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Rotational and translational positions determine the structural and dynamic impact of a single ribonucleotide incorporated in the nucleosome

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Abstract

Ribonucleotides misincorporated by replicative DNA polymerases are by far the most common DNA lesion. The presence of ribonucleotides in DNA is associated with genome instability, causing replication stress, chromosome fragility, gross chromosomal rearrangements, and other mutagenic events. Furthermore, nucleosome and chromatin assembly as well as nucleosome positioning are affected by the presence of ribonucleotides. Notably, nucleosome formation is significantly reduced by a single ribonucleotide. Single ribonucleotides are primarily removed from DNA by the ribonucleotide excision repair (RER) pathway via the RNase H2 enzyme, which incises the DNA backbone on the 5'-side of the ribonucleotide. While the structural implications of a single ribonucleotide in free duplex DNA have been well studied, how a single ribonucleotide embedded in nucleosomal DNA impacts nucleosome structure and dynamics, and the possible consequent impact on RER, have not been explored. We have carried out 3.5 microsecond molecular dynamics simulations of a single ribonucleotide incorporated at various translational and rotational positions in a nucleosome core particle. We find that the presence of the 2'-OH group on the ribose impacts the local conformation and dynamics of both the ribonucleotide and nearby DNA nucleotides as well as their interactions with histones; the nature of these disturbances depends on the rotational and translational setting, including whether the ribose faces toward or away from the histones. The ribonucleotide's preferred C3'-endo pucker is stabilized by interactions with the histones, and furthermore the ribonucleotide can cause dynamic local duplex disturbance involving an abnormal C3'-endo population of the adjacent deoxyribose pucker, minor groove opening, ruptured Watson-Crick pairing, and duplex unwinding that are governed by translation-dependent histone-nucleotide interactions. Possible effects of these disturbances on RER are considered.

Graphical Abstract

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Conflicts of interest

The authors do not have conflicts of interests.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version.

Keywords

Ribonucleotide; Nucleosome core particle; Molecular dynamics simulation; Ribonucleotide excision repair

1. Introduction

Ribonucleotides are the most prevalent genotoxic lesions in eukaryotic genomes [1–5]. For example, during DNA replication ~ 1 ribonucleotide is incorporated per 1250 dNTPs by the replicative DNA polymerase Pol ϵ in budding yeast [1, 5], a typical order of magnitude for polymerases in humans [3]. In the mouse genome, during each cell division more than 1 million ribonucleotides are incorporated [2]. This is true despite the fact that ribonucleotide incorporation is inhibited by a steric gate residue [6–10] that is present in most DNA polymerases. Ribonucleotide incorporation results from the fact that the rNTP concentration in the cell greatly exceeds that of the dNTPs [10, 11]. Ribonucleotides that are not repaired are very deleterious, causing mutations, double strand breaks, chromatin rearrangements, and other forms of genome instability [5, 12, 13], although they may also function positively to discriminate strands in mismatch repair [5]. Recently, genomic ribonucleotides have been implicated as a source of DNA lesions relevant for cancer progression [14].

Repair of ribonucleotides embedded in DNA is primarily performed by the ribonucleotide excision repair (RER) pathway; this process is initiated by RNase H2-mediated recognition of the ribonucleotide. Subsequently, RNase H2 incises the DNA backbone immediately 5' to the ribonucleotide, followed by strand displacement synthesis and ligation [15–17]. A recent study has shown that the complete loss of RNase H2 in the epidermis results in spontaneous DNA damage and squamous cell carcinoma of the skin [18]. In humans, partial loss-of-function mutations in the RNase H2 gene cause a severe neuroinflammatory disorder, Aicardi-Goutières syndrome [19, 20].

The basic unit of DNA packaging in chromatin is the nucleosome, in which 145–147 base pairs of DNA are wrapped in ~ 1.7 left-handed superhelical turns around a core of eight histone proteins (Figure 1A). The presence of ribonucleotides inhibits nucleosome formation, and even a single ribonucleotide has a significant impact [21, 22]. Hovatter *et. al* [22], determined that one ribonucleotide per 125 base pairs of DNA reduces nucleosome formation to ~ 80%; 2 or 3 ribonucleotides reduce it by more than 50%, and more than 5% ribonucleotides completely inhibit nucleosome formation and distort the DNA. Thus, a ribonucleotide incorporated in DNA causes local perturbations that account for its impact on nucleosome formation.

