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Differential Repair and Replication of Damaged DNA in Ribosomal RNA Genes in Different CHO Cell Lines

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We studied the repair of psoralen adducts in the pol I–transcribed ribosomal RNA (rRNA) genes of excision repair competent Chinese hamster ovary (CHO) cell lines, their UV sensitive mutant derivatives, and their UV resistant transformants, which express a human excision repair gene. In the parental cell line CHO-AA8, both monoadducts and interstrand crosslinks are removed efficiently from the rRNA genes, whereas neither adduct is removed in the UV sensitive derivative UV5; removal of both adducts is restored in the UV resistant transformant CHO-5T4 carrying the human excision repair gene ERCC-2. In contrast, removal of psoralen adducts from the rRNA genes is not detected in another parental CHO cell line CHO-9, neither in its UV sensitive derivative 43-3B, nor in its UV resistant transformant 83-G5 carrying the human excision repair gene ERCC-1. In contrast to such intergenomic heterogeneity of repair, persistence of psoralen monoadducts during replication of the rRNA genes occurs equally well in all CHO cell lines tested. From these data, we conclude that: 1) the repair efficiency of DNA damage in the rRNA genes varies between established parental CHO cell lines; 2) the repair pathways of intrastrand adducts and interstrand crosslinks in mammalian cells share, at least, one gene product, i.e., the excision repair gene ERCC-2; 3) replicational bypass of psoralen monoadducts at the CHO rRNA locus occurs similarly on both DNA strands.

Key words: hamster, transcription, psoralen

Carcinogens that damage DNA are known to initiate genetic alterations in specific regions of mammalian genomes. Such subgenomic alterations can lead to gene mutation, protooncogene activation, and probably also antioncogene inactivation [1]. It is thus essential to understand the mechanism(s) by which mammalian cells repair or, alternatively, tolerate potentially pathogenic damage in specific genomic regions.

A particularly intriguing problem is to establish the relationship between DNA repair, DNA replication, and transcription. Using psoralen photoaddition as prototype chemical damage to DNA, we have previously demonstrated effective repair and

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replication of psoralen adducts in an RNA polymerase II–transcribed gene of normal human cells [2]. Using our recently developed RAGE (Renaturing Agarose Gel Electrophoresis) procedure [3], we have now compared the repair and replication of psoralen adducts in the ribosomal RNA genes (rRNA) of DNA damage resistant and DNA damage sensitive Chinese hamster ovary (CHO) cells. Mammalian rRNA genes, which are specifically and uniquely transcribed by RNA polymerase I, form a distinct category of housekeeping genes. rRNA genes are naturally reiterated, with several hundred copies per haploid genome distributed as clustered tandem repeats on several autosomal chromosomes and are selectively transcribed in a subchromosomal structure, the nucleolus. We report that the repair efficiency of psoralen adducts in the rRNA genes of CHO cells varies between different cell lines; in contrast, the replicational bypass of psoralen monoadducts in the hamster rRNA genes happens to a similar extent in all these cell lines.

MATERIALS AND METHODS

Cell Cultures

The repair competent parental line CHO-AA8, its UV sensitive line CHO-UV5, and its UV resistant transformant CHO-5T4-12 containing the ERCC-2 gene (kindly provided by L. Thompson, Lawrence Berkeley National Laboratory, CA), the repair competent parental line CHO-9, its UV sensitive line CHO-43-3B, and its UV resistant transformant CHO-83-G5 containing the ERCC-1 gene (kindly provided by J.H. Hoeijmaker, Erasmus Universiteit Rooterdam, The Netherlands) were grown as a monolayer in Earle's modified minimal essential medium (GIBCO, modified, autoclavable) supplemented with 10% fetal calf serum, antibiotics, and 2 mM glutamine, and for the transformants, in medium modified as published [4,5]. Exponentially growing cells were used in all experiments to ensure active transcription of the rRNA genes.

RAGE Assay

The conditions of cell treatment with the psoralen derivative 4′-hydroxymethyl-4,5′,8-trimethyl-psoralen (HMT) and UVA light, purification of DNA, repair, and replication analysis were carried out as previously described [3].

