The etiology of uracil residues in the *Saccharomyces cerevisiae* genomic DNA

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Abstract
Non-canonical residue in DNA is a major and conserved source of genome instability. The appearance of uracil residues in DNA accompanies a significant mutagenic consequence and is regulated at multiple levels, from the concentration of available dUTP in the nucleotide pool to the excision repair for removal from DNA. Recently, an interesting phenomenon of transcription-associated elevation in uracil-derived mutations was described in *Saccharomyces cerevisiae* genome. While trying to understand the variability in mutagenesis, we uncovered that the frequency of uracil incorporation into DNA can vary depending on the transcription rate and that the non-replicative, repair-associated DNA synthesis underlies the higher uracil density of the actively transcribed genomic loci. This novel mechanism brings together the chemical vulnerability of DNA under transcription and the uracil-associated mutagenesis, and has the potential to apply to other non-canonical residues of mutagenic importance.

Keywords DNA repair · Non-canonical nucleotides · Transcription-associated mutagenesis · Uracil · dUTPase

Introduction
Uracil, a very frequent form of endogenous DNA modification, can appear in DNA via two different mechanisms: the incorporation into DNA in place of thymine and the deamination of cytosine. Many DNA polymerases including eukaryotic replicative DNA polymerases cannot distinguish between a uracil and a thymine base, and will readily incorporate uracil in place of thymine during replication and repair, depending on the [dUTP]/[dTTP] ratio, resulting in a stable U:A base pair (Bessman et al. 1958; Warner et al. 1981). If uracil persists to the subsequent round of replication, adenine can be incorporated in the complementary strand; U incorporation in place of T, therefore, is not mutagenic in itself. The cytosine deamination can occur either through a spontaneous or an enzymatic process creating U:G mispairs, which potentially is highly mutagenic. If uracil persists in a U:G mispair, the subsequent round of replication would result in a C:G-to-T:A conversion. On the other hand, if an abasic site is generated through the removal of uracil by a uracil DNA glycosylase, it can either be repaired in error-free manner by base excision repair machinery or result in further mutagenic event mediated by the error-prone translesion synthesis DNA polymerases (Boiteux and Jinks-Robertson 2013). Repeated cycles of uracil excision by glycosylases also come with the risk of the accumulation of abasic sites leading to DNA strand breaks and, ultimately, cell death (el-Hajj et al. 1988; Gadsden et al. 1993). It is, thus, important to maintain the minimal level of uracil in DNA to prevent genomic instability. Here, we discuss the wider implication of a novel mechanism of uracil occurrence in DNA that is recently reported by our lab.

The sources of uracil in DNA

Even though uracil in DNA has been indicated to be a major source of genomic instability, we still lack information on
the number of uracil DNA residues derived from either deamination and/or incorporation. Various quantification methods aimed at measuring the genome-wide level of uracil in DNA have yielded inconsistent results and have the added complication that the quantification methods cannot distinguish U:A and U:G base pairs, arising from uracil incorporation and cytosine deaminations, respectively (Galashkevskaya et al. 2013; Horvath and Vertessy 2010). Spontaneous deamination of cytosines is estimated to occur about 70–500 times per cell per day (Kavli et al. 2007; Lindahl 1993). In addition, enzymes that deaminate cytosines to uracil, such as Alipoprotein B mRNA-editing enzyme catalytic polypeptide-like family proteins (APOBECs) including Activation Induced Deaminase (AID), have been identified in many metazoan species (Siriwardena et al. 2016). These enzymes are critical to antibody-diversification and innate immunity against retroviruses. It is, therefore, possible that the number of uracil residues that are derived from cytosine deamination in metazoa is significantly higher than others without clearly characterized cytosine deaminases.

