Available online at www.sciencedirect.com
SCIENCE DIRECT
DNA Repair 4 (2005) 1306–1313
DNA REPAIR
www.elsevier.com/locate/dnarepair

Sirtuins (histone deacetylases III) in the cellular response to DNA damage—Facts and hypotheses

Marcin Kruszewski *, Irena Szumiel

Department of Radiobiology and Health Protection, Institute of Nuclear Chemistry & Technology, Dorodna 16, 03-195 Warszawa, Poland

Received 3 June 2005; received in revised form 24 June 2005; accepted 25 June 2005
Available online 3 August 2005

Abstract

Histone deacetylases (HDAC) are an important member of a group of enzymes that modify chromatin conformation. Homologues of the yeast gene *SIR2* in mammalian cells code type III histone deacetylases (HDAC III, sirtuins), dependent on NAD^+ and inhibited by nicotinamide. In yeast cells, Sir2 participates in repression of transcriptional activity and in DNA double strand break repair. It is assumed that certain sirtuins may play a similar role in mammalian cells, by modifying chromatin structure and thus, altering the accessibility of the damaged sites for repair enzymes. A relation between poly(ADP-ribosylation) and sirtuin function in cells with damaged DNA has been also postulated.

Interconnections between NAD^+ metabolism, poly(ADP-ribosylation), DNA repair and gene expression should allow to modulate the cellular response to agents that damage DNA. Preliminary results, reviewed in this paper indicate that such possibility exists. We propose a hypothetical mechanism of sirtuin participation in DSB repair. It is based on the assumption that activation of PARP at the sites of DNA strand breaks leads to a local increase in nicotinamide concentration. Nicotinamide then inhibits sirtuins exactly at the site of DNA strand break. At present, however, there are no data directly confirming the effect of sirtuin inhibition on DSB repair processes in mammalian cells. Nevertheless, a connection between the acetylation status of histones and repair of DNA breaks has recently been found, indicating that all HDAC classes may modulate DNA repair processes. In addition, sirtuins exert an anti-apoptotic action in various cell types. Hence, it is possible to sensitise cells to apoptosis-inducing agents by sirtuin inhibitors.

Keywords: DNA double strand breaks; Apoptosis; Poly(ADP-ribose) polymerase

1. Introduction

In diagrams of functions of various DNA repair systems, DNA usually is presented as a straight line. This, of course, is a greatly simplified picture. Although DNA is a linear molecule, in eukaryotic cells it is present as a complicated structure complexed with numerous proteins, chromatin. Double-stranded DNA is wound around core histones H2A, H2B, H3, H4, two of each per nucleosome "core", to form the basic chromatin fibre (reviewed in [1]). Between nucleosomes, a stretch of DNA—linker DNA—binds histone H1. Such is the "beads on a string" model of chromatin. This "basic fibre" is further coiled and supercoiled and stabilised by other, non-histone proteins. Chromatin is a highly dynamic structure, and the degree of condensation locally varies depending on whether the particular DNA fragment is to be stored as transcriptionally inactive, or is—on demand—available to be transcribed, replicated or repaired. The condensation–decondensation transition depends on var-

Abbreviations: CBP, CREB-binding protein; DNA-PK, DNA-dependent protein kinase; DSB, DNA double strand break; FOXO, forkhead box class O; Gadd45, growth arrest and DNA damage response gene; HAT, histone acetyltransferase; HDAC, histone deacetylase; Mn-SOD, manganese superoxide dismutase; NHEJ, non-homologous end joining; p27/Kip1, cyclin-dependent kinase inhibitor; PARG, poly(ADP-ribose) glycohydrolase; PARP-1, poly(ADP-ribose) polymerase-1; PCAF, p300/CBP-associated factor; Sir, silent information regulator; SIRT1, human homologue of Sir2; SWI/SNF, ATP-dependent chromatin remodeling factors; TRAIL, tumor necrosis factor apoptosis related ligand

* Corresponding author. Tel.: +48 22 811 07 36; fax: +48 22 811 07 36.
E-mail address: marcinkr@orange.ichtj.waw.pl (M. Kruszewski).

1568-7864/$ – see front matter © 2005 Elsevier B.V. All rights reserved.
doi:10.1016/j.dnarep.2005.06.013

ious factors, the essential ones being the post-translational modifications of histone molecules.

From every nucleosome there extend eight flexible polypetide "tails", one for each histone molecule of the nucleosomal core. These "tails" are modified: acetylated, methylated, ADP-ribosylated, ubiquitinated or phosphorylated, depending on the functional needs. Acetylation is a way to decondense the chromatin fibre and it opens a further possibility to remodel the structure, e.g. by the ATP-dependent chromatin remodeling factors (known as SWI/SNF remodelers) that permit to access the promoter sequences (reviewed by [2]). Deacetylation opens the way to histone methylation and formation of the inactive ("silenced") chromatin—heterochromatin.

The opposing actions of two types of enzymes that carry out histone modifications are shown in Fig. 1. Histone acetyltransferases add acetyl groups to the numerous lysine residues in histone "tails". Thus, the positive charges that interact with DNA are neutralised and the nucleosome fiber becomes loosened. Histone deacetylases remove acetyl groups and thus, a more compact structure is formed. There are numerous members of both these enzyme families and they are included into the "transcriptosome" complex. The complex not only produces gene transcripts, but also contains some proteins co-operating with excision repair enzymes in the transcription-coupled excision repair system.