RESULTS

Figure 1 illustrates the RAGE method used to measure psoralen adducts in the rRNA genes of the CHO cell lines. Briefly, the assay combines the capacity of rapid renaturation of DNA containing interstrand crosslinks with the separation by electrophoresis in nondenaturing agarose gels of double- and single-stranded DNA. The distribution between single- and double-stranded DNA of specific restriction fragments in either parental or newly synthesized DNA is then determined by Southern transfer of the DNA on a nylon membrane and hybridization with nick-translated DNA probes. DNA interstrand crosslinks are measured directly, whereas monoadducts are measured indirectly through their photoconversion in vitro into interstrand crosslinks.

Repair of HMT Adducts in the Hamster rRNA Locus

We have examined the removal of HMT adducts from the 22 kb HindIII fragment that encompasses the CHO rRNA locus by using a 1.8 kb human 28 S

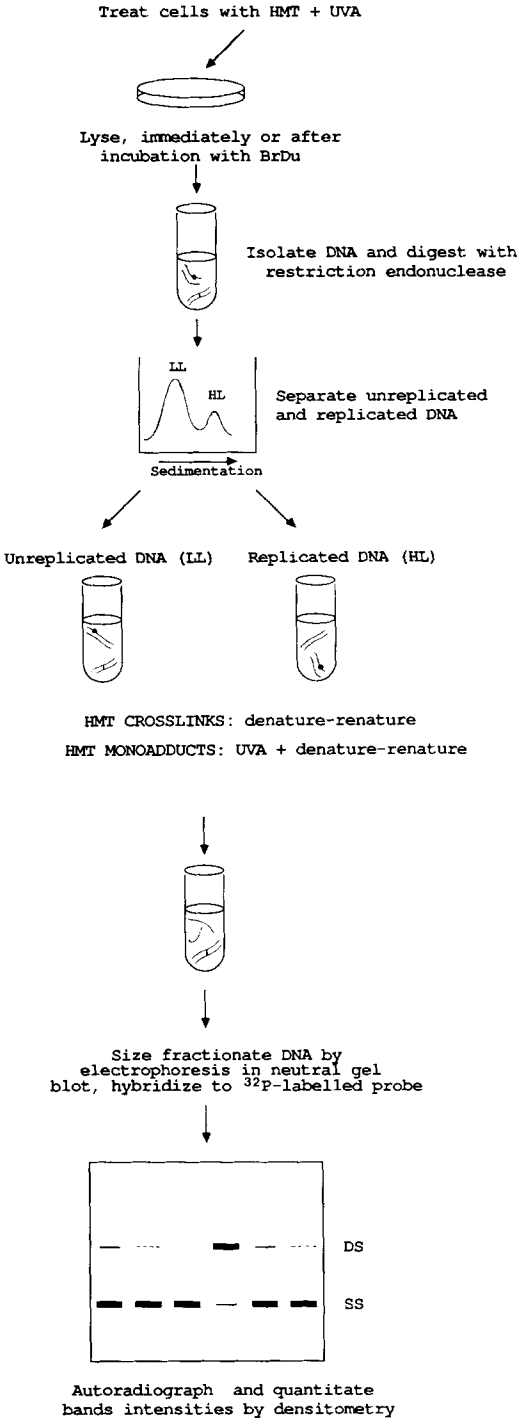

Fig. 1. Schematic illustration of the RAGE method.

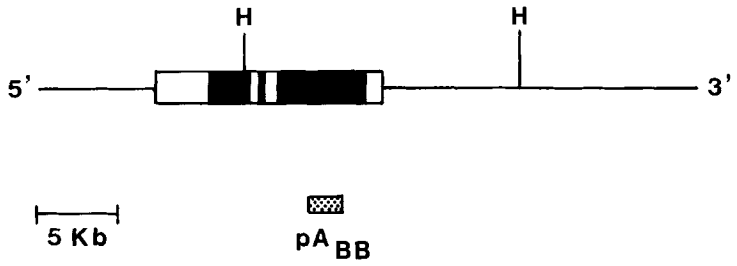

Fig. 2. Genomic maps of the functional Chinese hamster ribosomal RNA genes. Boxes: region coding for the rRNA precursors with the 5S, 18S, and 28S shown as filled boxes. The locations of HindIII (H) restriction sites and of the 28S homologous human probe pA_{BB} are indicated.

