was not investigated in this study [64].

AP endonuclease 1

The AP endonuclease 1 (APE1, also known as redox factor 1, REF-1) is a multifunctional protein involved in BER and in transcription [58]. In fact, APE1 is able to repress the parathyroid hormone gene by binding to negative calcium response element (nCaRE) [65], or to activate MDR1 gene transcription by binding to the Y-box binding protein [66]. The gene-expression-related activity, but not the endonuclease activity of APE1, is regulated by p300-mediated acetylation of K6 and K7 [65, 66]. These residues are located in the N-terminal region not involved in the catalytic function of the protein [67]. However, additional lysines, including K27, K31, K32 and K35 have been found acetylated in HeLa cells, and these modifications resulted in the inhibition of the interaction with nucleophosmin and RNA, but also in the modulation of the endonuclease activity [68]. Although the KAT activity responsible for acetylation of these residues was not investigated in that study, the post-translational modification occurred after genotoxic stress [68]. Interestingly, cells expressing K to A mutants of K27, K31, K32 and K35 residues were more resistant to treatment with methylmethanesulfonate (MMS) and showed an impaired proliferation [69]. The same mutations mimicking the acetylated form of the protein by abolishing the charge of lysine residues showed a catalytic activity higher than the WT protein. As an explanation, acetylation of these residues was suggested to induce a conformational change in APE1 structure. In addition, the charged status of these residues modulated the acetylation of K6/K7 residues, suggesting a crosstalk between different lysine residues in response to genotoxic damage [69]. Acetylation of K6 and K7 residues was shown to occur in chromatin, once the enzyme was bound to the AP sites, and this binding was necessary for acetylation to proceed. Accordingly, blocking this binding with methoxamine induced the inhibition of chromatin association and concomitantly abrogated APE1 acetylation [70]. Consequence of APE1 acetylation by p300 in vitro was the enhancement of its catalytic efficiency, probably by inducing in the protein a conformational change. In addition, acetylation was able to promote the interaction with downstream BER factors (e.g., DNA pol β, XRCC1 and DNA ligase III), as also indicated by specific co-localization of acetylated APE1 with XRCC1 in chromatin [70]. As a further proof of the role of acetylation in the response to DNA damage, cells expressing APE1 acetylation-defective mutants showed a higher sensitivity to agents inducing DNA lesions repaired through BER [70].

APE1 is overexpressed in various types of tumors, including colon, lung, and pancreatic cancers, and higher levels of acetylated APE1 were found in these tumors, which consequently showed an enhanced efficiency of DNA repair of AP sites [71] and an increased stability of the protein [72]. Therefore, APE1 acetylation, by stimulating DNA repair activity in tumor cells may contribute to protect them from both drug-induced as well as endogenous DNA damage. In fact, overexpression of APE1 was associated with enhanced proliferation and resistance to chemotherapeutic agents [72].

Deacetylation of APE1 by HDAC1 was suggested by their interaction [65], while another study indicated that K6 and K7 are deacetylated by SIRT1 [73]. Remarkably, SIRT1 knockdown induced an increase in AP sites, suggesting that APE1 deacetylation is required for protecting cells from DNA damage-induced cell death [73]. This is in apparent contrast with the findings indicating that acetylation stimulates its activity and, therefore, DNA repair. A possible explanation of this paradox could be provided if an acetylation–deacetylation cycle of K6 and K7 was required to shift APE1 from transcription (nCaRE binding) to DNA repair

mode, since a crosstalk between K6 and K7 with acetylation of the other residues has been suggested [69]. This interpretation would be in agreement with the observed protective effect of SIRT1 upon genotoxic stress, as also supported by the evidence that SIRT1 promoted the interaction between APE1 and XRCC1 [73].