The perturbations stem from the key conformational feature of ribonucleotides in duplexes: their preference for the A-form C3'-endo sugar pucker domain (Figure 1B, C); this preference stems from steric crowding between the 2'-OH group on the sugar ring and nearby DNA residues (Figure 1C) when the pucker is C2'-endo as in B-DNA [6, 8–10]. In duplexes, deoxyribose can adopt both B-form C2'- and A-form C3'-endo domains [23, 24].

In free duplex DNA, an NMR study by DeRose *et al.* [25] showed that a single ribonucleotide embedded in the DNA sequence of the Dickerson dodecamer [26] adopts C3'-endo pucker without changing the C2'-endo pucker of adjacent deoxynucleotides. However, although the overall B-form is maintained, there are significant local disturbances in backbone torsion angles, helical parameters, and minor groove widths adjacent to the ribonucleotide and on the nearby partner strand. In another study, Evich *et al.* [27] investigated by high resolution NMR a single ribonucleotide embedded in three 9-mer DNA duplexes with different nearest neighbor sequences adjacent to the ribonucleotide. They analyzed the sugar pucker conformations of the ribonucleotide and the adjacent deoxyribonucleotides and found that the ribonucleotide was ~ 40–60 % C3'-endo, and the impact on the adjacent deoxynucleotides was sequence-dependent, with asymmetric 3' perturbation of the duplex primarily on the ribonucleotide-containing strand. These studies in free DNA suggest that such perturbations, stemming from intrinsic steric effects imposed by an embedded ribonucleotide would also be manifested in a nucleosome core particle.

Despite the importance of ribonucleotides in genomic DNA, there is still a gap in our knowledge concerning the molecular structures and dynamics of these lesions embedded in nucleosomal DNA. Even a single ribonucleotide has a significant impact on nucleosome formation [22] and the incorporation of consecutive ribonucleotides by DNA polymerases is likely to be rare [3, 5] with the exception of Okazaki fragment primers. Hence, our interest here is on how a single ribonucleotide embedded in a nucleosome core particle affects the structure and dynamics of the local nucleosome region. Furthermore, we wished to understand how the impact of the ribonucleotide is modulated by its position in the nucleosome. The position of a ribonucleotide in a NCP is described by the translational and rotational setting; translational positioning is the displacement from the central base at the dyad and the rotational positioning is the orientation relative to the center of the histone core (Figure 1A).

We utilized ~ 3.5 μ s molecular dynamic simulations to investigate six different positions of the ribonucleotide in the NCP (Figure 1A); three translational positions: at the dyad (SHL 0) which has unusual DNA periodicity [28, 29], near the DNA end (SHL 5.5) where spontaneous unwrapping is observed [30], and a position between them (SHL 2.5) where there are histone-DNA interactions that are important sites for nucleosomal interactions with proteins required for biological transactions such as transcription [31, 32]. These were combined with two rotational positions where the ribonucleotide interacts maximally with either histones (facing inward) or solvent (facing outward). In this way, we could explore unique interactions of the ribonucleotide and its neighboring DNA with histones. Our results showed a strong impact of the translational and rotational position of the ribonucleotide on local structure and dynamics. The origin of the ribonucleotide's effect stems from the preferred C3'-endo conformation of the sugar 2'-OH group; however, the specific nature of

the impact is governed by the local environment: histones or solvent. In addition, we consider how RER by RNase H2, the enzyme that incises a single ribonucleotide within a DNA duplex, might be modulated by the position of the ribonucleotide in a NCP. We also consider how access to the DNA for biological transactions, such as replication, transcription, and repair could be affected by the presence of a ribonucleotide.

2. Materials and Methods

2.1 Molecular Modeling

Our NCP model is based on PDB [33] ID 1KX5 [34], which contains 14 crystallographic Mn^{2+} that we retained (Figure 1A). All the histone tails were truncated according to trypsin digestion experiments [35] (supplementary Figure S1). Ribonucleotides were placed at three SHLs: near the dyad (SHL 0, the 3rd base pair from the dyad, namely BP 3), the 24th base pair from the dyad (SHL 2.5, BP 24), and the 55th base pair from the dyad (SHL 5.5, BP 55). At each of these BPs, a ribonucleotide was positioned so that it was maximally oriented toward the histones or toward the solvent, respectively (supplementary Figure S2). These models provided a total of six different ribonucleotide-containing NCPs. In addition, we simulated an all-DNA NCP as a control (ribose-free NCP). Local duplex sequences containing a single ribonucleotide in the center are displayed in supplementary Figure S2.