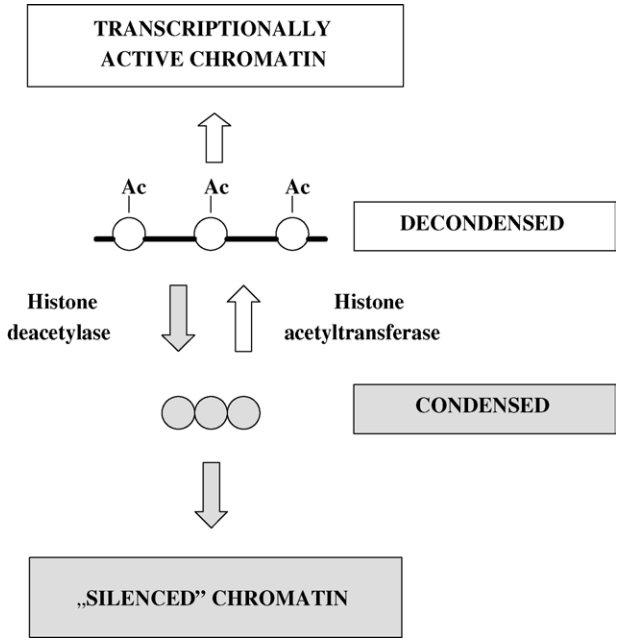

Fig. 1. The histone acetylation switch. HAT and HDAC determine the condensation status and in consequence, the potential transcriptional activity of chromatin. Acetylation "opens" the structure and permits SWI/SNF chromatin remodeling factors to access promoter sequences and thus, allows initiating transcription. Deacetylation, especially when followed by histone methylation, forms a condensed ("silenced") chromatin conformation (heterochromatin), which is transcriptionally inactive. Acetylated histone tails are shown as lines with Ac.

2. Histone deacetylases

HDACs are globular molecules with conical "pockets" that fit acetylated lysine residues. The enzyme "clips off" the acetyl groups and thus, unmasks the positive charge of the amino acid.

There are 11 isoforms of human HDACs, divided into three classes, according to their similarity to yeast enzymes. Class I HDACs are similar to the yeast (y) transcriptional repressor, yRPD3; class II HDACs, to yHDA1 and class III HDACs, to ySIR2. They are present in almost all tissues, both normal and malignant, with tissue-specific expression patterns. Their activity is related to such diverse and interrelated cellular processes as transcription activation, gene silencing, cell-cycle progression and DNA replication and repair (reviewed [3–5]).

The class I enzymes (HDAC1, HDAC2, HDAC3, HDAC8) have smaller size (ca. 500 amino acid residues), whereas the class II enzymes (HDAC4, HDAC5, HDAC6, HDAC7, HDAC9) are about twice larger. It is not clear what are the functional differences between individual HDACs. Probably, some of these differences come from co-operation with distinct sets of transcription regulatory factors and hence, HDAC act specifically as co-repressors of gene expression from different promoters [6–9]. Inhibitors of class I and II HDACs have been developed that show great promise as anticancer drugs, although the molecular basis of the anticancer effect is not fully understood [10–13], reviewed by [14–16]. HDAC inhibition not only results in acetylation of histones but also transcription factors such as p53 and estrogen receptor-alpha [15]. Altogether, it seems to act very selectively, as it affects the expression of only 2% of mammalian genes [17].

The ySIR2 homologues or type III HDACs in mammalian cells form a gene family consisting of seven sirtuins which are NAD^+-dependent histone/protein deacetylases (reviews in [18,19]). Due to the NAD^+ dependence, sirtuins might link metabolic activity and gene expression by means of the histone/protein acetylation activity. Their mechanism of action has recently been revealed [20–22]. The deacetylating activity of sirtuins depends on NAD^+ and is inhibited by the reaction product, nicotinamide ($IC_{50} < 50\ \mu M$, [23].

$$\text{Acetylprotein} + NAD^+ \xrightarrow{\text{Sirtuin}} \text{nicotinamide} + \textit{O}\text{-acetyl-ADP-ribose} + \text{protein}$$

There are indications that *O*-acetyl-ADP-ribose is a signalling molecule [24]. The inhibitory effect of nicotinamide explains the calorie restriction effect, in which Sir2 is implicated. Prolongation of lifespan of both budding yeast and the nematode worm, *Caenorhabditis elegans*, by restricted access to nutrients occurs by increasing the activity of Sir2. This effect is due to activation of the PNC1 gene, coding nicotinamidase. Thus, the concentration of nicotinamide and its inhibitory effect on Sir2 decrease [25,26].

3. Histone deacetylation and DNA repair

3.1. Yeast cells

Both deacetylation and acetylation of histones seem important for DSB repair in yeast cells. Several HDACs have been identified as partners in the DSB repair machinery. In *Saccharomyces cerevisiae* cells, Sir2 affects DNA double strand break (DSB) repair of the NHEJ type [27–29]; recent findings, however, indicate that the effect may be indirect (cf. [28]). On the other hand, deacetylation by the Sin3p/Rpd3p complex is required for repair of phleomycin-induced DSB by the NHEJ system in *Saccharomyces cerevisiae* [28]. In particular, Sin3p-dependent hypoacetylation of lysine 16 of histone H4 occurs in the vicinity of DSBs. Lack of Sin3p activity does not affect the homologous recombination repair pathway and some breaks still are repaired by NHEJ, although with reduced efficiency. As shown by Boulton et al. [30], the *Caenorhabditis elegans* homologue of Rpd3p, Hda-3, is required for DNA repair after X-ray-exposure.

In contrast with the above-described observations, the importance of histone acetylation for DSB repair in yeast cells is emphasised by other authors. For example, the whole lysine cluster in the C-terminal tail of histone H4 must be acetylated in order to support NHEJ [31]. Consistently, lack of the ESA1 histone acetyl transferase also is the cause of DSB repair defect. This enzyme is part of the multiprotein complex recruited to DSB, that binds to H4 histone and acetylates lysine residues [31]. It seems meaningful that acetylation and deacetylation of the same lysine 16 is necessary for efficient DSB repair. On the basis of these and other results, concerning other post-translational modifications, it was assumed that a correct histone modification is established for each step of the DNA repair process [28]. In addition, Fernandez-Capetillo and Nussenzweig [29] suggest that the specific pattern of histone modifications serves to trigger a compaction of chromatin around DSB and thus concentrate the NHEJ proteins and limit the mobility of free DNA ends.