DNA polymerase β

DNA polymerase (pol) β is another important BER player contributing to genome integrity maintenance, as also indicated by the lethality of knockout mice for this gene, and the cancer-prone phenotypes of pol β variants [74, 75]. In the BER process, DNA pol β catalyzes both the lyase reaction of the 5′-deoxyribose phosphate (dRP) moiety remaining after cleavage of the AP site by APE1, and the gap filling of the missing nucleotide [58]. Acetylation of pol β by the activity of p300 was demonstrated to occur predominantly on a single lysine residue (K72) [75]. The modification resulted in a significant reduction in the ability of pol β to participate in a BER reaction in vitro, due to the inhibition of the dRP-lyase activity residing in the N-terminal portion of the protein. No significant effect on AP lyase, on the gap filling activity, or on the DNA binding ability, was observed. Acetylation of pol β was also verified in vivo, and it was suggested to regulate the pathway choice of either short- or long-patch BER, or to inactivate the dRP-lyase activity after completion of the repair process [76]. Very recently, additional sites of modification (K5, K35, K47, K67, K81, K113, K141, K206, K209, K220 and K230) were observed by MS after in vitro reaction with purified pol β and p300. However, K72 and K81 were the most represented [77]. Although no significant differences among the acetylated and the deacetylated form was observed when the pol β activity was assayed on nucleosomal substrates, acetylated pol β enhanced strand displacement synthesis, while the inhibition of dRP-lyase activity was confirmed [77].

Acetylation of NER factors

Nucleotide excision repair is an important DNA repair mechanism removing complex and helix-distorting lesions. Nevertheless, the interaction and acetylation by p300/CBP have been investigated only for a few factors specifically participating in this process.

DNA damage binding (DDB) protein complex

After UV exposure, UV–DDB complex, formed by p127 (DDB1) and p48 (DDB2) subunits, is rapidly recruited to chromatin where it recognizes UV-induced lesions to initiate global genome-NER [78]. Some early experiments demonstrated that both subunits were able to interact with p300 and CBP, in vitro and in vivo [79]. However, further experiments revealed that the p127 subunit is able to associate with p300 independent of the p48 [80]. Given that the UV–DDB complex plays a role in chromatin, the interaction was suggested to keep the p300/CBP–DDB complex anchored to chromatin, and to promote DNA repair in less accessible chromatin [79, 80]. A large-scale proteomic study by MS showed that residue K278 in DDB2, and K1067 in DDB1 are possibly acetylated in vivo [29]. However, no direct evidence confirming that p300 and/or CBP are responsible for acetylation of DDB proteins has been reported so far.

XPA

Xeroderma pigmentosum group A (XPA) protein plays an important role in NER by interacting and positioning core NER factors around the lesion [81]. Specific acetylation of XPA protein by CBP and p300 was identified, both in vitro and in vivo, at lysine K63 and K67, after labeling with ^{14}C-acetyl CoA [82]. After cell exposure to UV-C radiation, XPA was deacetylated by SIRT1 to ensure correct NER since silencing of SIRT1 resulted in a reduced DNA repair and an increased sensitivity to UV radiation. XPA-deficient cell lines complemented with mutants mimicking hypoacetylated XPA (K63,67R) rescued cell sensitivity to UV radiation, while expression of a mutant (K63, 67Q) mimicking the acetylated form did it only partially. In addition, XPA deacetylation led to an increased interaction between XPA and RPA, further supporting the importance of XPA deacetylation for efficient NER [82]. Confirmation of XPA acetylation was obtained in liver extracts, although the effect on NER process appeared to be negligible, probably because of low acetylation levels of the protein [83]. Further studies are needed to clarify the significance of XPA modification by p300/CBP.

XPG

XPG protein is another core NER factor endowed with 3′ endonuclease activity necessary for DNA incision and lesion removal [81]. In a large-scale proteomic study by MS, XPG protein was found acetylated at K6, a residue located in the catalytic region [28]. A search for p300 and PCNA interactors during NER showed that XPG protein does interact not only with PCNA but also with p300 and CBP. The interaction increased after UV-C irradiation, and acetylation of XPG protein was detected both in vivo and in vitro; the acetylated form was preferentially associated with chromatin [84]. Depletion of both p300 and CBP by RNAi, or chemical inhibition by curcumin, induced a decrease in XPG acetylation with a concomitant increase in the chromatin-bound

protein. A similar increase was observed in p21-null fibroblasts, suggesting that p21 may influence XPG acetylation by displacing PCNA interaction with p300. In fact, PCNA reduced XPG acetylation in vitro, probably by inhibiting p300 activity [84–86]. These results suggested that p300-mediated acetylation promotes XPG release from chromatin after DNA repair, and are supported by MS analysis (our unpublished results) that in vitro p300 acetylates XPG at the C-terminal, on a residue different than K6 located in the N-terminal catalytic region. Therefore, additional studies are required to establish whether acetylation may affect XPG catalytic activity and/or the DNA binding.