On the other hand, in yeast, a large fraction of genomic uracil is thought to originate from the dUTP incorporation by DNA polymerases rather than from cytosine deamination. Accordingly, the excision of uracil base-paired to an adenine rather than to a guanine is a major source of abasic sites in the yeast genome (Guillet and Boiteux 2003). Our lab has contributed toward estimating the number of uracils in DNA by studying the mutations originating from cytosine deamination and uracil incorporation in the S. cerevisiae model system using several well-defined mutation reporters, which can be expediently screened for mutation via a change in nutrient requirement (Kim and Jinks-Robertson 2009; Kim et al. 2011b; Owiti et al. 2018). One of these reporters, the pTET-lys2-TAG, reverts by changes to the TAG stop codon inserted in-frame into the LYS2 gene, which encodes an enzyme essential in lysine biosynthesis. The AP sites, which are generated by the excision of uracil base by Ung1 DNA glycosylase, are bypassed with the insertion of mostly Cs in the complementary strand by the TLS polymerases. At a TAG stop codon, the overall mutational output resulting from uracil, incorporated in place of thymine on either DNA strand or resulted from the cytosine deamination, are T>G/A>C or G>C, respectively (Fig. 1a). The abasic sites generated by the spontaneous loss of cytosine base can also be a contributing factor for G>C mutations. In apn1Δ background, where the error-free repair of AP lesions is largely disabled, the rate of uracil-dependent (T>G/A>C) mutations is ~20-fold higher than the cytosine-dependent (G>C) mutations (Fig. 1b, c). This dramatic difference between the uracil and the cytosine-associated mutations was consistently observed when the deoxyuridine monophosphate deaminase, Dcd1, or the putative cytosine deaminase, Fcy1, was disrupted in apn1Δ dcd1Δ or apn1Δ fcy1Δ strain.

![Fig. 1 Uracil- or cytosine-dependent mutations at the pTET-lys2-TAG reporter. a Excision of uracil from U:A base pairs (U incorporated in place of thymine) or from U:G base pairs (U generated by deamination of C) result in T>G/A>C or G>C mutations, respectively (Fig. 1a). The abasic sites generated by the spontaneous loss of cytosine base can also be a contributing factor for G>C mutations. In apn1Δ background, where the error-free repair of AP lesions is largely disabled, the rate of uracil-dependent (T>G/A>C) mutations is ~20-fold higher than the cytosine-dependent (G>C) mutations (Fig. 1b, c). This dramatic difference between the uracil and the cytosine-associated mutations was consistently observed when the deoxyuridine monophosphate deaminase, Dcd1, or the putative cytosine deaminase, Fcy1, was disrupted in apn1Δ dcd1Δ or apn1Δ fcy1Δ strain.](image-url)
respectively. In fact, the disruption of Fcy1, which was recently reported to facilitate deamination of cytosines that are located in the chromosomal DNA (Freudenreich 2018, Su and Freudenreich 2017), did not significantly change the rate of G>C mutations in apn1Δ or apn1Δ dcd1Δ background. The rates of G>C mutations were also not significantly affected by the disruption of the uracil DNA glycosylase, Ung1, whereas the rates of T>G/A>C mutations were greatly reduced in all backgrounds. Together, these data suggest that, in the context of our reporter assay, a majority of genomic uracil in yeast originate from the incorporation of uracil into DNA by DNA polymerases rather than from cytosine deamination. This finding is rather restricted by the limitation of the pTET-lys2-TAG system and needs further experiments to see if it generally applies. For example, in the reporter system used here, the only surveyed cytosine base is located on the transcribed DNA strand annealed to the reporter system used here, the only surveyed cytosine experiments to see if it generally applies. For example, in yeast cytosine deaminases. Chaudhuri et al. 2003), and this might be also true for the context of single-stranded DNA (Bransteitter et al. 2003; has a strong preference for the cytosines located within the ing transcription. The mammalian cytosine deaminase AID limitation of the of uracil into DNA by DNA polymerases rather than from cytosine deamination. This finding is rather restricted by the limitation of the pTET-lys2-TAG system and needs further experiments to see if it generally applies. For example, in the reporter system used here, the only surveyed cytosine base is located on the transcribed DNA strand annealed to the nascent mRNA and, therefore, not single-stranded during transcription. The mammalian cytosine deaminase AID has a strong preference for the cytosines located within the context of single-stranded DNA (Bransteitter et al. 2003; Chaudhuri et al. 2003), and this might be also true for the yeast cytosine deaminases.