2.2 Molecular Dynamics Simulations

Construction of molecular topology and coordinate files for each NCP model was performed using the *tleap* module of AmberTools14 [36]. We used the force field *ff14SB* [37] for histones, the backbone torsion modifications including *parmbsc1* [38] and *OL1* [39] for nucleic acids, the Joung-Cheatham [40] model for the K^+ ions and divalent ion parameters [41] for Mn^{2+} ions. Hydrogen atoms were added to both nucleic acids and protein and the systems were explicitly solvated with the TIP3P [42] water model with at least a 10 Å buffer. We used K^+ ions to neutralize the system, and then added approximately 115 K^+/Cl^- ion pairs to reach a salt concentration of ~ 0.15 M salt. The final solvated systems contained 294 K^+ ions and 115 Cl^- ions, and ~ 40,000 water molecules, resulting in ~ 140,000 atoms in total.

In all NCP simulations, the cutoff distances for van der Waals and short-range electrostatic interactions were set to 9 Å. The long-range electrostatic interactions were treated with the particle-mesh Ewald (PME) method [43, 44]. The SHAKE [45] algorithm was used to constrain the covalent bonds involving hydrogen atoms, using a time step of 2 fs for equilibration and production runs. We conducted ~ 3.5 μs MD simulations using the AMBER14 [36] package. The details of the MD protocols are given in Methods in Supporting Information. All simulations were performed with the CUDA-enabled graphics processing units (GPUs) [46, 47] version of PMEMD in the AMBER16 [48] program package on NVIDIA Tesla K80 and P40 cards.

All molecular images and movies were generated with PyMOL (The PyMOL Molecular Graphic System, version 1.3x Schrödinger, LLC). Post-processing of all simulations was carried out using the CPPTRAJ [49] module of AMBER14 [36], 3DNA [50] and Curves

+ [51]. All structural analyses were obtained from these MD simulations with the first 400 ns discarded.

3. Results

We are interested in the impact that incorporation of a single ribonucleotide at different translational and rotational positions has on the structural and dynamic properties of the nucleosome. Accordingly, we have carried out ~ 3.5 μ s MD simulations of NCPs containing a single ribonucleotide positioned translationally at SHL 0, 2.5 and 5.5 with a ribonucleotide placed facing inward toward the histone core or outward toward the solvent for each SHL; this provides a total of six different ribonucleotide-containing positions (Figure 1A and supplementary Figure S2), and we also investigated a ribose-free NCP control. At and near each ribonucleotide, we analyzed the sugar pucker conformations, minor groove widths, local dynamics, twist angles, hydrogen bonding interactions involving the ribose 2'-OH group, as well as nucleotide interactions with histones.

3.1 A single ribonucleotide embedded in a NCP alters the local sugar pucker conformations

DNA in the ribose-free NCP is predominantly C2'-endo but occasionally visits the C3'-endo domain.—Our MD simulation of the ribose-free NCP showed that the DNA sugar puckers are predominantly in the C2'-endo domain (South) (supplementary Figure S3). However, we also observed lesser populations of C3'-endo pucker (North), that vary depending on the SHL location: at several sites (BP 8, 21, 23, 44, 51, and 69 in Chain I as shown in supplementary Figure S3A and BP 6 and 20 in Chain J as shown in supplementary Figure S3B), the deoxyribose adopts puckering characteristics in the North region of the pseudorotation cycle in over 20% of the MD simulation. Moreover, we observed that those sites where the deoxyribose has significant populations of C3'-endo pucker, there are dynamic interactions with histones. For example, the deoxyribose at BP 23 on Chain I becomes C3'-endo when the distance between phosphates at BP 23 and 22 shortens due to interactions between these phosphates and ARG72 from histone H3 helix (supplementary Figure S4). A shortened phosphate-phosphate distance is characteristic of C3'-endo pucker [24, 52]. Similar phenomena are observed at a number of other sites where the deoxyribose C3'-endo puckering occupancies are higher than 20%. Another example occurs at BP 44, where interactions of ARG17 from H2A with both BP 44 and BP 43 similarly shorten the phosphate-phosphate distance and cause the sugar at BP 44 to repucker to C3'-endo (supplementary Figure S5). These interactions and the correlated repuckerings of the deoxyribose are dynamic during the simulations.