fragment homologous to the hamster sequence (Fig. 2). The CHO cell lines studied include the parental, repair competent CHO-AA8 and CHO-9, their UV sensitive derivatives CHO-UV5 and CHO-43-3B, and their UV resistant transformants CHO-5T4-12 and CHO-83-G5, respectively. The cells were treated with 1 μg/ml of HMT and 5 min UVA irradiation (1.2 kJ/m^2/min.), a dose that produces an average of one HMT adduct per fragment. The results obtained indicate that the removal of both psoralen interstrand crosslinks and intrastrand monoadducts from the rRNA locus occurs efficiently in CHO-AA8 cells, but poorly, if at all, in CHO-9 cells (Fig. 3A,D). In contrast, there is no evidence of HMT adduct removal from the rRNA locus of the UV sensitive CHO-UV5 and CHO-43-3B cell lines (Fig. 3B,D). However, efficient removal is restored in the UV resistant transformant CHO-5T4-12 expressing the human excision repair gene ERCC-2, but not in the UV resistant transformant CHO-83-G5 expressing the human excision repair gene ERCC-1 (Fig. 3C,D). Thus, the differential rates of psoralen removal between the rRNA locus of the parental cell lines CHO-AA8 and CHO-9 are reproduced between their ERCC containing derived cell transformants.

Replication of HMT Adducts in the Hamster rRNA Locus

We have previously demonstrated that DNA containing HMT monoadducts in the proficiently repaired, pol II–transcribed, dihydrofolate reductase gene was replicated with high efficiency [2,3]; we suggested that such translesion replication is an alternative

Fig. 3. Comparison of repair and replication of HMT adducts in the ribosomal RNA genes of CHO cells. RAGE analysis of (**A**) parental CHO-AA8 (**upper**) and CHO-9 (**lower**), (**B**) UV sensitive derivatives CHO-UV5 (**upper**) and CHO-43-3B (**lower**), and (**C**) UV resistant transformants CHO-5T4-12 (**upper**) and CHO-83-G5 (**lower**). Growing cultures of CHO cells were either untreated (A, **lanes a, e,** and **i**) or treated with 1 μg/ml of HMT and 6 KJ/M^2 (**other lanes**) and genomic DNA was isolated immediately (0) or 24 h (24) after treatment. After HindIII restriction and separation by density gradient centrifugation of unreplicated (24_P) and replicated (24_R) DNA, samples were loaded on a non-denaturing agarose gel either native, alkali-denatured, or alkali-denatured after UVA irradiation (indicated at the bottom of the figures). After electrophoresis, Southern transfer, and hybridization with radiolabelled pA_{BB}, the filter was exposed to XR film. Positions of the double-stranded (DS) and single-stranded (SS) probed fragments are indicated on the side. **D:** The level of HMT adducts, either interstrand crosslinks (XL) or crosslinkable monoadducts (MA), detected in the unreplicated (open histograms) and replicated (shaded histograms) of the HindIII fragment of rRNA genes of the parental (CHO-AA8 and CHO-9) and UV transformants (CHO-5T4-12 and CHO-83-G5) was calculated by densitometric analysis of the above autoradiograms, respectively, A and C. This level is expressed in arbitrary units relative to the initial level of HMT adducts.