One more factor that points to the importance of histone acetylation status is the choice of DSB repair pathway. In yeast it depends on the "histone code", that is, the specific pattern of post-translational modifications, including acetylation. Histone H2A acetylation and phosphorylation and H4 deacetylation marks commitment for NHEJ, whereas H4 acetylation, H2A phosphorylation and H3 acetylation directs the DSB repair to homologous recombination pathway [32].

As with many other features of DNA repair, also in that case mammalian cells probably resemble yeast, although histone acetylation status is not the only factor that affects the choice of DSB repair [33].

3.2. Mammalian cells

In contrast with other eukaryotes, yeast cells are devoid of poly(ADP-ribosylation) reactions. In mammalian cells, sirtuins, in addition to other HDACs, may play a similar role in DSB repair as NAD^+-independent HDACs in yeast. This assumption is based on the fact that DNA breaks activate PARP-1. This enzyme and another, closely related one, PARP-2, use up NAD^+ and produce nicotinamide, similarly to sirtuins.

$$\text{protein} + n\,\text{NAD}^+ \xrightarrow{\text{PARP-1}} n\,\text{nicotinamide} + (\text{ADP-ribosylated})n\,\text{protein}$$

Recently, a hypothesis has been published by Zhang [34] which assumes a relation between poly(ADP-ribosylation) and sirtuin function in mammalian cells. Interconnections between NAD^+ metabolism, poly(ADP-ribosylation) and sirtuin activity are based on a similarity of PARP and sirtuin catalysed reactions. Accordingly, inhibition or activation of sirtuins may profoundly alter the cellular response to DNA damage, since the effects also concern the competing ADP-ribosylation system.

In mammalian cells, sirtuins can affect the cellular response to DNA damage in two ways, not mutually exclusive: (i) by modifying chromatin structure at the damaged site and thus, altering the access for repair enzymes and (ii) preventing hyperacetylation of p53, thus affecting the p53-dependent apoptosis [35,36]. As discussed below, experimental data support both mechanisms; since the cell lines used in the in vitro experiments were of various origin, it is plausible to assume that the choice of response type depends on the cellular context.

4. Sirtuins and DSB repair

Activation of PARP at the sites of DNA strand breaks (review in [37]) leads to a local increase in nicotinamide concentration, hundreds of molecules are generated by one enzyme molecule, since the poly(ADP-ribose) polymers are synthesised at multiple sites and consist of ADP-ribose monomer units ranging from several tens to about 200. This means that sirtuins may be locally inhibited by nicotinamide *exactly at the site of DNA strand break*. So, apart from the decondensing effect of ADP-ribose polymers, local generation of nicotinamide opens the way to histone acetylation and a further decondensing of chromatin. The inhibition would be transient, as the polymers—after reaching a certain size—are quickly degraded by poly(ADP-ribose) glycohydrolase. Inhibition of the glycohydrolase should give the same biological effect as sirtuin activation. Before degradation, the polymers facilitate recruitment of poly(ADP-ribose) binding proteins and "reprogram" domain functions of various target proteins [37].

Both single strand breaks (SSB) and DSB are effective activators of PARP; however, SSB rejoining is very effective and very soon completed; thus, the time interval for the possible inhibitory effect on sirtuins is very short. We are not aware of any data that would indicate the effect of sirtuin inhibitors on SSB repair. On the other hand, at least a sector

of DSB is repaired more slowly than SSB. This means that PARP activation is prolonged.

The local chromatin decondensation step may facilitate recruitment of repair enzymes and affect their function. Recent models of DNA repair in the chromatin context include HAT and HDAC activities [38,39]. HAT activity was shown to be indispensable for DNA repair [40]. The role of reversible histone acetylation is supported by the observation of Park et al. [41] that DNA-PK, the main actor of NHEJ, phosphorylates the C-terminal tail of histone H2AX with increased efficiency in an acetylation-dependent manner. This dependence is observed only when the histone H2AX molecules are in acetylated nucleosomes and not in the free form. It should be added that binding of Ku subunits to DSB and the subsequent activation of DNA-PK catalytic subunit is not enhanced by histone acetylation. Phosphorylation of histone H2AX by ATM or DNA-PK is an early step in formation of DSB repair foci (cf. review by [42]). Also, HDAC inhibition increases radiosensitivity of tumor cells in vitro and in vivo and prolongs the expression of γH2AX foci in X-irradiated human tumor cells in vitro; the latter effect suggests a slower DSB repair [43–45].

Altogether, the reversible acetylation-deacetylation of histone tails seems to be an important factor in DSB repair. As proposed by Jazayeri et al. [28] for yeast, at each repair step there is a specific histone modification pattern ("epigenetic pattern") and acetylation is part of it. This statement seems to be fully valid for mammalian cells. Furthermore, it is probable that all HDAC classes participate in creating the proper "epigenetic pattern". For example, HDAC1 co-immunoprecipitates with ATM protein [46], a key component in generation of the alarm signalling after infliction of DNA breaks, and with Hus1, a member of the 9-1-1 sensor/cell cycle checkpoint complex [47]. At the same time, HDAC1 is bound to the nuclear matrix [46], site of transcription, replication and DNA repair (reviewed by [48–50]).