In the ribose-containing NCPs, the ribonucleotide facing inward adopts a stable C3'-endo conformation and perturbs adjacent deoxynucleotides.—In our simulations with a ribonucleotide facing inward, the ribonucleotide stably adopts the preferred C3'-endo conformation in over 90% of the population (supplementary Table S1) at all three SHLs (Figure 2A and supplementary Figure S6A). Furthermore, the C3'-endo ribonucleotide facing inward perturbs nearby deoxyribonucleotides on the ribonucleotide-containing strand by affecting their sugar pucker preferences and dynamics. The specifics of

this impact differ somewhat in the three SHL positions and for up to two deoxynucleotides on either side of the ribonucleotide. In general, the local DNA region embedding the ribonucleotide, displays enhanced dynamics in sugar pucker compared to the ribose-free NCP; there are generally more transitions to the C3'-endo domain that are usually brief and non-persistent. However, a ribonucleotide at SHL 2.5 shows a unique impact on its 5'-side adjacent deoxyribonucleotide, whose sugar repuckering to C3'-endo occurs twice, persisting for ~ 0.5 μ s in each occurrence (supplementary Figure S6A). As discussed below, at this SHL position the ribonucleotide imposes unique interactions between histones and local nucleotides.

With ribonucleotide facing outward, its C3'-endo conformation is unstable and adjacent deoxynucleotides are scarcely affected.—

When the ribonucleotide is facing outward, the C3'-endo conformation is unstable (Figure 2B and supplementary Figure S6B): it either fluctuates between C3'- and C2'-endo or becomes predominantly C2'-endo. At SHL 0 and 5.5, the C3'-endo pucker represents only 60% and 52% (Table S1), respectively, of the population, with rapid interconversion between C3'- and C2'-endo states throughout the simulations. At SHL 2.5, the ribose sugar pucker is ~ 90 % in the C2'-endo domain, similar to the deoxyribose sugar pucker (~ 98 % in the C2'-endo state) in the ribose-free NCP. At all three SHLs, the ribose's pucker is more dynamic than the corresponding site in the ribose-free NCP (supplementary Figure S6B). However, the C2'-endo deoxynucleotides adjacent to the ribonucleotide are scarcely affected in pucker conformation or dynamics because the ribonucleotide is somewhat disposed toward C2'-endo.

The preferred C3'-endo sugar pucker of a ribonucleotide facing outward is destabilized due to a rigid, narrow minor groove and 2'-OH-specific hydrogen bonds.—The minor-groove widths of the DNA in the crystal structure of the NCP (PDB ID 1KX5 [34]) are periodic along the SHLs [53]: they are maximal when the groove faces solvent, and are minimal where it faces the histones and are mainly penetrated by arginine residues (supplementary Figure S7A). In addition to this periodicity, our MD simulations of the ribose-free NCP reveal periodic dynamics of the minor grooves (supplementary Figure S7B). The dynamics is greatest where the groove width is maximal, and least where the width is minimal. Furthermore, among the minima, the width of the groove is more dynamic when arginine is absent or is positioned away from the DNA groove. Thus, our results show that arginine plays an important role in establishing the dynamics of the minor groove.

In the ribonucleotide-containing NCPs, we observe that a ribonucleotide always enlarges the minor groove (supplementary Figure S7C), particularly when its pucker is C3'-endo (Figure 2A, B); this permits accommodation of the 2'-OH without crowding. However, the favorable C3'-endo pucker is not always achieved; the minor groove width and its dynamics plays an important role in governing this phenomenon. Specifically, a minimally dynamic and narrow minor groove inhibits the C3'-endo state. We found this particularly to be the case at SHL 2.5 with the ribonucleotide on the outer strand (Figure 2C); the ribonucleotide principally adopts an unfavorable C2'-endo conformation (~ 90%). The interactions between the groove and ARG83 from the H3 histone core stabilize the groove and prevent it from widening to

accommodate to C3'-endo pucker; hence, this observation supports the concept that a rigid, narrow minor groove can inhibit the preferred C3'-endo pucker.

