Free Radical Biology and Medicine (xxxx) xxxx–xxxx

Contents lists available at ScienceDirect

Free Radical Biology and Medicine

journal homepage: www.elsevier.com/locate/freeradbiomed

Chromatin associated mechanisms in base excision repair - nucleosome remodeling and DNA transcription, two key players

Hervé Menoni[a,d,*], Paolo Di Mascio[b], Jean Cadet[c], Stefan Dimitrov[d], Dimitar Angelov[a,**]

[a] Laboratoire de Biologie et Modélisation de la Cellule (LBMC) CNRS/ENSL/UCBL UMR 5239 and Institut NeuroMyoGène – INMG CNRS/UCBL UMR 5310, Université de Lyon, Ecole Normale Supérieure de Lyon, 69007 Lyon, France
[b] Departamento de Bioquímica, Instituto de Química, Universidade de Sao Paulo, CP 26077, CEP 05508-000 Sao Paulo, SP, Brazil
[c] Département de Médecine Nucléaire et de Radiobiologie, Université de Sherbrooke, Sherbrooke, Québec, Canada J1H 5N4
[d] Université de Grenoble Alpes/INSERM U1209/CNRS UMR 5309, 38042 Grenoble Cedex 9, France

ARTICLE INFO

Keywords:
DNA oxidative damage
Base excision repair
OGG1
8-oxoG
Transcription coupled repair
Nucleosome
Chromatin
Chromatin remodeling

ABSTRACT

Genomic DNA is prone to a large number of insults by a myriad of endogenous and exogenous agents. The base excision repair (BER) is the major mechanism used by cells for the removal of various DNA lesions spontaneously or environmentally induced and the maintenance of genome integrity. The presence of persistent DNA damage is not compatible with life, since abrogation of BER leads to early embryonic lethality in mice. There are several lines of evidences showing existence of a link between deficient BER, cancer proneness and ageing, thus illustrating the importance of this DNA repair pathway in human health. Although the enzymology of BER mechanisms has been largely elucidated using chemically defined DNA damage substrates and purified proteins, the complex interplay of BER with another vital process like transcription or when DNA is in its natural state (*i.e.* wrapped in nucleosome and assembled in chromatin fiber is largely unexplored. Cells use chromatin remodeling factors to overcome the general repression associated with the nucleosomal organization. It is broadly accepted that energy-dependent nucleosome remodeling factors disrupt histones-DNA interactions at the expense of ATP hydrolysis to favor transcription as well as DNA repair. Importantly, unlike transcription, BER is not part of a regulated developmental process but represents a maintenance system that should be efficient anytime and anywhere in the genome. In this review we will discuss how BER can deal with chromatin organization to maintain genetic information. Emphasis will be placed on the following challenging question: how BER is initiated within chromatin?

1. Introduction

Constant formation of DNA lesions resulting from aerobic metabolism, environmental conditions or spontaneous hydrolysis of weak chemical bounds are major challenges to the maintenance of genome stability in mammalian cells [1]. Several reactive oxygen species (ROS) including the highly reactive hydroxyl radical (•OH) that are formed as byproducts of cellular metabolic processes can modify the bases and 2-deoxyribose moieties of the DNA [2–5]. Oxidation of proteins or lipids, which can be submitted to the same kinds of radical attack, can be discarded and replaced by non-damaged ones. In contrast, DNA damage jeopardizes the integrity of the genetic information, an essential element for the stability of the genome and cell viability [6].

Suitable repair pathways for numerous kinds of DNA lesions have been described. Within the vast majority of DNA damage events, the information coded by the undamaged complementary strand can be utilized to maintain genome integrity. Cells have selected sophisticated mechanisms to detect and excise the damaged base, or a patch of nucleotides surrounding the lesion. The base excision repair (BER) and the nucleotide excision repair (NER) are the major excision repair pathways described in mammals. Strikingly, a complete elimination of the BER system is not compatible with life and leads to an early embryonic lethality [6,7]. For historical reasons NER deficiencies have been linked to a broad spectrum of clinical outcomes from mild sun sensitivity to premature death and various neurodegenerative diseases, segmental premature ageing syndromes or cancer proneness. A link of BER and human diseases is not so extensively characterized but BER is a DNA repair pathway involved in cancer and ageing [8].