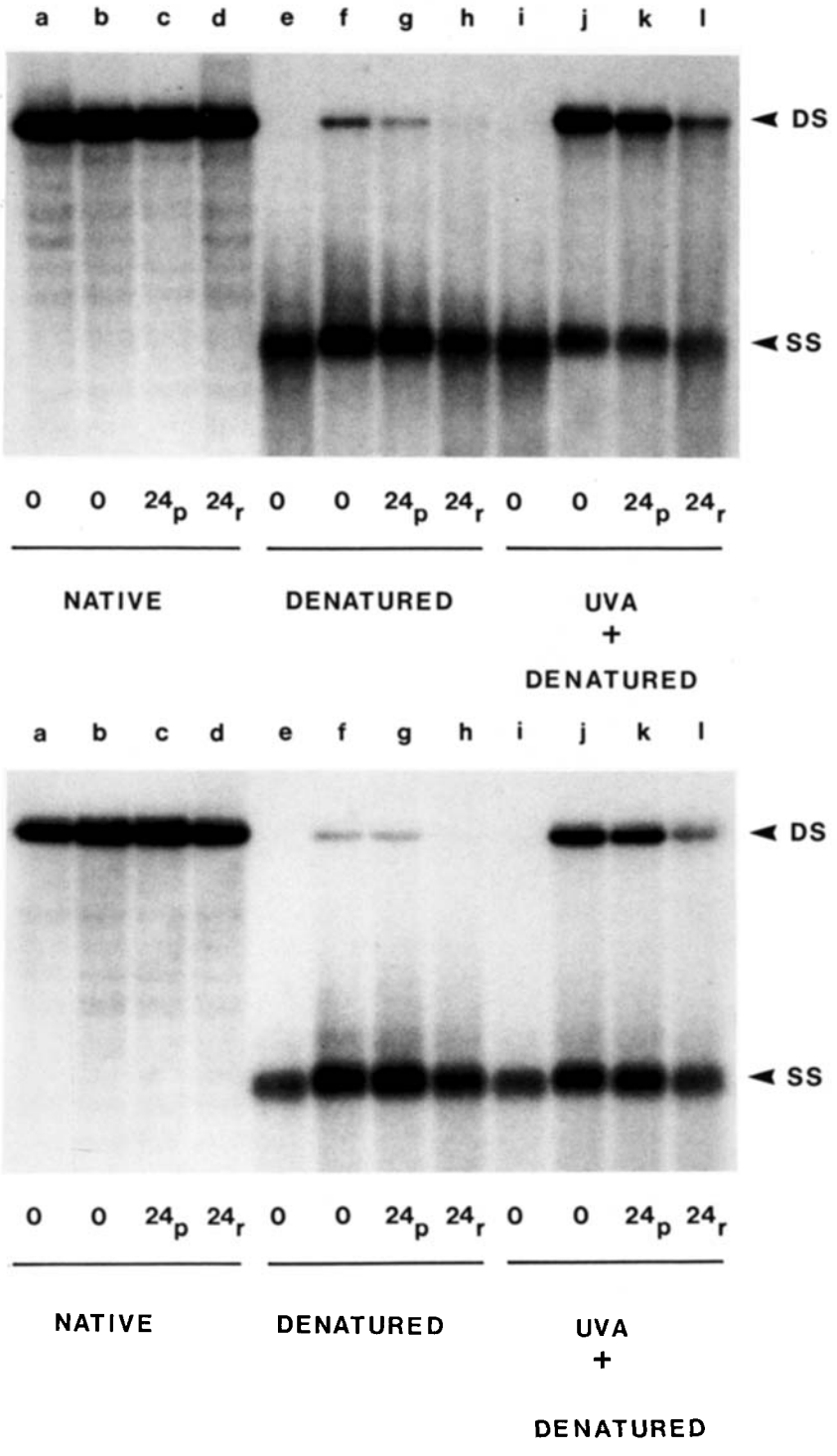
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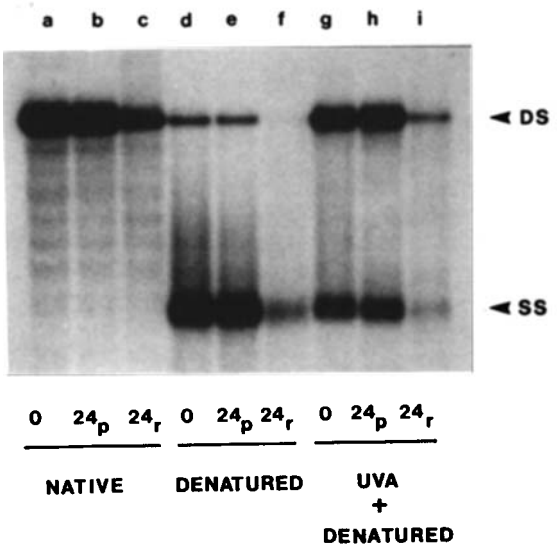
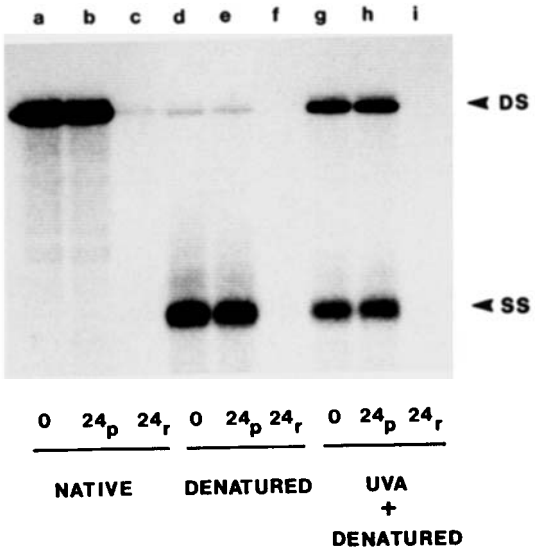
Figure 3





