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Efficient repair of bleomycin-induced double-strand breaks in barley ribosomal genes

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Abstract

Ability of barley ribosomal genes to cope with damage produced in vivo by the radiomimetic agent bleomycin was investigated. Repair kinetics of bleomycin-induced double-strand breaks in ribosomal and total genomic DNA was compared. Induction and repair of double-strand breaks in defined regions of the ribosomal genes was also analyzed. Preferential sensitivity of barley linker DNA towards bleomycin treatment in vivo was established. Relatively higher yield of initially induced double-strand breaks in genomic DNA in comparison to ribosomal DNA was also found. Fragments containing intergenic spacers of barley rRNA genes displayed higher sensitivity to bleomycin than the coding sequences. No heterogeneity in the repair of DSB between transcribed and non-transcribed regions of ribosomal genes was detected. Data indicate that DSB repair in barley rDNA, although more efficient than in genomic DNA, does not corelate with the activity of nucleolus organizer regions.

Keywords: Ribosomal genes; DSB repair; Barley; Bleomycin

1. Introduction

Eukaryotic cells are continuously exposed to a number of substances with mutagenic nature capable to induce different types of DNA damage. Amongst the variety of DNA lesions double-strand breaks (DSB) are considered to be the most deleterious ones as they severely disrupt genome integrity. It is established that DSB arise in cellular DNA after exposure to exogenous agents such as ionizing radiation, bleomycin or restriction endonucleases. They can also occur naturally in any stage of the cell cycle during replication, transcription, recombination or repair. It is widely acknowledged that generation of DSB is the initial event leading to the formation of chromosomal rearrangements, unequivocally associated with increased genomic instability [1]. Proper maintenance of the genetic information, being essential for the cell survival and normal development of the organisms, has to rely on the effective rejoining of DNA DSB [2].

Non-random distribution of primary lesions and repair after treatment with various mutagenic agents in distinct genomic and chromatin locations has been a matter of substantial interest. Various aspects of the problem have been approached, including the role of mutagen type, chromatin compactness, higher-order chromatin structure, nucleosome positioning, gene and sequence specificity, transcriptional activity as well as cell and cell cycle dependence [3–9]. Crucial

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breakthrough in the topic of differential repair was the finding that actively transcribed genes are more quickly repaired by nucleotide excision repair than non-expressed ones [10]. It was further demonstrated that such a preferential recovery of active genes has to be confined to the accelerated repair of lesions in the transcribed DNA strand termed "transcription-coupled repair" (TCR) [11]. Moreover, intragenic repair heterogeneity, reflecting chromatin alterations along the genes was also established [12,13]. After the initial observation for strand-specific repair of cyclobutane pyrimidine dimers (CPD), other types of DNA lesions that interfere with transcription elongation like chemically induced DNA adducts and oxidative damage were approached and the link between DNA repair and transcription has been extensively followed [14–16]. It was recently demonstrated that intragenomic localization of TCR resembles uneven distribution of transcriptome, CpG islands and histone acetylation, thus pointing towards the close association between DNA repair, transcription and chromatin structure in human cells [17,18].

Phenomenon of transcription-mediated repair heterogeneity has been attributed exclusively to the genes transcribed by RNA polymerase II. Early studies on the repair of rRNA genes whose transcription is driven by RNA polymerase I did not reveal such a tendency [19]. When the repair of UV-induced pyrimidine dimers was assessed in mammalian ribosomal genes it was suggested that ribosomal DNA (rDNA) recovery is accomplished by transcription-independent repair system. No evidence for transcription repair coupling of this particular damage in the individual strands of active or total rDNA was detected [20–22], with repair efficiency being somewhat lower than in other constitutively expressed genes. Recently, however a transcription-dependent repair of UV-induced CPD in yeast rRNA genes executed by NER and photoreactivation has been demonstrated [23,24].

It is noteworthy that the data on the repair heterogeneity of DNA strand breaks are not abundant. Combined application of restriction endonucleases and gene probing by Southern hybridization for detection of DNA DSB induced by ionising radiation in mammalian cells [25] proved to be sensitive enough and reliable approach to analyze their non-random distribution and rejoining in various genomic and chromatin domains [26–28]. Studies on intragenomic heterogeneity of ionizing radiation-induced DNA DSB assayed in regions of different gene density and activity in comparison to the genome overall did not reveal any specificity for the active HPRT gene [29], while faster repair rate has been observed in the *c-myc* locus [30]. When the gene-specific repair of alkaline-sensitive sites and DNA strand breaks after γ-irradiation of human cancer cells was measured, no difference in the repair between the two individual DNA strands of the DHFR gene and no preferential repair in comparison to the inactive, X-linked gene was found [31]. It appears that repair of DNA strand breaks in mammalian genome is relatively fast, uniform and such a trend is also typical for the ribosomal genes [32].

In a contrast to the impeded repair of CPD an efficient recovery of single-strand breaks (SSB) induced by bleomycin in human rDNA has been detected [33]. Relatively high repair potential for the other major UV-induced lesion, namely 6-4 photoproducts, was also reported for rDNA [34]. These data favour the notion that repair efficiency in rDNA might have rather lesion-specific character.

Although a subject of intensive research in yeast and mammalian cells, the problem of gene-specific repair in plants and in rRNA genes in particular is still open and underestimated. rRNA genes are suitable model for repair studies in individual plant genes due to their highly repeated character. Availability of barley karyotypes with appropriate reconstruction concerning the expression of ribosomal gene clusters allowed us to analyse the influence of transcriptional activity on their repair efficiency. Our previous observations, that nucleolus organizer regions (NORs), where ribosomal genes are located, behave as prominent aberration hotspots after treatment with restriction endonucleases [35,36] urged us to analyze further the induction and repair of double-strand breaks in barley rRNA genes. Recently, we have demonstrated effective recovery of DSB in barley ribosomal DNA after treatment with ionizing radiation [37]. Here, we report data on the induction and repair of DSB produced by bleomycin in ribosomal genes with normal and increased transcriptional activity as well as in barley genomic DNA as a whole. As the ribosomal repeat comprises sequences with different chromatin organization and transcriptional status, we were also interested to compare the induction and repair of bleomycin-induced DSB within the defined regions of ribosomal DNA. Elevated sensitivity of the fragments containing non-transcribed intergenic spacers in comparison to the coding regions was found in line T-1586. On the other hand, the repair rate of DSB within the transcribed and non-transcribed regions was of similar magnitude in both karyotypes despite their different NOR expression. It appears that repair efficiency of bleomycin-induced DSB in barley ribosomal genes does not correlate with their transcriptional activity.