Free dUTP pools and incorporation of uracil into DNA

In general, circumstances that would increase [dUTP]/[dTTP] ratio would increase the incorporation of uracil into DNA. Studies have shown that exogenously treating yeast cells with 5-fluorouracil (5-FU), a thymidylate synthase (TS) inhibitor, increase the amount of uracil incorporated into DNA (Owiti et al. 2018; Seiple et al. 2006). By inhibiting TS, 5-FU blocks dTTP synthesis and, therefore, increases the [dUTP]/[dTTP] ratio. Modulating the levels of enzymes involved in the dTTP biosynthesis process can also affect the [dUTP]/[dTTP] ratio and facilitate uracil incorporation. Although the dUTP pyrophosphatase Dut1 is the major enzyme responsible for the synthesis of dUMP, the obligate precursor of dTTP synthesis, dUMP can also be synthesized by another highly conserved enzyme deoxyuridylate monophosphate deaminase, Dcd1, which converts dCMP to dUMP (Wang and Weiss 1992). The dUMP production by Dcd1, albeit much less robust than that by Dut1, is sufficient in generating adequate dTTP to sustain replication (McIntosh et al. 1986). Studies in S. cerevisiae indicated that deletion of DCD1 led to a significant increase in dCTP and reduction in dTTP pool without affecting the viability of the cells (Kohalmi et al. 1991; Sanchez et al. 2012). An increase in mutagenesis derived from uracil following the deletion of DCD1 gene was previously reported and confirmed by our own investigation using the pTET-Lys2-TAG reporter (Fig. 1b, c) (Kohalmi et al. 1991; Owiti et al. 2018). The level of uracil-associated mutations in yeast cells is, therefore, highly sensitive to the fluctuation in the [dUTP]/[dTTP] ratio and likely correlates with the frequency of dUTP usage by DNA polymerases.

Non-uniform distribution of uracil in DNA

Recent studies suggest that the uracil distribution in the genome is unexpectedly non-uniform with several factors dictating what parts of the genome serve as hotspots of ura- cil. (Bryan and Hesselberth 2015; Kim and Jinks-Robertson 2009; Owiti et al. 2018; Shu et al. 2018). Earlier work using yeast genetic approach showed an increase in the rate of mutations with a distinct uracil-associated signature (A:T- to-C:G transversions). These mutations were dramatically reduced when the uracil DNA glycosylase, Ung1, was disabled or when the yeast dUTPase, Dut1, was overexpressed further supporting the hypothesis that the rate of A:T-to-C:G mutations can be correlated with the frequency of uracil in DNA. More interestingly, these mutations were almost eliminated when the transcription of the reporter gene was suppressed, indicating that transcription can dictate the extent of uracil content in the genomic DNA in a locus-specific manner.

Our recent work extended the investigation by directly quantifying uracil in DNA at several different loci in the yeast genome to confirm the previous mutagenesis experiments (Owiti et al. 2018). Because the measurement of ura- cil in DNA is complicated by the similarity of uracil and thymine, we adapted the long-amplicon qPCR approach that was successfully used to quantify DNA damage at different parts of the genome (Ayala-Torres et al. 2000; Horvath and Vertessy 2010; Hunter et al. 2010). Using the long-ampli- con qPCR technique, we revealed that, at a single gene with regulatable promoter, the uracil density varied by > twofold depending on the transcription rate (Table 1). Comparing the highly transcribed TDH3 gene to the moderately transcribed CAN1 gene also reiterated the correlation between transcription rate and the uracil density in the yeast genome. This observation of non-random distribution of uracil content has also been implied by other reports. Replication timing was indicated to be a determinant of uracil DNA content in yeast and E. coli (Bryan et al. 2014). This study showed that the early and late replication origins are completely depleted of uracil residues and that altering nucleotide bio- synthesis disrupts the regulation of uracil incorporation into DNA. Another genome-wide study of uracil content in the human genome found that uracil is not randomly distrib- uted throughout the genome and enriched in the centromere regions (Shu et al. 2018). Together with our recently published result, these studies show that a non-uniform distribution of uracil across the genome is a conserved feature from prokaryotes to metazoans. The significance of this
disproportionate pattern of uracil density and specifically
the correlation with transcription rate at the genome-wide
scale warrant further investigation.