Acetylation of other DNA repair factors

Among other important DNA repair pathways, such as homologous recombination (HR), NHEJ, and interstrand cross-link (ICL) repair [28], only a few reports have indicated factors that are acetylated by p300 and/or CBP.

Ku70

Ku70 is, together with Ku80, a protein binding damaged DNA during the repair process of double-strand breaks by NHEJ, and also during V(D)J recombination [28]. Ku70 acetylation by CBP was demonstrated both in vitro and in vivo by autoradiography and MS analysis. At least eight residues were identified as targets for modification in vivo [87]. Five of them, i.e., K542, K544, K553, and K556 are located in the C-terminal region of the protein adjacent to the Bax interaction domain. Acetylation of at least two residues was necessary to inhibit the ability of Ku70 to suppress Bax-induced apoptosis, since interaction between the two proteins was disrupted. The single substitution of lysine with glutamine (K539Q or K542Q), mimicking the acetylated form, resulted in the complete block of the ability to inhibit Bax-induced apoptosis, while the K to R substitution had no effect [87]. Ku70 acetylation increased following DNA damage by UV radiation, in concomitance with cytoplasmic translocation of CBP, thus implying that Ku70 acetylation might occur in the cytoplasm [87]. A site-directed mutagenesis study investigated the role of lysine residues K282, K317, K331, K338, K539, and K542, given that many of them are acetylated in vivo, and also implicated in DNA binding. Acetylation-mimicking mutants (K > Q) resulted in reduced DNA binding and impaired cell ability to repair DNA DSBs [88]. Nuclear Ku70 was also acetylated in neuroblastoma cells in response to IR and reduction in CBP-mediated Ku70 acetylation resulted in an increased DNA repair activity [89]. This result was explained by the reduced affinity of acetylated Ku70 for binding to DNA ends [89]. Thus, Ku70 acetylation following DNA damage may be a signal for reducing nuclear DNA repair to promote apoptotic cell death. In fact, the HDAC inhibitor TSA impaired NHEJ after IR-induced DNA damage, while the p300/CBP-specific inhibitor C646 reversed this effect [38].

WRN

The Werner protein (WRN) is a member of the RecQ family, playing important roles in the maintenance of genome stability [90]. Defects in *WRN* gene are associated with Werner syndrome characterized by premature aging. WRN protein shows both DNA helicase and exonuclease activities, which are required for DNA replication and repair [90]. In particular, WRN is involved in recovering stalled forks after replication stress, in connection with the replication checkpoint [91]. After DNA damage, WRN protein is translocated from nucleolus to the nucleus and the HDAC inhibitor TSA enhanced this translocation [92]. WRN was acetylated in vivo and this reaction was stimulated by p300 overexpression. In support of these findings, p300-mediated acetylation of WRN protein was detected in vitro by radiolabeling both at the N-terminal (1–368), where the exonuclease domain is located, and at the C-terminal region (1072–1432) containing the NLS of the protein [93]. WRN acetylation increased in response to DNA damage induced by UV radiation, hydroxyurea (HU), MMS, mytomycin C (MMC), and cisplatin [94]. However, WRN acetylation modified DNA binding and catalytic activity depending on DNA structures, suggesting that its role was enhanced only for physiological substrates [94]. For MMS-induced lesions, acetylation stimulated the catalytic activity of the enzyme both in vitro and in vivo during BER [93]. Interestingly, p300-mediated WRN acetylation stimulated the strand displacement DNA synthesis by DNA pol β and long-patch BER [93]. Further studies by MS identified acetylation at residues K366, K887, K1117, K1127, K1389, and K1413. Both CBP and p300 acetylated WRN protein, yet acetylation by CBP was found to stabilize WRN protein by inhibiting ubiquitination. In addition, a WRN mutant in which all six lysines were changed to arginine showed an increased sensitivity to MMC [95]. All available lines of evidence support the importance of acetylation in the regulation of multiple WRN functions in response to DNA damage, with a clear positive effect in promoting DNA repair.

