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.

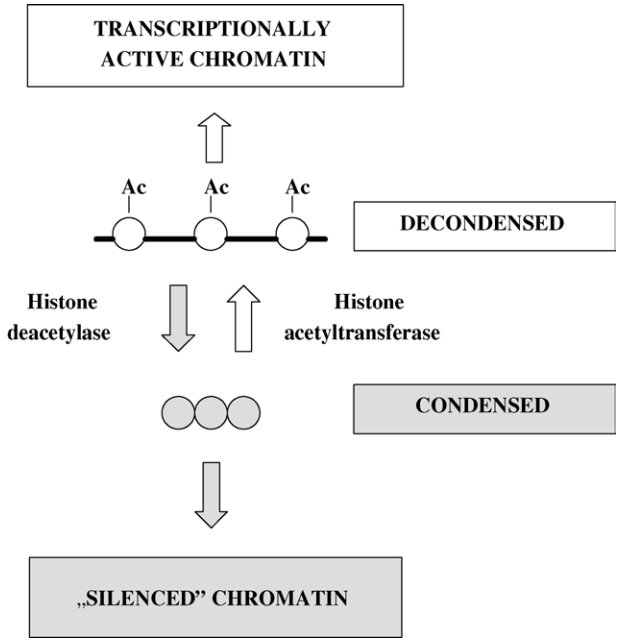

Fig. 1. The histone acetylation switch. HAT and HDAC determine the condensation status and in consequence, the potential transcriptional activity of chromatin. Acetylation "opens" the structure and permits SWI/SNF chromatin remodeling factors to access promoter sequences and thus, allows initiating transcription. 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].