We have also gained some insight into the mechanism
of transcription-associated elevation in uracil density. The
dUTP pool in G1 and G2 is significantly higher compared
to S phase, because the expression of dUTPase-encoding
gene is significantly induced in the S-phase, ensuring mini-
mal [dUTP] during replication (Cho et al. 1998; Ladner and
Caradonna 1997; Pardo and Gutierrez 1990). For the DNA
synthesis occurring outside S-phase, such as that associated
with repair, the available nucleotide pool has a relatively
higher [dUTP]/[dTTP], which translates into the higher risk
of incorporating uracil into DNA during the repair synthe-
sis occurring during G1 or G2. Interestingly, reducing the
[dUTP]/[dTTP] ratio by overexpressing dUTPase from G1- or
G2-specific promoters significantly reduced the rate of uracil-
associated mutations only at the highly transcribed mutation
reporter. At highly transcribed genomic loci, that is, more
frequent rounds of DNA repair synthesis occurring in G1 and
G2 could be the key events leading to the higher uracil DNA
content (see model in Fig. 2). This model of uracil incorpora-
tion during repair synthesis is further supported by the eleva-
tion in uracil-dependent mutations and uracil residues in the
genome observed when cells are treated with DNA damaging
agent CPT or 4NQO, without any reported connection to the
dUTP/dTTP metabolic pathway. Underlying this model is the
assumption that active transcription will lead to higher level of
DNA damage necessitating DNA repair synthesis in G1 and
G2. Although we were not able to directly demonstrate such
repair synthesis occurs, there are many previous reports, indi-
cating that DNA under active transcription is generally more
susceptible to base damages and other chemical modifications
(reviewed in (Jinks-Robertson and Bhagwat 2014; Kim and
Jinks-Robertson 2012)). Active transcription is also reported
to facilitate the formation of non-canonical secondary struc-
tures such as G4-DNA or R-loops, which can be recognized
as DNA damages and elicit DNA damage response (Fan et al.
2018). It would be important to determine whether DNA syn-
thesis associated with resolving such structures could also
contribute to the increased incorporation of uracil into DNA.
In addition, there are reports of a close connection between
DNA damage response/DNA repair pathways and co-tran-
scriptional, nascent RNA processing mechanisms (Mikolas-
kaova et al. 2018). Nonetheless, the analysis of unscheduled
DNA synthesis occurring at actively transcribed regions of the
genome in cells not treated with exogenous genotoxic agents
is an important and necessary next step.

### Non-uniform distribution of other non-canonical
residues in the genome

Another type of non-canonical nucleotides of interest
for the genome maintenance is ribonucleotides. Despite
the significant rNTP vs dNTP discrimination inherent in
many DNA polymerases (Williams and Kunkel 2014), the
high abundance of rNTP in the cell culminates in frequent
incorporation of ribonucleotide during replication with the
estimated rate of ~ 1,000,000 insertions per mammalian
genome (Reijns et al. 2012). Failure to remove ribonucleo-
tides from DNA, mainly carried out by RNase H2 endo-
nuclease, has been associated with an increase in genome
instability. In yeast, RNase H-deficient strain exhibited a
greatly elevated rate of spontaneous mutations consisting
of 2–5 bp deletions in repeat sequences in a Top1-depend-
ent manner (Kim et al. 2011a; Nick McElhinny et al.
2010). In addition, the accumulation of ribonucleotides in
DNA has been correlated with elevation in recombination
and gross chromosomal rearrangements in yeast and increased levels of micronuclei, chromosomal rearrangements, interchromosomal translocations, and embryonic lethality in mice (Allen-Soltero et al. 2014; Potenski et al. 2014; Reijns et al. 2012). Most recently, impairment in the ribonucleotide excision repair has been shown to be linked to the increased cytotoxicity of PARP inhibitors (Zimmermann et al. 2018). It would be of particular interest to determine whether a mechanism analogous to the uracil incorporation at highly transcribed genes through unscheduled DNA synthesis could explain the elevated levels of ribonucleotide-dependent mutations observed at highly transcribed genomic regions (Kim et al. 2011a; Nick McElhinny et al. 2010). This is especially intriguing and promising, because, similar to dUTPase, RNR1, the gene encoding a subunit of ribonucleotide reductase is tightly regulated in a cell-cycle dependent manner to ensure the maintenance of optimal (rNTP/dNTP) ratio during replication (Elledge and Davis 1990). In considering the role of ribonucleotides in promoting genome instability, their abundance in the genome, and the potential target of ribonucleotide excision repair pathway, it would be useful to understand the factors influencing their density and the distribution patterns in the genome.