Fig. 2 illustrates our hypothesis concerning the contribution of sirtuins to the sequential steps of DSB repair. The diagram is considerably simplified and assumes a linear sequence of events, whereas in reality, some of them take place in parallel, in a concerted way and there are several feedback loops ommited for clarity (only one is shown). It should be noted that activity of other HDACs is presumed. Also, there usually is a redundancy of cellular functions, which assures a flexibility of cellular responses. It may be expected that the expression pattern of various HDACs, which may vary depending on cell type, will define the relative contribution of sirtuins to DNA breakage repair. Another factor affecting the contribution would be the expression and subcellular localisation of PARP1 and PARP2. Vigorous activation of PARP takes place at the sites of DNA breakage and hence, generation of nicotinamide also occurs in the close vicinity to DNA breaks. Thus, nicotinamide will inhibit SIRT1 exactly where histone acetylation is necessary. Nevertheless, the same role in shifting the equilibrium between acetylation and deacetylation at the early step of DSB repair may be

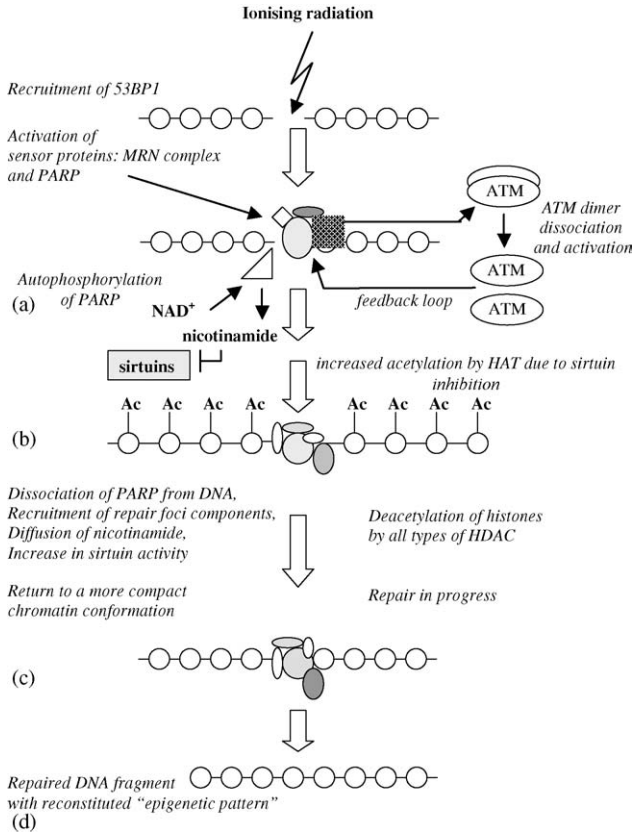

Fig. 2. The possible sequence of steps following induction of a DSB. (a) Sensor proteins become activated: the MRN complex (Mre11-Rad50-nibrin) activates the ATM dimer; the active monomers phosphorylate numerous repair proteins, including MRN; PARP undergoes automodification, using up NAD^+ and producing nicotinamide. (b) Local increase in nicotinamide concentration inhibits sirtuins; this shifts the HDAC-HAT equilibrium to establish enhanced acetylation of histones. Decondensation of the nucleosome fiber follows and opens sites for other post-translational modifications and for binding various components of repair foci. (c) After the poly(ADP-ribose) polymers reach critical size, the PARP molecules dissociate from DNA and "reprogram" domain functions of various target proteins [37]; alternatively, the polymers become degraded by PARG. Nicotinamide stops being produced and its inhibitory action on sirtuins ceases. At this point, various post-translational modifications can follow, including that of HDAC-catalysed deacetylation. (d) DSB repair is completed and the original post-translational modification pattern ("epigenetic pattern") is restored. Note, that the diagram is considerably simplified and assumes a linear sequence of events, whereas in reality, some of them take place in parallel.

played by highly active HAT and weakly expressed class I or II HDACs. Hence, sirtuins probably are not unconditionally necessary for the early repair steps and may be replaced by other HDACs; at least, the absence of SIRT1 does not radiosensitise embryo fibroblasts from SIRT1-deficient mice [49,51]. The contribution of other nuclear sirtuins was not examined.

5. Sirtuins and apoptosis: p53

There are several paths to apoptosis; in the case of cell with damaged DNA, the pathway of choice is the p53-

dependent one. Regulation of p53 functions is very complex. The molecule is reversibly phosphorylated, ADP-ribosylated and acetylated. Acetylation of lysine residues, (including K320, K373 and K382 in human p53) favours stabilisation of the molecule and its transactivation function [52], reviewed by [53]. SIRT1, the human Sir2 homolog, acts as a negative regulator of the transactivation function of p53 [54].

This role of SIRT1 is supported by further observations. SIRT1 is recruited to the promyelocytic leukemia protein (PML) nuclear bodies where it binds and deacetylates p53 [55]. To study the function of SIRT1, Cheng et al. [51] generated two different SIRT1 mutations in mice, one which expressed a mutant protein lacking part of the catalytic domain, and one which completely eliminated protein expression. There was a significant increase in p53-dependent, γ-ray-induced apoptosis in SIRT1-deficient thymocytes isolated from 2 to 3-week-old mutant mice. This finding was consistent with p53 hyperacetylation observed after infliction of DNA damage by irradiation with 5 Gy γ-rays. There was no change in p53 stability, as judged from Western blotting, in contrast with the increased stability after inhibition of HDAC1. On the other hand, embryo fibroblasts from mutant mice were similarly γ-ray-resistant as wild type cells. So, it may be assumed that cellular context decides on the effect of SIRT1 inactivation on the response to ionising radiation. Additionally, Cheng et al. [51] provided evidence that other HDAC cannot fully replace SIRT1 in deacetylation of p53. Further support for sirtuin role in p53-dependent apoptosis was supplied by Luo et al. [56], who found that the murine homologue of ySir2, Sir2alpha, physically interacted with p53 and repressed apoptosis induced by DNA damage.

We propose that this feature of SIRT1 action may explain the relation observed in X-irradiated cells between the extent of DNA breakage, rejoining rate and apoptosis. Persistent breaks keep PARP in an activated state and nicotinamide thus produced inhibits SIRT1. This leads to hyperacetylation of p53 and to its enhanced transactivation function. In consequence, increase in transcription of pro-apoptotic genes and in the amount of the corresponding proteins contribute to the observed increase in apoptosis.

The Ku70 subunit of DNA-PK has recently been shown to suppress apoptosis by sequestering Bax from mitochondria. Increased acetylation of cytoplasmic Ku70 disrupts the Ku70–Bax interaction whereas HDAC inhibitors abolish the ability of Ku70 to suppress Bax-mediated apoptosis [35,57]. It remains to be established to what extent sirtuin inhibitors would induce apoptosis by this mechanism.