RECQL4

RECQL4 is another member of the RecQ family, endowed with helicase activity, that has been associated with at least three different diseases: Rothmund-Thomson, RAPADILINO and Balled–Gerold syndromes, all characterized by genome instability, cancer predisposition and developmental abnormalities [90]. The RECQL4 protein

was shown to interact with p300 both in vitro and in vivo, and acetylation of five lysine residues was identified at positions 376, 380, 382, 385, 386 by mutational analysis and detection with anti-acetyl-lysine antibody [96]. Since these residues are located in the same regions containing a nucleolar and nuclear localization signals, the functional role of acetylation was investigated with K > A and K > R mutants. The results indicated that the positive charge of the residue is important for the nuclear localization, since the K > A mutant showed a cytoplasmic localization. Overexpression of a catalytic dead mutant form of p300 (due to mutation in HAT domain), or co-expression of a K > R mutant, resulted in the nuclear residence of the helicase [96]. Cytoplasmic localization was also induced by cell treatment with the deacetylase inhibitors TSA and nicotinamide, thus confirming the role of acetylation in regulating the cellular localization of RECQL4. It was suggested that the cytoplasmic protein might regulate the interaction with UBR1 and UBR2 E3 ubiquitin ligases, thus pointing to a proteasomal degradation of RECQL4 [96].

FANCJ

Fanconi anemia (FA) complementation group J (FANCJ) is a 5′–3′ helicase also known as BRCA1-associated C-terminal helicase 1 (BACH1) [97]. By interacting with BRCA1, FA proteins participate in the response to DNA-damaging agents that induce lesions such as interstrand cross-links (ICL) whose processing promotes HR [97]. In fact, when FANCJ activity is missing due to inactivation or failure to localize to DNA damage sites, cells show defects in DSBR repair and are hypersensitive to ICL inducing agents (e.g., cisplatin). Acetylation of FANCJ helicase has been reported in a study investigating the regulation of this protein, in which lysine K1249 was identified by mutational analysis and confirmed by MS [98]. CBP was the unique KAT able to perform this modification. DNA-damaging agents including zeocin, camptothecin and HU enhanced FANCJ acetylation, which contributed to lesion processing. However, cells expressing the mutant form K1249R were functional since catalytic activity of the protein was not modified and their expression in FA cells was able to restore MMC resistance. Interestingly, mutation mimicking constitutive acetylation (K1249Q) contributed to a repair mechanism through HR processing, while the mutation preventing acetylation (K to R) favored a process of DNA damage tolerance [98]. Therefore, FANCJ acetylation may be required to promote DNA resection-associated events, facilitating HR repair and limiting translesion DNA synthesis.

p300/CBP interaction with DDR factors

Although several works have reported the involvement of p300 and CBP in DNA repair processes, their requirement as KAT-modifying DDR factors has been demonstrated in a limited number of studies. In fact, for other important DDR players, the information available indicates that p300/CBP may interact with them, while there is no evidence of their acetylation.

ATR

The ATM and Rad3-related (ATR) proteins are the apical kinases responsible for cell cycle checkpoint activation [99]. ATR is particularly involved in the response when a genotoxic stress occurs during DNA replication. p300 and CBP interact with ATR and their association was found to increase after a replication stress induced by HU treatment [100]. After replication block, other proteins (e.g., WRN) are recruited to stalled forks; therefore, it was suggested that ATR interaction recruits p300/CBP that will then acetylate WRN, thereby regulating its transition from the nucleolus to the nucleoplasm [92]. Depletion of either or both p300 and CBP resulted in the failure to activate the replication checkpoint, suggesting that KAT activity is required in this pathway [100].

Chromodomain helicase DNA binding protein 4 (CHD4)

CHD4 protein is a helicase involved in chromatin remodeling in an ATP-dependent process, and in coordination of the DDR [101, 102]. CHD4 is recruited to DNA damage sites, and facilitates recruitment of other DNA repair factors. CHD4 or p300 knockdown reciprocally influenced their assembly at DNA repair sites, and both proteins physically interacted indicating a cooperative function for DNA repair of DSBs [103].