### Concluding remarks

The variation in DNA content with non-canonical residues such as uracil and ribonucleotides is a major component of genomic instability. The recent finding that uracil is incorporated into DNA during non-replicative DNA synthesis likely initiated by the transcription-induced endogenous DNA damage leads to the possibility that other non-canonical DNA nucleotides such as ribonucleotides could be incorporated into the DNA by a similar mechanism. This model presents a novel mechanism to account for the variability in the chemical makeup of DNA and suggests that such replication-independent mechanism of incorporation of non-canonical residues could be an important source of mutations in non-proliferating, stationary phase cells or terminally differentiated cells such as the neurons.

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Fig. 2 Model of repair-associated incorporation of uracil into DNA
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Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

Human rights and animal participants This study did not involve any human or animal subjects and followed all ethical standards of research.

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References

Allen-Soltero S, Martinez SL, Putnam CD, Kolodner RD (2014) A Saccharomyces cerevisiae RNase H2 interaction network functions to suppress genome instability. Mol Cell Biol 34:1521–1534. https://doi.org/10.1128/MCB.00960-13
Ayala-Torres S, Chen Y, Svoboda T, Rosenblatt J, Van Houten B (2000) Analysis of gene-specific DNA damage and repair using quantitative polymerase chain reaction. Methods 22:135–147. https://doi.org/10.1006/meth.2000.1054
Bessman MJ, Lehman IR, Adler J, Zimmerman SB, Simms ES, Kornberg A (1958) Enzymatic synthesis of deoxyribonucleic acid. III. The incorporation of pyrimidine and purine analogues into deoxyribonucleic acid. Proc Natl Acad Sci USA 44:633–640
Boiteux S, Jinks-Robertson S (2013) DNA repair mechanisms and the bypass of DNA damage in Saccharomyces cerevisiae. Genetics 193:1025–1064. https://doi.org/10.1534/genetics.112.145219
Bransteitter R, Pham P, Scharff MD, Goodman MF (2003) Activation-induced cytidine deaminase deaminates deoxycytidine on single-stranded DNA but requires the action of RNase. Proc Natl Acad Sci USA 100:4102–4107. https://doi.org/10.1073/pnas.0730835100
Bryan DS, Hesselberth JR (2015) Temporal regulation of dUTP biosynthesis limits uracil incorporation during early DNA replication. bioRxiv. https://doi.org/10.1101/027508
Bryan DS, Ransom M, Adane B, York K, Hesselberth JR (2014) High resolution mapping of modified DNA nucleobases using excision repair enzymes. Genome Res 24:1534–1542. https://doi.org/10.1101/gr.174052.114
Chaudhuri J, Tian M, Khuong C, Chua K, Pinaud E, Alt FW (2003) Transcription-targeted DNA deamination by the AID anti-CD3e transmembrane domain. J Immunol 170:1069–1075
Cho RJ et al (1998) A genome-wide transcriptional analysis of the mitotic cell cycle. Mol Cell 2:65–73
el-Hajj HH, Zhang H, Weiss B (1988) Lethality of a dut (deoxyuridine triphosphatase) mutation in Escherichia coli. J Bacteriol 170:1069–1075
Elledge SJ, Davis RW (1990) Two genes differentially regulated in the cell cycle and by DNA-damaging agents encode alternative regulatory subunits of ribonucleotide reductase. Genes Dev 4:740–751
Fan Q, Xu X, Zhao X, Wang Q, Xiao W, Guo Y, Fu YV (2018) Rad5 coordinates translesion DNA synthesis pathway by recognizing specific DNA structures in Saccharomyces cerevisiae. Curr Genet 64:889–899. https://doi.org/10.1007/s00294-018-0807-y