* Corresponding author at: Laboratoire de Biologie et Modélisation de la Cellule (LBMC) CNRS/ENSL/UCBL UMR 5239 and Institut NeuroMyoGène – INMG CNRS/UCBL UMR 5310, Université de Lyon, Ecole Normale Supérieure de Lyon, 69007 Lyon, France.
** Corresponding author.
E-mail addresses: herve.menoni@ens-lyon.fr (H. Menoni), dimitar.anguelov@ens-lyon.fr (D. Angelov).

http://dx.doi.org/10.1016/j.freeradbiomed.2016.12.026
Received 27 September 2016; Received in revised form 13 December 2016; Accepted 19 December 2016

This review focuses on BER mechanisms with some reference to other DNA repair pathways for which further information could be found [9–11]. BER is the main pathway for the repair of most small DNA base lesions caused by oxidation, deamination and alkylation. These modifications are in general not helix-distorting and they do not strongly interfere with transcription [12]. BER is initiated when a specific DNA glycosylase recognizes a modified base. In a subsequent step the glycosylase cleaves the *N*-glycosylic bond between the damaged base and the 2-deoxyribose moiety of the nucleotide. This creates an abasic site called either apurinic or apyrimidinic site (AP-site). There are at least 11 mammalian glycosylases that can detect in a more or less specific way different types of damaged bases, making BER a widely usable repair pathway that can deal with a wide variety of modified bases. DNA glycosylases are small monomeric enzymes, grouped according to motifs conserved from prokaryotes to humans [13]. Glycosylases can be classified as bi- or mono-functional depending on their capacity to cleave DNA or only the *N*- glycosidic bond of a damaged base. In metazoans, AP sites are processed by the AP endonuclease APE1, which generates a single strand break (SSB) in the DNA [12]. The resulting gap is filled by DNA polymerase β, which uses the undamaged strand as a template. The final step of BER is ligation of the nicked DNA strand, by DNA ligase III that forms a complex with XRCC1. XRCC1 plays a major role in SSB repair and the completion of BER by interacting with various partners either by increasing (*e.g.* PNK) or reducing (*e.g.* PARP-1) their activities (reviewed in [14]. A redundancy with other ligases present in the nucleus may be possible suggesting that XRCC1 complexed with ligase III is not required for BER to proceed [15]. A very wide plasticity exists in cells for the requirement of ligase in nucleus as well as in mitochondria [16]. In the same way, it has been speculated since many years that glycosylases may have redundant function with their overlapping activities on some DNA lesions [17]. This could explain the small increase of the steady state level of specific modified bases in various knockout mice for single DNA glycosylases.

The amount of DNA lesions and their location can vary a lot from cell to cell according to changes in physiological or environmental conditions (*e.g.* exposure to chemicals, agents generating ROS, sunlight and other processes including spontaneous chemical bond breakage, activation-induced deaminase during B-cell maturation *etc.*) [18,19]. The steady-state level of DNA damage that is observed results from their continuous formation and removal dynamic by the repair machinery present in the cell. The accumulation of DNA lesions, when failure in repair occurs, can have various outcomes depending on the intrinsic properties of the lesions: i) mutagenesis in the case of 8-oxo-7,8-dihydroguanine (8-oxoG) [20] or uracil [21], ii) replication block for 5,6-dihydroxy-5,6-dihydrothymine also called “thymine glycols” (Tg) [22] and complete transcription block for various helix-distorting DNA lesions such as ultraviolet light (UV)–induced cyclobutane pyrimidine dimers (CPDs) [23,24]. Therefore, DNA damage can trigger a complex response with various consequences on cellular physiology. This enlightens the importance for cells to accurately handle DNA repair together with transcription (and other cellular processes) in order to avoid more deleterious effects [25]. Even though, transcription through DNA lesions (*e.g.* 8-oxoG) can induce transcriptional mutagenesis [18,26], uncontrolled BER initiation can be more dangerous by triggering cell death and cellular sensitivity to DNA damaging agents [27,28]. Imbalances in the execution of normally regulated repair steps may be at origin of harmful effects observed on cells upon over-expression of proteins involved in repair pathways (*e.g.* glycosylases) [29]. Whereas 8-oxoG is not a strong block for transcription [30], the OGG1 glycosylase induced nick that are generated upon the lesion processing can cause transcription blockage [31]. Strikingly, elongation by RNA Pol II in a purified reconstituted transcription system can be blocked to different extents, illustrating that depending on the type of DNA lesion a complex spectrum of transcription impediment can occur in the nucleus [32]. Certainly, *in vivo* many other parameters such as chromatin context, promoter strength, or simply “who is first” on the lesion create wide options for the type of DNA Damage Response (DDR) generated by a same DNA insulting agent.