Consequences of p300/CBP-mediated acetylation of DDR factors

Several lines of evidence have indicated that p300/CBP activity is enhanced by DNA damage [45, 48, 104]. The functional role of acetylation in DDR factors occurs at multiple levels. Lysine acetylation may regulate catalytic activity, the cellular localization, the DNA binding affinity, or influence protein interaction with consequences affecting the stability and turnover (degradation) of the protein of interest. However, only in particular cases protein

Table 1 Functional consequences of p300/CBP-mediated acetylation of reported proteins

Protein	Catalytic activity	DNA/protein binding[a]	Localization	Protein stability
PARP-1		↑ (38)		
NBS1		↑ (40)		↑ (40)
PCNA		↑ Pol δ, β (46)		↓ (47, 48)
FEN-1	↓ Nicked flap (51, 52)	↓ (52)		
DNA-2	↑ 53	↑ 53		
TDG		↓ APE1 (56)		
OGG1	↑ (59)	↓ (59)		↓ (62)
NEIL2	↓ (63)			
APE1	↑ K27, 31, 32, 35 (68)	↑ K6, 7 (65) ↑ Chromatin, DNA Lig III, XRCC1 (70)	Nucleolus (69)	↑ (72)
DNA Pol β	↓ dRP-lyase (76) ↑ Strand displacement (77)			
XPA		↓ RPA (82)		
XPG		↑ Chromatin (84)		
Ku70		↓ Bax (87) ↓ DNA ends (88)	Cytoplasm → Nucleus (89)	
WRN	↑ (93)	↑(93)	Nucleolus → Nucleus (92)	↑ (95)
hSSB1				↑ (43)
RECQL4			Nucleus (96)	

[a]Influence on binding to DNA, or to a relevant protein when this is indicated

acetylation results in a clear positive effect for the whole pathway (Table 1). In the case of BER, the first step (lesion recognition and formation of an AP site) is mediated by a DNA glycosylase, whose activity may be stimulated (e.g., OGG1), or inhibited by acetylation (e.g., NEIL2). Under physiological conditions, NEIL2 has been shown to take care of pre- or post-replicative BER [58, 105]. However, upon DNA damage an increase in p300/CBP activity inhibiting NEIL2 may switch the initial BER reaction to a sub-pathway where other enzymes will be stimulated (e.g., OGG1 and APE1) [106]. In addition, acetylation-mediated inhibition of FEN1, as well as of the dRP-lyase activity of pol β, have been indicated to switch the reaction from the short-patch to the long-patch BER sub-pathway [53]. This shift, in concomitance with the stimulation of Dna2 activity by acetylation, will result in strand displacement synthesis, and longer flaps removal which will favor fidelity of DNA synthesis [53, 104]. Furthermore, strand displacement synthesis will be also promoted by acetylation of WRN protein [93]. Thus, the overall acetylation of multiple BER factors may result in an improvement of the fidelity in the repair mechanism and indicate a genome-protecting role for p300 and CBP [104].

It is difficult to envisage a similar interconnection for other DNA repair mechanisms, given that the number of factors currently known to be acetylated by p300/CBP are less numerous, while DNA repair processes are complex, requiring a substantial number of factors.

When the acetylation results in the inhibition of a key enzyme, or when it regulates protein function by promoting its degradation, the resultant effects in the DNA repair are less obvious. It may be worth considering that DNA repair is a process that must be terminated and factors have to be removed from DNA once their activity is not needed any more. Thus, acetylation-driven localization changes or protein degradation may provide the relevant signal to avoid persistent and unnecessary activation of DNA repair factors [107, 108]. From this point of view, it is interesting to note that protein turnover from chromatin is a process requiring protein ubiquitination, and linked to protein acetylation [109]. Such condition may be exemplified by the effect of acetylation on PCNA degradation, or on chromatin residence of XPG [48, 84].

Conclusions

The requirement of full p300/CBP activity for DNA repair is supported by studies showing DDR defects in *CREBBP*$^{+/-}$ mice [110], with a reduction in the efficiency of NER or BER, when both CBP and p300 are silenced by RNA interference [84, 111], or when both acetyl transferases are specifically inhibited with small molecules [104, 112].

In conclusion, the available lines of evidence indicate that p300 and CBP are direct regulators of DDR, and their impairment may contribute to loss of genome integrity and

tumorigenesis, as a consequence of inefficient DNA repair, and/or inactivation of checkpoint functions. Although more studies are required to complete the view of the effect of protein acetylation in DDR, and particularly in the different DNA repair processes, the participation in these pathways provides another relevant mechanism contributing to the tumor suppressor functions of p300 and CBP acetyl transferases.

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