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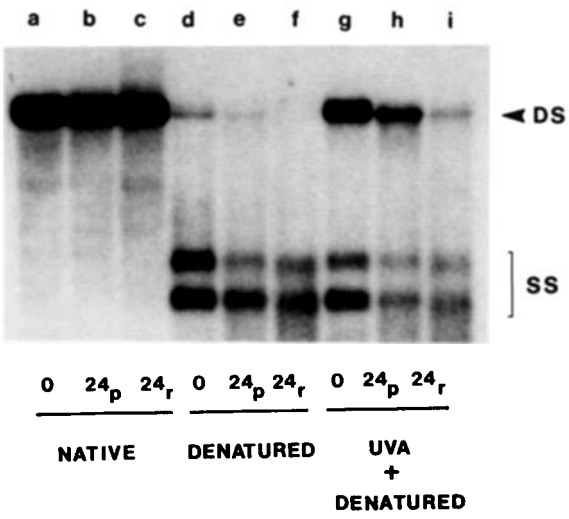

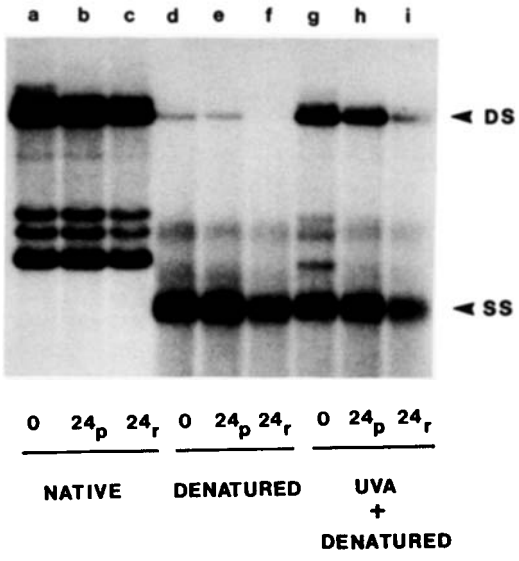

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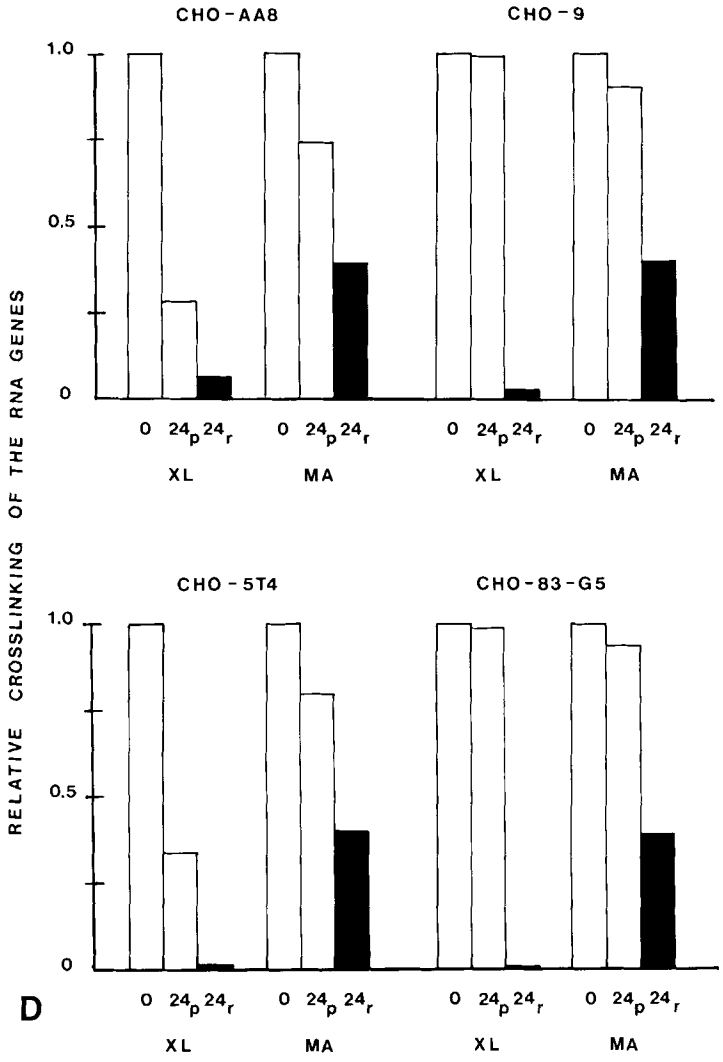

Figure 3 (continued)

mechanism to excision repair to circumvent transcription blockage in a damaged gene (Fig. 4). To determine whether such mechanism of recovery is also operational in the differentially repaired, pol I–transcribed, rRNA locus, we studied the persistence of psoralen adducts in the replicated DNA of the rRNA locus of the various CHO cell lines (Fig. 3A–D). HMT monoadducts are clearly visible in the rRNA locus of all cell lines, although the UV sensitive mutants had very limited amounts of replicated DNA (see Discussion). Moreover, the relative monoadduct