Freudenreich CH (2018) R-loops: targets for nuclelease cleavage and repeat instability. Curr Genet 64:789–794. https://doi.org/10.1007/s00294-018-0806-z
Gadsden MH, McIntosh EM, Game JC, Wilson PJ, Haynes RH (1993) dUTP pyrophosphatase is an essential enzyme in Saccharomyces cerevisiae. Embo J 12:4425–4431
Galashevskaya A, Sarno A, Vagbo CB, Aas PA, Hagen L, Slupphaug G, Krokan HE (2013) A robust, sensitive assay for genomic uracil determination by LC/MS/MS reveals lower levels than previously reported. DNA Repair (Amst) 12:699–706. https://doi.org/10.1016/j.dnarep.2013.05.002
Guillet M, Boiteux S (2003) Origin of endogenous DNA abasic sites in Saccharomyces cerevisiae. Mol Cell Biol 23:8386–8394
Horvath A, Vertessy BG (2010) A one-step method for quantitative determination of uracil in DNA by real-time. PCR Nucleic Acids Res 38:e196. https://doi.org/10.1093/nar/gkq815
Hunter SE, Jung D, Di Giulio RT, Meyer JN (2010) The QPCR assay for analysis of mitochondrial DNA damage, repair, and relative copy number. Methods 51:444–451. https://doi.org/10.1016/j.ymeth.2010.01.033
Jinks-Robertson S, Bhagwat AS (2014) Transcription-associated mutagenesis. Annu Rev Genet 48:341–359. https://doi.org/10.1146/annurev-genet-120213-092015
Kaval B, Otterlei M, Slupphaug G, Krokan HE (2007) Uracil in DNA—general mutagen, but normal intermediate in acquired immunity. DNA Repair (Amst) 6:505–516. https://doi.org/10.1016/j.dnarep.2006.10.014
Kim N, Jinks-Robertson S (2009) dUTP incorporation into genomic DNA is linked to transcription in yeast. Nature 459:1150–1153. https://doi.org/10.1038/nature08033
Kim N, Jinks-Robertson S (2012) Transcription as a source of genome instability. Nat Rev Genet 13:204–214. https://doi.org/10.1038/nrg3152
Kim N et al (2011a) Mutagenic processing of ribonucleotides in DNA by yeast topoisomerase I. Science 332:1561–1564. https://doi.org/10.1126/science.1205016
Kim N, Mudrak SV, Jinks-Robertson S (2011b) The dCMP transferase activity of yeast Rev1 is biologically relevant during the bypass of endogenously generated AP sites. DNA Repair (Amst) 10:1262–1271. https://doi.org/10.1016/j.dnarep.2011.09.017
Kohalmi SE, Glattke M, McIntosh EM, Imbal BA (1991) Mutational specificity of DNA precursor pool imbalances in yeast arising from deoxyguanylidylate deaminase deficiency or treatment with thymidylate. J Mol Biol 220:933–946
Ladner RD, Caradonna SJ (1997) The human dUTPase gene encodes both nuclear and mitochondrial isoforms. Differential expression of the isoforms and characterization of a cDNA encoding the mitochondrial species. J Biol Chem 272:19072–19080
Lindahl T (1993) Instability and decay of the primary structure of DNA. Nature 362:709–715. https://doi.org/10.1038/362709a0
McIntosh EM, Gadsden MH, Haynes RH (1986) Transcription of genes encoding enzymes involved in DNA synthesis during the cell cycle of Saccharomyces cerevisiae. Mol Gen Genet 204:363–366
Mikolaskova B, Jurcik M, Cipakova I, Kretova M, Chovanec M, Cipak L (2018) Maintenance of genome stability: the unifying role of interconnections between the DNA damage response and RNA-processing pathways. Curr Genet 64:971–983. https://doi.org/10.1007/s00294-018-0819-7
Nick McElhinny SA et al (2010) Genome instability due to ribonucleotide incorporation into DNA. Nat Chem Biol 6:774–781. https://doi.org/10.1038/nchembio.424
Owiti N, Wei S, Bhagwat AS, Kim N (2018) Unscheduled DNA synthesis leads to elevated uracil residues at highly transcribed