Another factor that impacts the ribonucleotide sugar pucker is specific hydrogen bonds involving the 2'-OH group with the DNA backbone and bases in the 3'-direction (supplementary Table S1). We observed these when the ribonucleotide is facing outward at SHL 5.5 (Figure 2D). The hydrogen bonds between the O2'-H and either O5' or O4' oscillate during the simulations and the H-bond, O2'-H...O5', is stronger with more ideal bond distance and angle and higher occupancy when the ribose sugar pucker is C2'-endo. Furthermore, we observed additional H-bonds which are only present when the pucker is C2'-endo: O2'-H hydrogen bonding with phosphate oxygen or base of DNA in the 3'-direction. These interactions dynamically regulate the sugar pucker so that it rapidly interchanges between C3'- and C2'-endo states, with more favorable hydrogen bonds supporting the C2'-endo state.

3.2 A ribonucleotide causes local DNA distortions that perturb histone-DNA interactions in a position-dependent manner.

Ruptured Watson-Crick base pairs.—Our simulations for the ribose-free NCP show uniformly stable Watson-Crick base pairing. However, when the ribonucleotide is present, the Watson-Crick pairs at the ribonucleotide or adjacent sites are episodically ruptured, revealed in analyses of the central hydrogen bond distance of each base pair (supplementary Figure S8). This ribonucleotide-induced disturbance differs at the various translational and rotational positions. At the dyad, a ribonucleotide facing outward has disturbed Watson-Crick pairing more frequently than when facing inward. This episodic ruptured Watson-Crick pairing occurs when the ribose sugar pucker is C3'-endo; in this pucker the 2'-OH can compete for hydrogen bonding with a nitrogen atom of the partner base (supplementary Figure S9). However, at SHL 2.5, a ribonucleotide facing inward has disturbed Watson-Crick pairing more frequently than when facing outward. In the latter case, the ribose pucker is predominantly C2'-endo (~ 90%, Figure 2B–C); this B-like pucker of the embedded ribonucleotide supports normal Watson-Crick base-pairing. Moreover, the extent of ribonucleotide-induced disturbance to adjacent Watson-Crick pairing also depends on position. At SHL 2.5, the disturbance is greatest when the ribonucleotide faces inward; there are dynamic new interactions between the backbone of BP 22 and ARG69 from the histone H3 helix: while ARG69 oscillates between BP 17 and 22 when the ribonucleotide is present (supplementary Figure S10A), it interacts stably with BP 17 in the ribose-free NCP (supplementary Figure S10B). The interactions between BP 22 and ARG69 open the groove and further cause episodic rupture of one Watson-Crick pair 3' to the ribonucleotide.

Duplex Unwinding.—Our results reveal that a ribonucleotide causes the trimer duplex with ribonucleotide at the center to be unwound; the unwinding is more prominent when the ribonucleotide faces inward, and it is most marked at SHL 2.5 (supplementary Figure S11). At this SHL, the ribonucleotide causes the local trimer duplex to be unwound in ~ 34 % of the population (Figure 3). Unwinding occurs when the 5'-side deoxyribose is C3'-endo or the 3'-side Watson-Crick pair is ruptured, and unwinding is greatest (~ 36°) when both occur at the same time. The local trimer duplex is mainly (~ 70 %) B-like (average twist around

74° over three base-pairs in the ribose-free NCP and 72° in B-form DNA [54]); however, it fluctuates between this B-like twist and unwound forms during the simulations.

At SHL 2.5, unique nucleotide-histone interactions cause severe and recurring distortions to the local duplex.—At SHL 2.5 with the ribonucleotide facing inward, the local dimer is A-like: the ribonucleotide is predominantly C3'-endo, and the 5'-side deoxynucleotide is also C3'-endo. In this A-like conformation, the minor groove is widened, and the local duplex is unwound. As a result, the DNA backbone is repositioned toward ARG69 of the histone H3 helix; the side-chain of this arginine occasionally rotates so that its positively charged guanidinium group interacts with the phosphate on the 3'-side of the ribonucleotide. These interactions then pull the ribonucleotide-containing strand away from its partner strand, causing the minor groove to widen greatly (Figure 4A). During these arginine-nucleotide interactions, one to two Watson-Crick pairs 3'- to the ribonucleotide rupture episodically and consequently further unwind the duplex (see supplementary Movie). This motion of the histone arginine occurs dynamically during the simulation; when it does it causes this correlated series of dynamic distortions that are not observed at any other SHL or in the ribose-free NCP at the same position. Thus, at this SHL 2.5, the nucleotide-histone interactions are unique among our investigated SHLs.