frequency in replicated DNA is comparable to that in the unreplicated DNA isolated at the same time (Fig. 3D). Thus, the efficient translesion replication is independent of the dramatic difference in the rates of repair of HMT monoadducts between the various CHO strains. In contrast, interstrand crosslinks are detected in replicated DNA at an extremely low level, if at all.

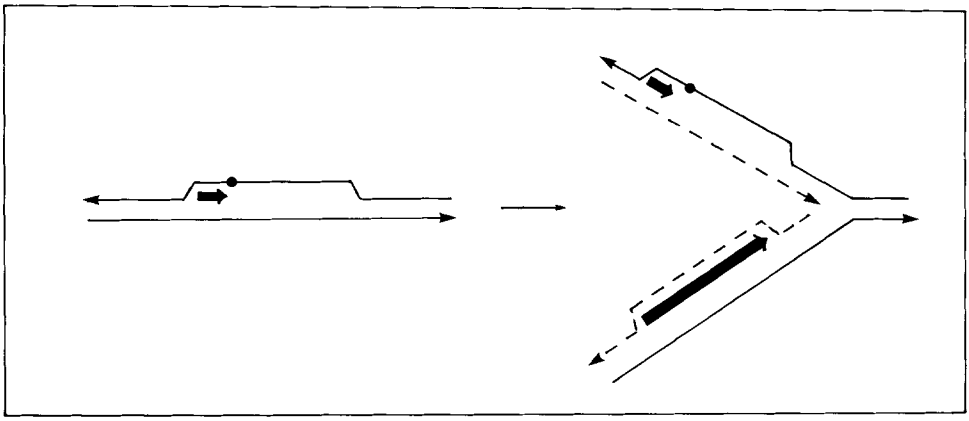

Fig. 4. Translesion replicational pathway for the restoration of transcription proficiency in a blocked gene. Before DNA replication, an actively transcribed gene carries a DNA adduct that blocks the progression of the RNA polymerase (**left side**); after DNA replication, one of the daughter chromatids is free of DNA damage and becomes transcription proficient (**right side**).

