DNA Repair 13 (2014) 55–60

Contents lists available at ScienceDirect

DNA Repair

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

Letter to the Editor

Ribonucleotides as nucleotide excision repair substrates

ARTICLE INFO

Keywords:
prokaryotic nucleotide excision repair
ribonucleotides
electrostatic and hydrogen bonding properties
lesion recognition and verification

ABSTRACT

The incorporation of ribonucleotides in DNA has attracted considerable notice in recent years, since the pool of ribonucleotides can exceed that of the deoxyribonucleotides by at least 10–20-fold, and single ribonucleotide incorporation by DNA polymerases appears to be a common event. Moreover ribonucleotides are potentially mutagenic and lead to genome instability. As a consequence, errantly incorporated ribonucleotides are rapidly repaired in a process dependent upon RNase H enzymes. On the other hand, global genomic nucleotide excision repair (NER) in prokaryotes and eukaryotes removes damage caused by covalent modifications that typically distort and destabilize DNA through the production of lesions derived from bulky chemical carcinogens, such as polycyclic aromatic hydrocarbon metabolites, or *via* crosslinking. However, a recent study challenges this lesion-recognition paradigm. The work of Vaisman et al. (2013) [34] reveals that even a single ribonucleotide embedded in a deoxyribonucleotide duplex is recognized by the bacterial NER machinery *in vitro*. In their report, the authors show that spontaneous mutagenesis promoted by a steric-gate pol V mutant increases in *uvrA*, *uvrB*, or *uvrC* strains lacking *rnhB* (encoding RNase HII) and to a greater extent in an NER-deficient strain lacking both RNase HI and RNase HII. Using purified UvrA, UvrB, and UvrC proteins in *in vitro* assays they show that despite causing little distortion, a single ribonucleotide embedded in a DNA duplex is recognized and doubly-incised by the NER complex. We present the hypothesis to explain the recognition and/or verification of this small lesion, that the critical 2′-OH of the ribonucleotide – with its unique electrostatic and hydrogen bonding properties – may act as a signal through interactions with amino acid residues of the prokaryotic NER complex that are not possible with DNA. Such a mechanism might also be relevant if it were demonstrated that the eukaryotic NER machinery likewise incises an embedded ribonucleotide in DNA.

Helix distorting and destabilizing DNA lesions are subject to repair by the nucleotide excision repair machinery in both prokaryotes and eukaryotes [1,2]. However, what constitutes a helix-distorting lesion may not be obvious. For example, *cis-syn* cyclobutane pyrimidine dimers (CPD<TT>), the most prevalent lesions produced by ultraviolet (UV) light are inefficiently repaired by the NER machinery *in vitro*, while the 6–4<TT> UV photoproduct, which occurs at about 25% lower frequency [3], is an excellent substrate for NER [4,5]. How these photoproducts differ in their impact on duplex DNA structure might not be obvious from the structures of the lesions themselves: the CPD<TT> dimer is shaped like an open book with the two thymines at an angle of about 50° (PDB [6] ID: 4A0A [7]), while in the 6–4<TT> lesion the pyrimidine/pyrimidone bases are near-perpendicular in a propeller-like orientation (PDB ID: 3EI1 [8]). However, NMR solution structures show that a 6–4<TT> modified duplex decamer is more distorted: Watson–Crick hydrogen bonding is disrupted at the 3′-side of the 6–4<TT> lesion but is present in the case of the CPD<TT> duplex which is much less bent [9].

Although the structural and mechanistic aspects of the prokaryotic and eukaryotic NER machineries are somewhat different, they share similar critical operational and functional features, particularly the recognition of the lesions and subsequent verification steps [1,2]. In both cases, there are two NER pathways, transcription coupled repair (TC-NER) [10–12] and global genomic repair (GG-NER). In TC-NER, the lesion is first recognized by transcription stalling, but subsequent steps are similar to GG-NER [2,13]. In GG-NER the damaged DNA is first recognized, in prokaryotes by UvrA [1] and in eukaryotes by XPC-RAD23B, with UV-DDB required upstream for UV-induced photoproducts [2,8,13]. Subsequently the damage is verified by UvrB in prokaryotes [1], and by TFIIH in cooperation with XPA and the helicase activity of XPD that is part of the multi-protein complex TFIIH in eukaryotes [2]. What follows in both cases is the excision of around ~13 and ~24–32 nucleotide-long oligonucleotide sequences containing the damage in the prokaryotic and eukaryotic systems, respectively, with subsequent gap-filling by one or more polymerases [1,2].

The factors that determine whether a given lesion is recognized for subsequent excision have elicited much interest, particularly since different lesions are excised with remarkably different efficiencies [5,13,14]. Dual incision experiments with DNA substrates

Abbreviations: rNTP, ribonucleotide; CPD<TT>, *cis-syn* cyclobutane pyrimidine dimer; 6-4<TT>, 6,4 pyrimidine-pyrimidone; dNTP, deoxyribonucleotide; RER, ribonucleotide excision repair; MD, molecular dynamics; NER, nucleotide excision repair; GG-NER, global genomic repair; TC-NER, transcription coupled repair.