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genomic loci in *Saccharomyces cerevisiae*. PLoS Genet 14:e1007516. [https://doi.org/10.1371/journal.pgen.1007516](https://doi.org/10.1371/journal.pgen.1007516)

Pardo EG, Gutierrez C (1990) Cell cycle- and differentiation stage-dependent variation of dUTPase activity in higher plant cells. Exp Cell Res 186:90–98

Potenski CJ, Niu H, Sung P, Klein HL (2014) Avoidance of ribonucleotide-induced mutations by RNase H2 and Srs2-Exo1 mechanisms. Nature 511:251–254. [https://doi.org/10.1038/nature13292](https://doi.org/10.1038/nature13292)

Reijns MA et al (2012) Enzymatic removal of ribonucleotides from DNA is essential for mammalian genome integrity and development. Cell 149:1008–1022. [https://doi.org/10.1016/j.cell.2012.04.011](https://doi.org/10.1016/j.cell.2012.04.011)

Sanchez A, Sharma S, Rozenzhak S, Roguev A, Krogan NJ, Chabes A, Russell P (2012) Replication fork collapse and genome instability in a deoxycytidy late deaminase mutant. Mol Cell Biol 32(21):4445–4454. [https://doi.org/10.1128/MCB.01062-12](https://doi.org/10.1128/MCB.01062-12)

Seiple L, Jaruga P, Dizdaroglu M, Stivers JT (2006) Linking uracil base excision repair and 5-fluorouracil toxicity in yeast. Nucleic Acids Res 34:140–151. [https://doi.org/10.1093/nar/gkj430](https://doi.org/10.1093/nar/gkj430)

Shu X et al (2018) Genome-wide mapping reveals that deoxyuridine is enriched in the human centromeric DNA. Nat Chem Biol 14:680–687. [https://doi.org/10.1038/s41589-018-0065-9](https://doi.org/10.1038/s41589-018-0065-9)

Siriwardena SU, Chen K, Bhagwat AS (2016) Functions and malfunctions of mammalian DNA-cytosine deaminases. Chem Rev 116:12688–12710. [https://doi.org/10.1021/acs.chemrev.6b00296](https://doi.org/10.1021/acs.chemrev.6b00296)

Su XA, Freudenreich CH (2017) Cytosine deamination and base excision repair cause R-loop-induced CAG repeat fragility and instability in *Saccharomyces cerevisiae*. Proc Natl Acad Sci USA 114:E8392–E8401. [https://doi.org/10.1073/pnas.1711283114](https://doi.org/10.1073/pnas.1711283114)

Wang L, Weiss B (1992) dcd (dCTP deaminase) gene of *Escherichia coli*: mapping, cloning, sequencing, and identification as a locus of suppressors of lethal dut (dUTPase) mutations. J Bacteriol 174:5647–5653

Warner HR, Duncan BK, Garrett C, Neuhard J (1981) Synthesis and metabolism of uracil-containing deoxyribonucleic acid in *Escherichia coli*. J Bacteriol 145:687–695

Williams JS, Kunkel TA (2014) Ribonucleotides in DNA: origins, repair and consequences. DNA Repair (Amst) 19:27–37. [https://doi.org/10.1016/j.dnarep.2014.03.029](https://doi.org/10.1016/j.dnarep.2014.03.029)

Zimmermann M et al (2018) CRISPR screens identify genomic ribonucleotides as a source of PARP-trapping lesions. Nature 559:285–289. [https://doi.org/10.1038/s41586-018-0291-z](https://doi.org/10.1038/s41586-018-0291-z)