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Redundancy in ribonucleotide excision repair: Competition, compensation, and cooperation

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ABSTRACT

The survival of all living organisms is determined by their ability to reproduce, which in turn depends on accurate duplication of chromosomal DNA. In order to ensure the integrity of genome duplication, DNA polymerases are equipped with stringent mechanisms by which they select and insert correctly paired nucleotides with a deoxyribose sugar ring. However, this process is never 100% accurate. To fix occasional mistakes, cells have evolved highly sophisticated and often redundant mechanisms. A good example is mismatch repair (MMR), which corrects the majority of mispaired bases and which has been extensively studied for many years. On the contrary, pathways leading to the replacement of nucleotides with an incorrect sugar that is embedded in chromosomal DNA have only recently attracted significant attention. This review describes progress made during the last few years in understanding such pathways in both prokaryotes and eukaryotes. Genetic studies in *Escherichia coli* and *Saccharomyces cerevisiae* demonstrated that MMR has the capacity to replace errant ribonucleotides, but only when the base is mispaired. In contrast, the major evolutionarily conserved ribonucleotide repair pathway initiated by the ribonuclease activity of type 2 Rnase H has broad specificity. In yeast, this pathway also requires the concerted action of Fen1 and pol δ, while in bacteria it can be successfully completed by DNA polymerase I. Besides these main players, all organisms contain alternative enzymes able to accomplish the same tasks, although with differing efficiency and fidelity. Studies in bacteria have very recently demonstrated that isolated rNMPs can be removed from genomic DNA by error-free nucleotide excision repair (NER), while studies in yeast suggest the involvement of topoisomerase 1 in alternative mutagenic ribonucleotide processing. This review summarizes the most recent progress in understanding the ribonucleotide repair mechanisms in prokaryotes and eukaryotes.

1. Introduction

The synthesis of vitally important macromolecules that encode and transmit genetic information in all living organisms relies on nucleic acid polymerases. The nucleotide substrate specificity separates these enzymes into two distinct groups: those that utilize ribonucleoside triphosphates (rNTPs) and those that utilize deoxyribonucleoside triphosphates (dNTPs). DNA polymerases; enzymes that are essential for genome duplication and repair synthesis; belong to the latter group. Deoxynucleotides; the building blocks of DNA; are synthesized by ribonucleotide reductases from ribonucleotides and are present throughout the cell cycle at much lower concentrations than their precursors. As a result; and since dNTPs and rNTPs are chemically and structurally very similar; it is imperative for DNA polymerases to exhibit a high degree of selectivity for deoxyribonucleotides over ribonucleotides.

Various structural, genetic and biochemical studies revealed that the major barrier for rNTPs is confined to a single residue in the active site of all DNA polymerases that is called the "steric gate" (for recent reviews see Refs. [1–3]). The side chain or backbone of this residue physically clashes with the 2′-OH group on the sugar ring of an incoming ribonucleotide and prevents its insertion. Moreover, most high-fidelity DNA replicases are equipped with $3' \rightarrow 5'$ exonucleolytic proofreading domains or subunits that are designed to improve enzymatic fidelity, and have the capacity to not only recognize and excise nucleotides with a wrong base, but also with a wrong sugar [4–6]. However, proofreading of errantly

Abbreviations: nt, nucleotide; dNTP, deoxyribonucleoside triphosphate; rNTP, ribonucleoside triphosphate; pol, DNA polymerase; RNase H, ribonuclease H; Top1, topoisomerase 1; Fen1, flap endonuclease 1; PCNA, proliferating cell nuclear antigen; Exo1, exonuclease 1; Cho, UvrC homologue; RER, ribonucleotide excision repair; NER, nucleotide excision repair; MMR, mismatch repair; BER, base excision repair; NHEJ, non-homologous end joining repair; *E. coli*, *Escherichia coli*; *S. cerevisiae*, *Saccharomyces cerevisiae*.

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incorporated ribonucleotides is relatively poor and as with other enzymatic processes, the overall discrimination against ribonucleotide insertion is not 100% efficient, even for high-fidelity DNA polymerases [2,3,7]. As a result, a significant number of ribonucleotides are incorporated into nuclear DNA during the normal process of genome duplication. Estimations of this value for replicative and repair DNA polymerases from a variety of organisms [1,3,8–14] has led to the realization that among all non-canonical nucleotides embedded in chromosomal DNA, rNMPs are the most abundant. These findings hint at the possibility that incorporation of rNMPs during DNA replication or repair, is not simply a result of failed attempts to prevent it, but rather is an evolutionarily conserved property of DNA synthesis that may be of important biological significance (for recent review see Refs. [15,16]). For example, this includes (i) marking the nascent DNA, thereby directing the mismatch repair (MMR) machinery to the correct strand [12,17], (ii) improving the efficiency and fidelity of pol μ-dependent non-homologous end joining in the course of double-strand break repair [9], or (iii) directing the recombination important for mating type switching in *Schizosaccharomyces pombe* [18]. However, when accumulated at excessive levels, rNMPs scattered throughout the chromosome might pose serious danger for a living cell, mainly due to the reduced stability [19] and altered structure of the nucleic acid backbone ([20,21] and references therein). This threat is imminent not only for dividing cells, but also for quiescent cells that have substantially lower dNTP:rNTP ratios. To prevent persistent ribonucleotide accumulation, cells rely on the help of repair systems with the capacity to monitor and excise rNMPs inadvertently incorporated by DNA polymerases into genomic DNA. Here, we review and summarize the most recent data that has led to the elucidation of ribonucleotide repair mechanisms with emphasis on our own *in vivo* and *in vitro* studies of prokaryotic pathways. We also present some previously unpublished data, which characterize specific features of excision/re-synthesis steps of the ribonucleotide repair pathway.