6. Sirtuins and apoptosis: FOXO

FOXO transcription factors control the expression of genes involved in apoptosis (Fas ligand; proapoptotic BH3-only proteins: Bim and TRAIL), cell cycle progression (cyclin D, Gadd45, p27/Kip1), and stress responses (Gadd45, Mn-SOD). As summarised in Fig. 3, similarly to p53, FOXO

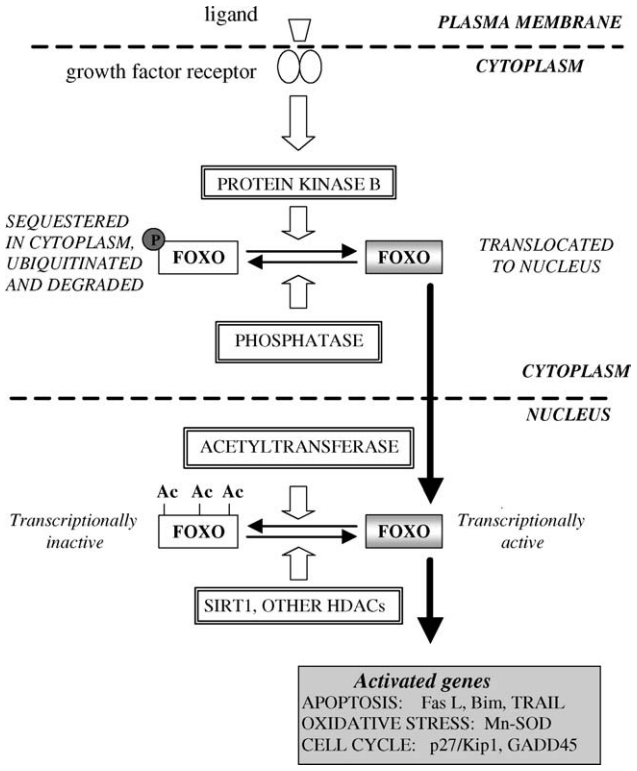

Fig. 3. Translocation and transactivation function of FOXO transcription factors, as dependent on post-translational modifications. Ac, acetyl group; P, phosphate group; other abbreviations listed in the abbreviation list.

factors' activity is regulated by phosphorylation and acetylation; furthermore, their functional interactions have recently been revealed and discussed (cf. [58]). When growth factors are present, protein kinase B (Akt) phosphorylates FOXO factors and thus, prevents their translocation into nucleus. When the growth factor signalling is switched off, FOXOs are located in the nuclei and act as transcription factors, unless acetylated. FOXO3 acetylation is carried out by protein acetyltransferases, PCAF and p300/CBP, in proportion to oxidative DNA damage. The acetylation degree is not altered by ultraviolet or ionising radiations [59]. This modification is the cause of loss of transactivation properties; the transcriptional activity of FOXO3 is restored by deacetylation carried out by SIRT1 [60,61] and also by class I and II HDACs. Thus, SIRT1 increases FOXO3's ability to induce cell cycle arrest and resistance to oxidative stress. According to Brunet et al. [59], in cells with damaged DNA such effect may contribute to cellular recovery by leaving more time for DNA repair and decreasing the amount of reactive oxygen species that act as pro-apoptotic signals.

However, SIRT1 does not enhance to the same extent the transcription of all genes targeted by FOXO factors [59]. Apparently, the anti-apoptotic effects of sirtuin inhibitors depends on the cellular context; specifically, on ligands available for binding to receptors that initiate the signalling to protein kinase B and on the pro-apoptotic genes targeted by FOXO factors in the particular cell type. Hence, the effect

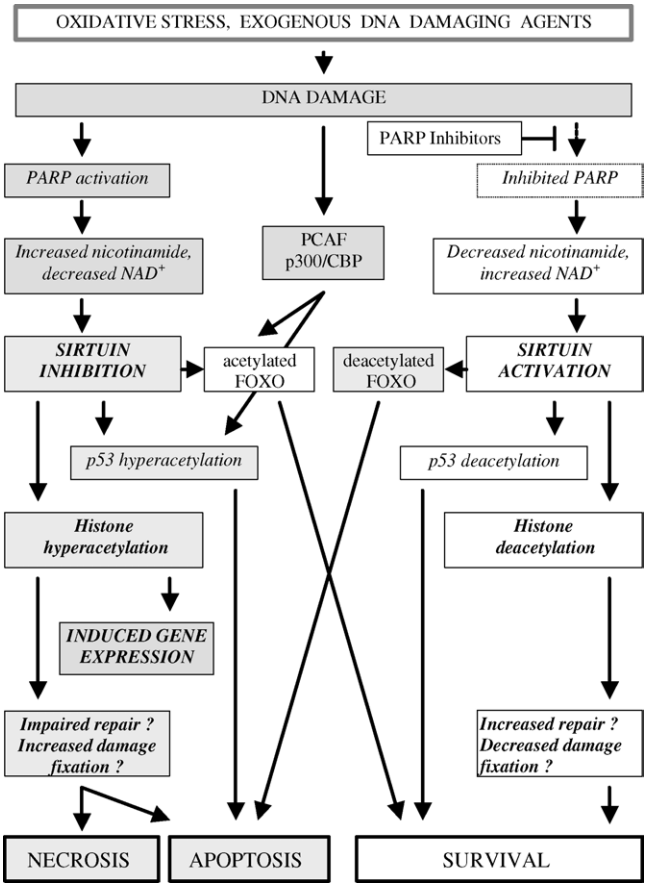

Fig. 4. Interactions between PARP and sirtuin-dependent processes in cells with damaged DNA. See text for discussion.

of sirtuins on FOXO-mediated apoptosis may be more limited than that on p53-dependent apoptosis. Nevertheless, in some cell types, especially in the absence of protein kinase B-targeted signalling, this effect may be important.