4. Discussion

The ribose sugar pucker and its impact on the adjacent deoxyribose puckers are modulated by rotational position that governs histone-nucleotide interactions.

In the present study we examined the structures and dynamics of a single ribonucleotide embedded in nucleosomal DNA and its impact on nearby DNA residues. We wished to characterize how the presence of the ribonucleotide imposes distortions to the DNA and to histone-nucleotide interactions. We investigated three different translational positions, with two rotational settings for each case. We found remarkable differences depending on the SHL and whether a ribonucleotide is facing inward or outward. Central to our interest is the sugar pucker of the ribonucleotide, which intrinsically favors the C3'-endo state; however, this preference is strikingly modulated by its rotational positioning in the NCP. When facing inward and directly interacting with histones, the ribonucleotide adopts a stable C3'-endo conformation, which also affects the adjacent deoxyribose puckers and their dynamics (Figure 2A). While the detailed nature and extent of this phenomenon is somewhat SHL-dependent, it affects no more than two nucleotides on either side of the ribonucleotide. By contrast, when the ribonucleotide faces outward toward the solvent, regardless of the SHL, the preferred C3'-endo ribose sugar pucker becomes unstable. It either fluctuates between C3'- and C2'-endo domains (SHL 0 and 5.5) or becomes predominantly C2'-endo (SHL 2.5) (Figure 2B). At SHL 5.5, fluctuating hydrogen bonds between the 2'-OH and the adjacent DNA backbone and bases cause the ribose sugar pucker to oscillate between C3'- and C2'-endo, rather than remaining stably C3'-endo (Figure 2C), emphasizing that base sequence can modulate the ribose's pucker. At SHL 2.5, a minor-groove arginine (ARG83) narrows the groove and thereby fosters the C2'-endo pucker (Figure 2D); at this position, a ribonucleotide is therefore much more like a deoxynucleotide. Furthermore, because the ribonucleotide facing outward has a proclivity toward C2'-endo pucker regardless of its

SHL, its impact on the adjacent deoxynucleotides is minimal (Figure 2B). Overall, direct histone-nucleotide interactions stabilize the preferred C3'-endo ribose pucker when the ribonucleotide faces inward, and thereby cause the adjacent DNA pucker to have a higher population of the C3'-endo state; however, in the absence of direct histone-nucleotide interactions when the ribonucleotide faces outward, the ribose pucker itself is dynamic but its impact on adjacent deoxynucleotides is minimal.

Inward facing ribonucleotides cause distortions to the local duplex, affecting histone-nucleotide interactions that are determined by translational position.

When the ribonucleotide faces inward, its stable, preferred C3'-endo pucker imposes distortions to the local duplex structure and dynamics, which are SHL-dependent. The greatest distortion occurs when the ribonucleotide is at SHL 2.5 where there are unique altered histone-nucleotide interactions (Figure 4A). Here the distortions include a persistent abnormal C3'-endo population of the adjacent deoxyribose pucker, minor groove opening, ruptured Watson-Crick pairing, and unwinding of the duplex. By contrast, at SHL 0 and 5.5, the ribonucleotide has only a modest impact and there are no striking changes in histone-nucleotide interactions. Thus, SHL-dependent histone-nucleotide interactions play an important role in the extent of distortion imposed by the ribonucleotide.

Implications for ribonucleotide excision repair.

Our results provoke some interesting speculations in relation to RER and its dependence on translational and rotational positioning of the ribonucleotide in the nucleosome. Ribonucleotide-imposed distortions may signal for the machinery responsible for remodeling and posttranslational modifications; this machinery opens chromatin for biological transactions, including repair, in line with the “access-repair-restore” paradigm [55, 56] for DNA repair, leading to recruitment of RNase H2. The signal for the remodeling and posttranslational modifications might be greater where the distortion is greater. One such location is at the dyad with ribonucleotide facing outward, where the distortion is localized to the ribonucleotide itself; the ribonucleotide facing outward has dynamic sugar pucker and frequent disturbance to its Watson-Crick pair. Another location is at SHL 2.5 with ribonucleotide facing inward where the distortion is greater and more extensive than when facing outward; the ribonucleotide facing inward imposes multiple dynamic distortions to the local duplex and to nucleotide-histone interactions (Figure 4A). By contrast, a ribonucleotide facing outward at SHL 2.5 is very much like a deoxynucleotide (Figure 2D), suggesting that at this position the ribonucleotide is disguised as a normal deoxyribonucleotide and may not be recognized. Since RNase H2, recognizes a C3'-endo ribonucleotide through hydrogen bonding interactions [57] (Figure 4B), one could speculate that RER would be most efficient where the ribonucleotide pucker is stably C3'-endo, which occurs when it faces inward, although, occlusion by histones is a barrier for contacting an inward-facing ribonucleotide. Thus, if inward-facing ribonucleotides are protected from RNase H2 and outward-facing ribonucleotides do not adopt a stable C3'-endo pucker, RER could be doubly inhibited by chromatin.