The basic subunit of chromatin, the nucleosome, 147 bp of DNA, wrapped in a left-handed toroidal helix around a histone octamer is a barrier to most of the factors interacting with DNA [33,34]. The next level of packaging of DNA into chromatin or the so-called 30 nm fiber and its higher level compaction forms represent other levels of obstruction.

In this review we will focus on the repair of non-helix-distorting lesions which are primarily substrates for the BER and discuss how chromatin associated mechanisms such as transcription and chromatin remodeling could play a role in the repair of such modified bases. First, we will describe *in vitro* studies on nucleosomal substrates. In the second part we will discuss the mechanisms that could favor access of BER factors to chromatin with a special mention to histone variant. Thirdly, results from live cell experiments will shed light on the connection between BER, transcription and chromatin remodeling. Finally we will give a more speculative view on BER initiation *in vivo* and conclude with some of the future challenges to address in the field.

2. BER *in vitro* on chromatin substrate

Most of the enzymes involved in BER have been extensively characterized (for structural targeted reviews, see [35,36]). Studies using short naked DNA substrates, in which a single modified base was site specifically inserted, gave insight in the repair substrates specificity of DNA glycosylases [37,38]. Interactions between various BER proteins have been described, especially with XRCC1, a scaffold protein with no catalytic activity, which is believed to favor optimal reaction [39]. However, much less is known on BER enzymes or scaffold proteins function in the context of chromatin. Here we will give a brief overview of the main findings and conclusions that can be drawn from relevant studies on three major BER lesions: uracil, Tg and 8-oxoG. For a more detailed information on various reconstituted systems used to study BER involving nucleosomal substrates we direct the reader to a recent review [40].

2.1. *Accessibility of various BER-lesions within* in-vitro *nucleosomal assemblies*

2.1.1. *Uracil*

Uracil in DNA results from either hydrolytic deamination of cytosine, creating a premutagenic U:G mispair, or misincorporation of dUMP instead of dTMP during replication, creating a U:A pair (reviewed in [13]). The mutagenic uracil base is recognized by a Uracil DNA Glycosylase (UDG) enzyme, which cleaves the *N*-glycosidic bond giving rise to a DNA abasic site. The removal of free uracil from *E. coli* DNA containing deaminated cytosine residues was the first glycosylase activity described by Lindahl [41]. The nucleosomal substrate reconstituted with the 5 S rRNA gene DNA (5 S), containing one single site-specific U:A base pairs, showed a rotational position independent 3- to 10-fold- decrease of SMUG1 and UNG2 efficiency compared to free DNA [42]. In disagreement with this initial study, a 10- to 30-fold rotational position dependent inhibition of UDG efficiency was reported [43,44]. These discrepancies might be due to the different DNA sequences used to position the nucleosome and also suggested that nucleosome was not such a strong barrier to BER initiation. However, another group using the same 5 S positioned nucleosomes reported a UDG activity inhibited 10^3–10^4-fold for inward facing sites, while for outward facing uracil reacted as efficiently as in free DNA [45]. Experimental difficulties in determining the initial rates of cleavage, requiring high-quality and naked-DNA free nucleosomes, could explain these variations inherent to assays on nucleosomal templates.

Restriction enzymes show a $\sim 10^4$-fold decrease in the cleavage