7. Concluding remarks

The interconnections between pathways involving PARP and sirtuins, as understood on the basis of research carried out so far and summarised by Zhang [34] are presented in the diagram (Fig. 4). Although the assumptions of Zhang seem convincing, they were not directly tested. As discussed in the previous sections, however, many studies implicate sirtuin participation in the response to DNA damage, in agreement with the presented concepts.

The web of interconnections presented in Fig. 4 is still more complicated in vivo. The net result, survival or death, depends on the equilibrium between specific pathways. The effect of PARP or sirtuin inhibition is not obvious, unless other intracellular conditions are defined. For example, in the case of FOXO-induced apoptosis, sirtuin inhibition will be anti-apoptotic, whereas p53-dependent apoptosis will be enhanced. However, active protein kinase B will prevent any influence of FOXO on apoptosis (see Fig. 3) and hence, also that of sirtuin inhibitors. Damaged cells with DNA breakage may be rescued from NAD^+ depletion and the consequent apoptosis by PARP inhibition. On the other hand, PARP is necessary for DNA repair (cf. [37]) and for preventing ionising radiation-induced damage fixation [62]. So, it is difficult to non-ambigously predict the effect of sirtuin inhibition in the damaged cell without additional knowledge of specific features of signalling and DNA repair in the particular cell type.

In spite of these reservations, most experimental data show that sirtuin activation counteracts pro-apoptotic signalling. It is speculated that in consequence, the cell with damaged DNA is not committed to die by apoptosis; this leaves time for DNA repair. Thus, high activity of sirtuins would be a pro-survival factor. Conversely, treatment with sirtuin inhibitors should sensitise to the DNA damaging agents as its consequence would be enhanced apoptosis, as, in fact, was observed under various experimental conditions. To apply sirtuin inhibitors in therapy, it is necessary to know the pre-requirements for obtaining the required result—a feasible task with the presently available techniques.

Acknowledgement

The authors were supported by the statutory grant of the State Committee for Scientific Research for the Institute of Nuclear Chemistry and Technology.

References

[1] R.D. Kornberg, Y. Lorch, Twenty-five years of the nucleosome, fundamental particle of the eukaryote chromosome, Cell 98 (1999) 285–294.
[2] L. Mohrmann, C.P. Verrijzer, Composition and functional specificity of SWI2/SNF2 class chromatin remodeling complexes, Biochim. Biophys. Acta 1681 (2005) 59–73.
[3] M.J. Carrozza, R.T. Utley, J.L. Workman, J. Cote, The diverse functions of histone acetyltransferase complexes, Trends Genet. 19 (2003) 321–329.
[4] A.J. de Ruijter, A.H. van Gennip, H.N. Caron, S. Kemp, A.B. van Kuilenburg, Histone deacetylases (HDACs): characterization of the classical HDAC family, Biochem. J. 370 (2003) 737–749.
[5] S. Milutinovic, Q. Zhuang, M. Szyf, Proliferating cell nuclear antigen associates with histone deacetylase activity, integrating DNA replication and chromatin modification, J. Biol. Chem. 277 (2002) 20974–20978.
[6] W. Fischle, S. Emiliani, M.J. Hendzel, T. Nagase, N. Nomura, W. Voelter, E. Verdin, A new family of human histone deacetylases related to Saccharomyces cerevisiae HDA1p, J. Biol. Chem. 274 (1999) 11713–11720.
[7] C.M. Grozinger, C.A. Hassig, S.L. Schreiber, Three proteins define a class of human histone deacetylases related to yeast Hda1p, Proc. Natl. Acad. Sci. U.S.A. 96 (1999) 4868–4873.
[8] E. Hu, Z. Chen, T. Fredrickson, Y. Zhu, R. Kirkpatrick, G.F. Zhang, K. Johanson, C.M. Sung, R. Liu, J. Winkler, Cloning and characterization of a novel human class I histone deacetylase that functions as a transcription repressor, J. Biol. Chem. 275 (2000) 15254–15264.