Insights on the role of translational and rotational position on repair of lesions in nucleosomes may be gained from other repair systems. For nucleotide excision repair with

Hela cell extracts, while access to a lesion would appear to be more facile when the lesion faces outward, recent studies have shown that dependence of repair efficiency on rotational position is lesion-dependent (Shafirovich, Broyde and Geacintov *et al.* submitted for publication). For base excision repair, lesions facing outward are repaired more efficiently than those facing inward in a number of cases. In addition, lesions with the same rotational orientation that are far from the dyad are repaired more efficiently than ones that are close to the dyad [58]. Furthermore, Delaney and coworkers have recently shown that human oxoguanine glycosylase activity is inhibited at the dyad axis, regardless of rotational position of the lesion [59]. In addition, this group has shown that when the lesion is positioned at about 20 base-pairs from the end of the DNA, a midway facing lesion (between facing inward and outward) has near quantitative glycosylase activity while inward or outward facing lesions display activity reduced by ~ half; they infer that distinct microenvironments of the NCP modulate the glycosylase activity [60]. This concept is in line with our findings that the structure and dynamics of a single ribonucleotide embedded in a NCP differ depending on the rotational and translational position.

In conclusion, our work points to the fertile ground for future experimental and computational explorations offered by ribonucleotide structure and repair in NCPs. The NCP is a complex substrate for RER; the detailed impact of lesion rotational and translational position and its interplay with nucleotide-histone interactions, with chromatin remodeling and post-translational modifications such as acetylation in a NCP, and possibly in higher order chromatin structures are some open challenges.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1.

(A) Overall structure of the ribose-free NCP (modeled based on PDB ID 1KX5 [34], see Methods). Super-helical locations (SHLs) and base pair (BP) positions relative to the dyad axis (SHL 0 and BP 0) are given. Ribonucleotides, introduced at BP 3, 24, and 55, are indicated by magenta stars. Each BP has two sites for ribonucleotide incorporation: one facing inward toward the histones and the second facing outward to the solvent. For clarity, half of the NCP with SHL < 0 is shown in transparent rendering. Chain I is shown in black and Chain J in blue (PDB ID 1KX5 [34]). (B) Pseudorotation cycle. Image adapted from Altona and Sundaralingam [61]. Pseudorotation phase angles (P) are given in multiples of 36° (solid lines) and corresponding sugar pucker conformations are designated. Here, we refer to the North (N), C3'-endo domain, for P values in the range of ~ -18 to $+72$ degrees and the South (S), C2'-endo domain, for P values in the range of $\sim +126$ to $+216$ degrees. (C) Orientation of the minor-groove positioned 2'-OH group (spheres) of a single ribonucleotide embedded in a B-DNA duplex in C3'- and C2'-endo conformations with P value designated. In the C3'-endo conformation, the 2'-OH group is directed toward the

minor groove and opens it; when in the C2'-endo conformation, the 2'-OH group crowds the backbone and base of the deoxyribonucleotide on the 3'-side. Zoomed-in view of a single ribonucleotide displays the C3'- and C2'-endo conformations: in the C3'-endo conformation, the C3' atom is out of plane and on the same side as the C5' atom and the base, while it is on the opposite side when the conformation is C2'-endo.

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Figure 2.