2. Approaches to study ribonucleotide repair

Ribonucleotide repair has been extensively investigated using *Saccharomyces cerevisiae* (*S. cerevisiae*) and *Escherichia coli* (*E. coli*) model systems. Studies in yeast were mainly performed using crude cell extracts from strains carrying a deletion of gene(s) encoding proteins implicated in RER and by reconstituting repair pathways *in vitro* using purified recombinant proteins [4,6,8,22,23]. We have elucidated RER in bacteria using biochemical and genetic approaches [24–27]. In particular, we have utilized low-fidelity *E. coli* pol V ($UmuD'_2C$) and a steric gate mutant (*umuC*_Y11A) that avidly misincorporates ribonucleotides into genomic DNA to investigate the mechanisms of prokaryotic ribonucleotide repair.

E. coli pol V is best characterized by its ability to promote translesion DNA synthesis with a concomitant increase in damage-induced mutagenesis [28,29]. However, in a *recA730 lexA*(Def) background, where pol V is maximally activated and the enzyme is able to compete with pol III for access to undamaged DNA, the low-fidelity pol V confers a significant spontaneous mutator phenotype in the absence of exogenous DNA damage. Biochemical characterization of wild-type pol V revealed that in addition to low base substitution fidelity, pol V readily incorporates ribonucleotides into DNA *in vitro* [24]. Furthermore, pol V is able to synthesize long RNA stretches *in vitro* when copying a DNA template in the presence of rNMPs. The *umuC*_Y11A steric gate mutant of pol V has an even greater propensity to incorporate ribonucleotides. This mutant is also characterized by reduced deoxyribonucleotide base specificity *in vitro*. We therefore expected that when expressed *in vivo*, the *umuC*_Y11A mutant would induce higher levels of spontaneous mutagenesis than wild-type pol V. However, the exact opposite phenotype was observed. Spontaneous mutagenesis in strain with the *umuC*_Y11A variant was only about 7% of spontaneous mutagenesis in strain with wild-type pol [24]. To explain this phenotype, we hypothesized that efficient and accurate repair specifically targeted to replace nucleotides with an incorrect sugar concomitantly replaces nucleotides with incorrect bases in the vicinity of the target ribonucleotide. In doing so, these repair pathways reduce the mutagenic consequences of DNA synthesis by the highly error-prone *umuC*_Y11A. Therefore by introducing the *umuC*_Y11A allele into a number of repair-deficient strains, we have been able to identify individual proteins and repair systems that make a significant contribution into the poorly mutable phenotype of *umuC*_Y11A-expressing cells, and thus have delineated both primary and back-up pathways of ribonucleotide repair in *E. coli*.

2.1. RNase H-dependent ribonucleotides excision repair

It should be noted that the accidental incorporation of ribonucleotides by DNA polymerases is not the major source of rNMPs embedded into chromosomal DNA. Initiation of DNA replication on both the leading and lagging strands in all organisms occurs through the synthesis of short RNA primers by primases, followed by primer elongation by replicative DNA polymerases. The RNA primers must then be replaced with deoxyribonucleotides before newly synthesized DNA can be ligated into an intact strand. Since replication of the lagging strand proceeds discontinuously, multiple Okazaki fragments form and the number of RNA primers that have to be removed during genome duplication is quite substantial, even in a bacterial chromosome, which has only a single origin of replication. Therefore, cells are equipped with an efficient system designed to detect and eliminate RNA patches from double-stranded DNA. Several distinct pathways have been implicated in RNA primer removal during Okazaki fragment maturation [30]. Naturally, at least one of these systems could be co-opted to remove errant ribonucleotides sporadically incorporated by DNA polymerases during replication and/or repair DNA synthesis. Indeed, it has been demonstrated in *E. coli* and *S. cerevisiae*, that the major pathway directed at the removal of isolated rNMPs from DNA is "RER" (Ribonucleotide Excision Repair), which is mechanistically very similar to the removal of ribonucleotide s during Okazaki fragment maturation. In principal, this pathway in prokaryotes and eukaryotes consists of the following key steps (Fig. 1): (i) cleavage of the phosphodiester bond at the RNA–DNA junction 5′ to the rNMP; (ii) DNA synthesis to replace excised nucleotides; (iii) a second cut is made 3′ to the ribonucleotide; (iv) sealing of the nick by a DNA ligase that ultimately completes the repair pathway.

As a general rule, DNA polymerases discriminate against rNMPs very efficiently and only rarely incorporate isolated ribonucleotides [2,7]. In contrast to multiple consecutive rNMPs forming the RNA primer of an Okazaki fragment, these ribonucleotides are randomly scattered across the genome. Therefore, initiation of the RER process requires a ribonucleotide-specific endonuclease that can recognize a single rNMP embedded in double-stranded DNA. The enzymes that can hydrolyze the 3′-O-P bond on such substrates are well-conserved in all domains of life and are called type 2 ribonuclease H (RNase H) (Fig. 1). Even though RNases of this type, such as eukaryotic RNase H2 encoded by the *rnh2* gene and prokaryotic RNase HII encoded by the *rnhB*, prefer to cleave the RNA moiety in DNA templates containing a single ribonucleotide, they are also able to incise templates containing multiple rNTPs [31–37].

In both bacterial and yeast strains expressing steric gate mutant DNA polymerases (pol V *umuC*_Y11A or pol ε M644G respectively), defects in type 2 RNase H have mutagenic consequences [8,22,26].