[9] N.R. Bertos, A.H. Wang, X.J. Yang, Class II histone deacetylases: structure, function, and regulation, Biochem. Cell Biol. 79 (2001) 243–252.
[10] J. Arts, S. De Schepper, K. Van Emelen, Histone deacetylase inhibitors: from chromatin remodeling to experimental cancer therapeutics, Curr. Med. Chem. 10 (2003) 2343–2350.
[11] L. Catley, E. Weisberg, Y.T. Tai, P. Atadja, S. Remiszewski, T. Hideshima, N. Mitsiades, R. Shringarpure, R. LeBlanc, D. Chauhan, N.C. Munshi, R. Schlossman, P. Richardson, J. Griffin, K.C. Anderson, NVP-LAQ824 is a potent novel histone deacetylase inhibitor with significant activity against multiple myeloma, Blood 102 (2003) 2615–2622.
[12] K. Fronsdal, F. Saatcioglu, Histone deacetylase inhibitors differentially mediate apoptosis in prostate cancer cells, Prostate 62 (2005) 299–306.
[13] S.B. Khan, T. Maududi, K. Barton, J. Ayers, S. Alkan, Analysis of histone deacetylase inhibitor, depsipeptide (FR901228), effect on multiple myeloma, Br. J. Haematol. 125 (2004) 156–161.
[14] R.W. Johnstone, Histone-deacetylase inhibitors: novel drugs for the treatment of cancer, Nat. Rev. Drug Discov. 1 (2002) 287–299.
[15] D.M. Vigushin, R.C. Coombes, Histone deacetylase inhibitors in cancer treatment, Anticancer Drugs 13 (2002) 1–13.
[16] M. Yoshida, A. Matsuyama, Y. Komatsu, N. Nishino, From discovery to the coming generation of histone deacetylase inhibitors, Curr. Med. Chem. 10 (2003) 2351–2358.
[17] J.R. Davie, Inhibition of histone deacetylase activity by butyrate, J. Nutr. 133 (2003) 2485S–2493S.
[18] G. Blander, L. Guarente, The Sir2 family of protein deacetylases, Annu. Rev. Biochem. 73 (2004) 417–435.
[19] S.W. Buck, C.M. Gallo, J.S. Smith, Diversity in the Sir2 family of protein deacetylases, J. Leukoc. Biol. 75 (2004) 939–950.
[20] M.T. Borra, M.R. Langer, J.T. Slama, J.M. Denu, Substrate specificity and kinetic mechanism of the Sir2 family of NAD+-dependent histone/protein deacetylases, Biochemistry 43 (2004) 9877–9887.
[21] M.D. Jackson, M.T. Schmidt, N.J. Oppenheimer, J.M. Denu, Mechanism of nicotinamide inhibition and transglycosidation by Sir2 histone/protein deacetylases, J. Biol. Chem. 278 (2003) 50985–50998.
[22] K. Zhao, R. Harshaw, X. Chai, R. Marmorstein, Structural basis for nicotinamide cleavage and ADP-ribose transfer by NAD(+)-dependent Sir2 histone/protein deacetylases, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 8563–8568.
[23] K.J. Bitterman, R.M. Anderson, H.Y. Cohen, M. Latorre-Esteves, D.A. Sinclair, Inhibition of silencing and accelerated aging by nicotinamide, a putative negative regulator of yeast sir2 and human SIRT1, J. Biol. Chem. 277 (2002) 45099–45107.
[24] M.T. Borra, F.J. O'Neill, M.D. Jackson, B. Marshall, E. Verdin, K.R. Foltz, J.M. Denu, Conserved enzymatic production and biological effect of O-acetyl-ADP-ribose by silent information regulator 2-like NAD+-dependent deacetylases, J. Biol. Chem. 277 (2002) 12632–12641.
[25] R.M. Anderson, K.J. Bitterman, J.G. Wood, O. Medvedik, D.A. Sinclair, Nicotinamide and PNC1 govern lifespan extension by calorie restriction in Saccharomyces cerevisiae, Nature 423 (2003) 181–185.
[26] C.M. Gallo, D.L. Smith Jr., J.S. Smith, Nicotinamide clearance by Pnc1 directly regulates Sir2-mediated silencing and longevity, Mol. Cell. Biol. 24 (2004) 1301–1312.
[27] L.K. Lewis, M.A. Resnick, Tying up loose ends: nonhomologous end-joining in Saccharomyces cerevisiae, Mutat. Res. 451 (2000) 71–89.
[28] A. Jazayeri, A.D. McAinsh, S.P. Jackson, Saccharomyces cerevisiae Sin3p facilitates DNA double-strand break repair, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 1644–1649.
[29] O. Fernandez-Capetillo, A. Nussenzweig, Linking histone deacetylation with the repair of DNA breaks, Proc. Natl. Acad. Sci. U.S.A. 101 (2004) 1427–1428.
[30] S.J. Boulton, A. Gartner, J. Reboul, P. Vaglio, N. Dyson, D.E. Hill, M. Vidal, Combined functional genomic maps of the C. elegans DNA damage response, Science 295 (2002) 127–131.
[31] A.W. Bird, D.Y. Yu, M.G. Pray-Grant, Q. Qiu, K.E. Harmon, P.C. Megee, P.A. Grant, M.M. Smith, M.F. Christman, Acetylation of histone H4 by Esa1 is required for DNA double-strand break repair, Nature 419 (2002) 411–415.
[32] J.D. Moore, J.E. Krebs, Histone modifications and DNA double-strand break repair, Biochem. Cell. Biol. 82 (2004) 446–452.
[33] Y. Aylon, B. Liefshitz, M. Kupiec, The CDK regulates repair of double-strand breaks by homologous recombination during the cell cycle, EMBO J. 23 (2004) 4868–4875.
[34] J. Zhang, Are poly(ADP-ribosyl)ation by PARP-1 and deacetylation by Sir2 linked? Bioessays 25 (2003) 808–814.
[35] H.Y. Cohen, S. Lavu, K.J. Bitterman, B. Hekking, T.A. Imahiyerobo, C. Miller, R. Frye, H. Ploegh, B.M. Kessler, D.A. Sinclair, Acetylation of the C terminus of Ku70 by CBP and PCAF controls Bax-mediated apoptosis, Mol. Cell 13 (2004) 627–638.
[36] H.Y. Cohen, C. Miller, K.J. Bitterman, N.R. Wall, B. Hekking, B. Kessler, K.T. Howitz, M. Gorospe, R. de Cabo, D.A. Sinclair, Calorie restriction promotes mammalian cell survival by inducing the SIRT1 deacetylase, Science 305 (2004) 390–392.
[37] M. Malanga, F. Althaus, DNA damage signaling through poly(ADP-robose), in: A. Bürkle (Ed.), Poly(ADP-Ribosyl)ation, Landes Bioscience, Georgetown, USA, 2004, pp. 484–493.
[38] P. Berardi, M. Russell, A. El Osta, K. Riabowol, Functional links between transcription, DNA repair and apoptosis, Cell. Mol. Life Sci. 61 (2004) 2173–2180.
[39] A. Verger, M. Crossley, Chromatin modifiers in transcription and DNA repair, Cell. Mol. Life Sci. 61 (2004) 2154–2162.
[40] T. Ikura, V.V. Ogryzko, M. Grigoriev, R. Groisman, J. Wang, M. Horikoshi, R. Scully, J. Qin, Y. Nakatani, Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis, Cell 102 (2000) 463–473.
[41] E.J. Park, D.W. Chan, J.H. Park, M.A. Oettinger, J. Kwon, DNA-PK is activated by nucleosomes and phosphorylates H2AX within the nucleosomes in an acetylation-dependent manner, Nucleic Acids Res. 31 (2003) 6819–6827.
[42] H. Lewandowska, I. Szumiel, Histone H2AX in DNA repair, Nukleonika 47 (2002) 127–131.
[43] K. Camphausen, T. Scott, M. Sproull, P.J. Tofilon, Enhancement of xenograft tumor radiosensitivity by the histone deacetylase inhibitor MS-275 and correlation with histone hyperacetylation, Clin. Cancer Res. 10 (2004) 6066–6071.
[44] K. Camphausen, W. Burgan, M. Cerra, K.A. Oswald, J.B. Trepel, M.J. Lee, P.J. Tofilon, Enhanced radiation-induced cell killing and prolongation of gammaH2AX foci expression by the histone deacetylase inhibitor MS-275, Cancer Res. 64 (2004) 316–321.
[45] K. Camphausen, D. Cerna, T. Scott, M. Sproull, W.E. Burgan, M.A. Cerra, H. Fine, P.J. Tofilon, Enhancement of in vitro and in vivo tumor cell radiosensitivity by valproic acid, Int. J. Cancer 114 (2005) 380–386.
[46] M.S. Kim, M. Blake, J.H. Baek, G. Kohlhagen, Y. Pommier, F. Carrier, Inhibition of histone deacetylase increases cytotoxicity to anticancer drugs targeting DNA, Cancer Res. 63 (2003) 7291–7300.
[47] R.L. Cai, Y. Yan-Neale, M.A. Cueto, H. Xu, D. Cohen, HDAC1, a histone deacetylase, forms a complex with Hus1 and Rad9, two G2/M checkpoint Rad proteins, J. Biol. Chem. 275 (2000) 27909–27916.
[48] J.R. Davie, S.K. Samuel, V.A. Spencer, L.T. Holth, D.N. Chadee, C.P. Peltier, J.M. Sun, H.Y. Chen, J.A. Wright, Organization of chromatin in cancer cells: role of signalling pathways, Biochem. Cell. Biol. 77 (1999) 265–275.
[49] S.K. Mauldin, R.C. Getts, W. Liu, T.D. Stamato, DNA-PK-dependent binding of DNA ends to plasmids containing nuclear matrix attachment region DNA sequences: evidence for assembly of a repair complex, Nucleic Acids Res. 30 (2002) 4075–4087.