DISCUSSION

Based on our results, we conclude that repair of psoralen adducts in rRNA genes varies significantly between different parental CHO cell lines. Such differential repair could be due to either a difference in the efficiency of repair of all sequences between these cell lines (overall repair) or to a difference in the repair efficiency between specific genomic loci, including the rRNA genes (intragenomic repair). The repair capacity of different CHO lines has been shown to vary over a wide range [6]; however, no comparison has been made in the distribution of intragenomic repair in these different cell lines. Our observation that 1) the amount of cellular DNA replicated 24 h after psoralen treatment is comparable between the two parental lines and between the two UV resistant transformants (approximately 50% of untreated cells) and that 2) such a level of replication is drastically reduced in both UV sensitive mutants (<5% of untreated cells) strongly suggests a similar overall repair capacity in the parental cell lines. Moreover, CHO-AA8 and CHO-9 present a similar resistance to several DNA damaging treatments, including DNA crosslinking agents [7,8], as measured by clonogenicity. The reasons for such intrastrain heterogeneity of repair at the rRNA locus are presently not understood. CHO-AA8 and CHO-9 are cell variants isolated through a series of independent selection procedures. Heritable variation affecting repair at the rRNA loci might be caused by genetic alterations, such as genomic rearrangements [9,10], and/or by epigenetic changes, such as DNA methylation [11,12], appearing spontaneously during prolonged subcloning from the original strain. More specifically, alteration in repair efficiency may be a general phenomenon of aging cells, as observed with primary mouse cells [13]. As a consequence, these results suggest caution in the interpretation of repair experiments on defined mammalian genes using established somatic cell cultures.

The comparison of repair of psoralen monoadducts and interstrand crosslinks in the parental CHO-AA8 line, the UV sensitive mutant CHO-UV5, and UV resistant transformant CHO-5T4-12 indicates that the repair of both types of adducts is under the

control of a gene analogous to the human excision repair gene ERCC-2. To our knowledge, this is the first demonstration that a defined mammalian gene is involved in the repair of both intrastrand and interstrand DNA lesions in human cells and, likely, other mammalian cells. The molecular function(s) of this gene is still unknown [5]. However, since the RAGE assay detects specifically incisions at DNA crosslinking sites [2], the gene ERCC-2 must be involved in the early steps of repair of both types of DNA adducts. By analogy to the bacterial uvrABC and SOS systems, the activity of the ERCC-2 gene product could be functional and/or regulatory.

HMT monoadducts were detected in the replicated rRNA genes at frequencies similar to those in unreplicated rRNA genes. More important, high persistence of psoralen monoadducts was observed in the absence of repair of psoralen adduct from these genes. We conclude that replicational bypass of monoadducts in the rRNA locus must occur at high efficiency on both strands of the DNA, i.e., transcribed and nontranscribed strands. As previously proposed [2,3], such replicational bypass of actively transcribed genes may be particularly important to restore transcription-competency for slowly repaired genes (Fig. 4). It should be stressed, however, that the mechanism and fidelity of such replicational bypass might be different on the opposite strands, depending for example on which strand is the leading and which the lagging strand.

In contrast to monoadducts, interstrand crosslinks were detected at extremely low levels, if at all, in the replicated rRNA genes. The poor repair of such DNA lesion in defined chromosomal regions such as the actively transcribed rRNA genes might create structural alterations prone to genomic rearrangements, particularly during DNA replication. DNA damage has been shown to enhance chromosomal recombination in mammalian cells [reviewed in 14]. Moreover, rRNA genes have been involved in various forms of chromosomal rearrangements [15]. Thus, differential repair of actively transcribed genes may induce differential susceptibility to chromosomal rearrangements.

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