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http://dx.doi.org/10.1016/j.dnarep.2013.10.010

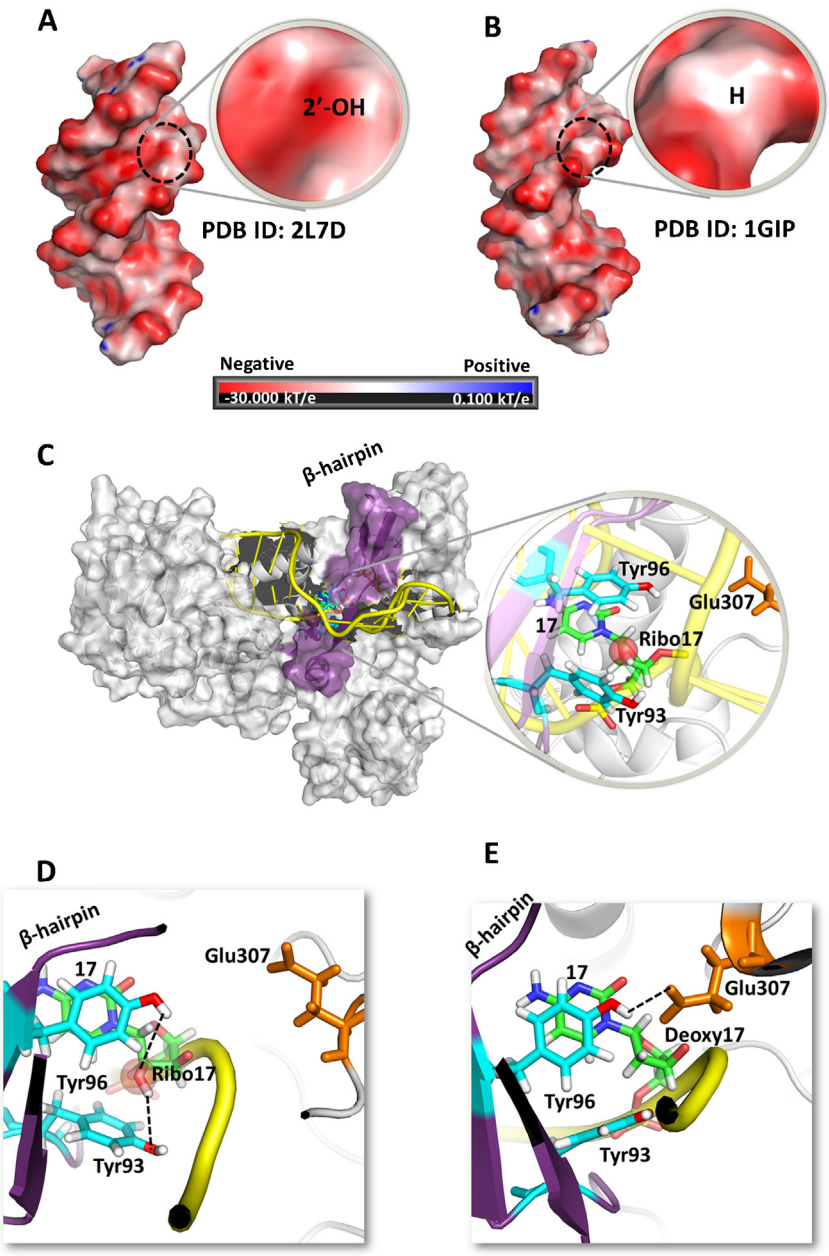

Fig. 1. (A and B) Electrostatic surfaces computed on the PDB2PQR Server (http://nbcr-222.ucsd.edu/pdb2pqr_1.8/). Panel (A) represents the electrostatic surface of the NMR solution structure of the Dickerson dodecamer containing an embedded ribonucleotide [39] and Panel (B) represents an NMR solution structure of the same normal dodecamer structure [56]. The negative (red) electrostatic patch due to the 2′-OH group depends little on the sugar pucker which is C3′-endo in (A). (C–E): Molecular dynamics simulations suggest how a ribonucleotide on the inner strand at the gate of the β-hairpin in UvrB could alter the interactions of nearby amino acid residues. Panel (C) is a view of the full UvrB structure together with the area of interest at the β-hairpin gate. These are also featured in the movie given in Supplementary Data. The ribonucleotide (D) as compared to the deoxyribonucleotide (E), provides opportunities for hydrogen bonding/electrostatic interactions with Tyr93 and Tyr96 that the 2′-deoxyribonucleotide lacks. These interactions disrupt the normal hydrogen bond between Tyr96 and Glu307. The black dashed lines indicate the relevant hydrogen bonding/electrostatic interactions. Electrostatic interactions are outside of hydrogen bonding distance but within the range of the Coulomb's law attractive potential. All graphics were prepared with PyMOL Molecular Graphics System Version 1.3. (Schrodinger, LLC).

containing variety of site-specifically inserted bulky lesions conducted with both prokaryotic and eukaryotic systems suggest that NER is promoted by local lesion-induced thermodynamic destabilization and enhanced dynamics [15–22]. These studies were focused mostly on DNA lesions resulting from polycyclic aromatic derivatives, other bulky lesions, or intrastrand crosslinks that distort the local DNA structure [23–27].

The incorporation of ribonucleotides in DNA has attracted considerable notice in recent years since the pool of ribonucleotides significantly exceeds that of the deoxyribonucleotides in prokaryotes [28] and in eukaryotes [29]. Hence single rNTP incorporation by DNA polymerases appears to be a common event, with human pol δ incorporating one rNTP per ~2000 dNTPs [29]. rNTPs are mutagenic [30] and lead to genome instability [31], and hence DNA polymerases have defenses against incorporating ribonucleotides, known as steric gates [32] or fences [33].

The intriguing observation suggesting NER susceptibility for ribonucleotides came to light in the studies of Vaisman et al. [34], who used a steric gate mutant of DNA polymerase V to elucidate the pathways involved in ribonucleotide repair. In an earlier study, the *umuC*_Y11A steric gate mutant was shown to exhibit low-fidelity DNA synthesis similar to wild-type pol V