(A and B): the stability of the C3'-endo pucker of a ribonucleotide depends on rotational position. (A) With the ribonucleotide facing inward, at all three SHLs, the sugar is stably C3'-endo. (B) When facing outward, the C3'-endo pucker is unstable: it fluctuates between C3'- and C2'-endo at SHL 0 and 5.5 and becomes predominantly C2'-endo at SHL 2.5 (see supplementary Figure S6). P value ensemble averages are shown as dots connected by a solid line and standard deviations are highlighted in shadows. The position of the single ribonucleotide is indicated by a red arrow. Base-pair numbers (BP) are the same as in Figure 1A. (C and D): the origins of inhibition and instability of the C3'-endo sugar pucker of the ribonucleotide facing outward in the ribose-containing NCPs. (C) ARG83 causes the minor groove to narrow, which inhibits the ribose C3'-endo pucker. Correlation of ribose sugar pucker and minor groove width (dotted line) is shown. (D) the 2'-OH of the ribonucleotide forms a hydrogen bond (distance $< 3.5 \text{ \AA}$) with the phosphate oxygen (OP2) of the DNA in the 3'-direction, which fosters its C2'-endo conformation. The H-bond between O2' and

OP2 is indicated in dotted line. Correlation of the ribose sugar pucker and the H-bond is shown.

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Figure 3.

A ribonucleotide facing inward at BP 24, SHL 2.5, causes unwinding and enhances dynamics of the local duplex. (A) Time-dependence of the twist angle over three nucleotide steps between BP 23 and 25. (B) Time-dependence of the deoxyribose sugar pucker (BP 25) on the 5'-side of the ribonucleotide. (C) Time-dependence of Watson-Crick base pair central distance, d_{N1N3} , at BP 23 (see Methods in Supporting Information). (D) Definition of twist angle [50] and structure of local trimer duplex containing the ribonucleotide at the center. Ribonucleotide is colored by atom with carbon in yellow. (E) Clustering of the BP 25 deoxyribose sugar pucker and Watson-Crick base pair central distance, d_{N1N3} , at BP 23 shows that unwinding of the trimer duplex occurs when the BP 25 deoxyribose sugar pucker adopts a C3'-endo pucker (C2 cluster) or the BP 23 Watson-Crick pair is ruptured ($d_{N1N3} > 4$ Å, C4 cluster) or both (C3 cluster). The average twist angle of the C1 cluster is $\sim 70^\circ$, which is B-like (twist angle 72° for a B-form DNA). The ensemble average twist angle is $\sim 74 \pm 6^\circ$ at the same position for the ribose-free NCP. The average untwist angle of each cluster is defined as follows: avg. twist (ribose-free NCP) – avg. twist (cluster). Average twist and untwist angles and populations of the four clusters are given in the table.

Figure 4.

(A) The ribonucleotide facing inward at SHL 2.5, BP 24 causes severe and recurring distortions to the local duplex and to nucleotide-histone interactions (see supplementary Movie). These distorted structures display C3'-endo puckers of the ribonucleotide and its adjacent 5'-side deoxynucleotide, a minor groove that is opened by $\sim 3.3 \text{ \AA}$, interrupted interactions between H3 residues 68 to 72 and nearby nucleotides, under-twisting of the local duplex trimer by ~ 36 degrees, and ruptured Watson-Crick pairing of BPs 22 and 23. Ensemble average values of these properties are given in the table. The twist angles are computed over the three nucleotides between BP 23 and BP 25, with the central ribonucleotide at BP 24. Minor groove widths **a** and Watson-Crick lengths **b** and **c** are designated by black dashed lines. Calculation of the Watson-Crick central distance (d_{NIN3}) given here is described in Methods in the Supporting Information. The Watson-Crick base pair is ruptured with $d_{NIN3} > 4 \text{ \AA}$. Pairs of phosphorus atoms designated by spheres are used for the measurement of minor groove widths. Interactions between nucleotides and histone residues 68 to 72 that are altered in the ribonucleotide-containing NCP are designated by red

dashed lines. The best representative structure from the C3 cluster (Figure 3D) is shown for the ribose-containing NCP; the best representative structure from the last 3 μ s of the MD simulation is shown for the ribose-free NCP. (B) Crystal structure of RNase H2 in complex with a ribonucleotide-containing DNA duplex (PDB ID 3O3F [57]). A single ribonucleotide is embedded in a 12-mer DNA duplex at residue 6 from the 5'-end (pink strand). The inset box illustrates specific interactions between RNase H2 and the 2'-OH of the ribonucleotide in the C3'-endo conformation. The 2'-OH group hydrogen bonds with the backbone amide groups of ARG22, GLY21 and 23, and the hydroxyl group of TYR163. The aromatic ring of TYR163 stacks with the deoxyribose ring of the DNA on the 3'-side of the ribonucleotide, which further stabilizes the ribose C3'-endo pucker. The ribonucleotide is colored by atom with the carbon in yellow in all structures.