[50] G.S. Stein, S.K. Zaidi, C.D. Braastad, M. Montecino, A.J. van Wijnen, J.Y. Choi, J.L. Stein, J.B. Lian, A. Javed, Functional architecture of the nucleus: organizing the regulatory machinery for gene expression, replication and repair, Trends Cell Biol. 13 (2003) 584–592.
[51] H.L. Cheng, R. Mostoslavsky, S. Saito, J.P. Manis, Y. Gu, P. Patel, R. Bronson, E. Appella, F.W. Alt, K.F. Chua, Developmental defects and p53 hyperacetylation in Sir2 homolog (SIRT1)-deficient mice, Proc. Natl. Acad. Sci. U.S.A. 100 (2003) 10794–10799.
[52] A. Ito, Y. Kawaguchi, C.H. Lai, J.J. Kovacs, Y. Higashimoto, E. Appella, T.P. Yao, MDM2-HDAC1-mediated deacetylation of p53 is required for its degradation, EMBO J. 21 (2002) 6236–6245.
[53] M. Gostissa, T.G. Hofmann, H. Will, G. Del Sal, Regulation of p53 functions: let's meet at the nuclear bodies, Curr. Opin. Cell Biol. 15 (2003) 351–357.
[54] H. Vaziri, S.K. Dessain, E.E. Ng, S.I. Imai, R.A. Frye, T.K. Pandita, L. Guarente, R.A. Weinberg, hSIR2(SIRT1) functions as an NAD-dependent p53 deacetylase, Cell 107 (2001) 149–159.
[55] E. Langley, M. Pearson, M. Faretta, U.M. Bauer, R.A. Frye, S. Minucci, P.G. Pelicci, T. Kouzarides, Human SIR2 deacetylates p53 and antagonizes PML/p53-induced cellular senescence, EMBO J. 21 (2002) 2383–2396.
[56] J. Luo, A.Y. Nikolaev, S. Imai, D. Chen, F. Su, A. Shiloh, L. Guarente, W. Gu, Negative control of p53 by Sir2alpha promotes cell survival under stress, Cell 107 (2001) 137–148.
[57] C. Subramanian, A.W. Opipari Jr., X. Bian, V.P. Castle, R.P. Kwok, Ku70 acetylation mediates neuroblastoma cell death induced by histone deacetylase inhibitors, Proc. Natl. Acad. Sci. U.S.A. 102 (2005) 4842–4847.
[58] H. You, T.W. Mak, Crosstalk between p53 and FOXO Transcription Factors, Cell Cycle 4 (2005) 37–38.
[59] A. Brunet, L.B. Sweeney, J.F. Sturgill, K.F. Chua, P.L. Greer, Y. Lin, H. Tran, S.E. Ross, R. Mostoslavsky, H.Y. Cohen, L.S. Hu, H.L. Cheng, M.P. Jedrychowski, S.P. Gygi, D.A. Sinclair, F.W. Alt, M.E. Greenberg, Stress-dependent regulation of FOXO transcription factors by the SIRT1 deacetylase, Science 303 (2004) 2011–2015.
[60] M.C. Motta, N. Divecha, M. Lemieux, C. Kamel, D. Chen, W. Gu, Y. Bultsma, M. McBurney, L. Guarente, Mammalian SIRT1 represses forkhead transcription factors, Cell 116 (2004) 551–563.
[61] A. van der Horst, L.G. Tertoolen, L.M. Vries-Smits, R.A. Frye, R.H. Medema, B.M. Burgering, FOXO4 is acetylated upon peroxide stress and deacetylated by the longevity protein hSir2(SIRT1), J. Biol. Chem. 279 (2004) 28873–28879.
[62] I.R. Radford, Transcription-based model for the induction of interchromosomal exchange events by ionizing irradiation in mammalian cell lines that undergo necrosis, Int. J. Radiat. Biol. 78 (2002) 1081–1093.