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The cutting edges in DNA repair, licensing, and fidelity: DNA and RNA repair nucleases sculpt DNA to measure twice, cut once

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ABSTRACT

To avoid genome instability, DNA repair nucleases must precisely target the correct damaged substrate before they are licensed to incise. Damage identification is a challenge for all DNA damage response proteins, but especially for nucleases that cut the DNA and necessarily create a cleaved DNA repair intermediate, likely more toxic than the initial damage. How do these enzymes achieve exquisite specificity without specific sequence recognition or, in some cases, without a non-canonical DNA nucleotide? Combined structural, biochemical, and biological analyses of repair nucleases are revealing their molecular tools for damage verification and safeguarding against inadvertent incision. Surprisingly, these enzymes also often act on RNA, which deserves more attention. Here, we review protein-DNA structures for nucleases involved in replication, base excision repair, mismatch repair, double strand break repair (DSBR), and telomere maintenance: apurinic/apyrimidinic endonuclease 1 (APE1), Endonuclease IV (Nfo), tyrosyl DNA phosphodiesterase (TDP2), UV Damage endonuclease (UVDE), very short patch repair endonuclease (Vsr), Endonuclease V (Nfi), Flap endonuclease 1 (FEN1), exonuclease 1 (Exo1), RNase T and Meiotic recombination 11 (Mre11). DNA and RNA structure-sensing nucleases are essential to life with roles in DNA replication, repair, and transcription. Increasingly these enzymes are employed as advanced tools for synthetic biology and as targets for cancer prognosis and interventions. Currently their structural biology is most fully illuminated for DNA repair, which is also essential to life. How DNA repair enzymes maintain genome fidelity is one of the DNA double helix secrets missed by James Watson and Francis Crick, that is only now being illuminated though structural biology and mutational analyses. Structures reveal motifs for repair nucleases and mechanisms whereby these enzymes follow the old carpenter adage: measure twice, cut once. Furthermore, to measure twice these nucleases act as molecular level transformers that typically reshape the DNA and sometimes themselves to achieve extraordinary specificity and efficiency.

Abbreviations: BER, base excision repair; MMR, mismatch repair; DSBR, double strand break repair; Ss, single stranded; Ds, double stranded; APE1, apurinic/apyrimidinic endonuclease 1; Nfo, Endonuclease IV; TDP2, tyrosyl DNA phosphodiesterase 2; UVDE, UV damage endonuclease; Vsr, very short patch repair endonuclease; Nfi, Endonuclease V; Mre11, meiotic recombination 11; FEN1, flap endonuclease 1; Exo1, exonuclease 1; Dcm, DNA-cytosine methyltransferase; 6-4PP, (6-4) photoproduct or (6,4) pyrimidine-pyrimidones; CPD, cyclobutane pyrimidine dimer; NIR, nucleotide incision repair; Lig1, ligase 1; DBD, DNA binding domain.

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1. Introduction

The discovery of the double helix transformed biology and opened the doors for molecular biology and the field of genetics. However, DNA repair was not considered. Francis Crick wrote in 1974, "We totally missed the possible role of enzymes in repair although due to Claud Rupert's early very elegant work on photoreactivation, I later came to realize that DNA is so precious that probably many distinct repair mechanisms would exist." [1]. DNA nucleases are essential players in DNA repair. For DNA, nucleases are a necessary evil. DNA damage needs to be trimmed off or removed, and this removal needs to be done both efficiently and accurately. Small errors in the substrate recognition or location of the incision can be deleterious to the cell and cause genomic instability. This review examines how nucleases ensure not only they have bound the correct substrate, but also that they do not bind and cut the wrong substrate. Here, we focus on DNA repair phosphoesterases that leave a 5′ phosphate and a 3′ hydroxyl suitable for polymerase extension and ligation. In particular, we analyze those whose structures have been determined with substrate and/or product DNA: apurinic/apyrimidinic endonuclease 1 (APE1), Endonuclease IV (Nfo), tyrosyl DNA phosphodiesterase (TDP2), UV Damage endonuclease (UVDE), very short patch repair endonuclease (Vsr), Endonuclease V (Nfi), Flap endonuclease 1 (FEN1), exonuclease 1 (Exo1), RNase T and Meiotic recombination 11 (Mre11). There is now a sufficient number of enzymes meeting this criteria that useful insights emerge, and these insights have general importance. For the eukaryotic enzymes, we also include an examination of motifs that can be used to identify mechanistically similar nucleases. These enzymes are central to cell biology: they act in replication, base excision repair (BER), mismatch repair (MMR) double strand break repair (DSBR), and telomere maintenance. Furthermore they are increasingly found to act on RNA as well as DNA, and these activities may well be important as well.

Some of these nucleases are endonucleases that make a single cut within the DNA and some are exonucleases that processively cut from a DNA end, but some fall into both categories. The "restriction nuclease" discovered by Stuart Linn and Werner Arber [2,3] provided breakthroughs in genetics because they provided enzymatic tools needed to "cut and paste" DNA molecules. Their specificity was based upon methylation or specific sequences, and thus they are site-specific nucleases. For damaged DNA, the discoveries of nucleotide excision repair and transcription-coupled repair pioneered by Phil Hanawalt and others sparked a dramatic evolution in our understanding of DNA and molecular biology by revealing the intriguing systems of DNA repair essential to life plus sets of nucleases needed for the cut-and-patch repair that are specific to DNA structure rather than sequence [4–8]. Thus, DNA damage repair nucleases have a different challenge than restriction nucleases with targeted sequences for incision. Although some recognize a modified base or phosphate backbone, others must recognize their substrates containing canonical nucleotides in an aberrant structure. The structure-specific nucleases in this review therefore provide a paradox of both extreme specificity and the lack of any sequence dependence with broad implications. For biotechnology, they can provide powerful tools to probe and modify DNA structure, as seen for FEN1 [9,10]. Biochemically, if misregulated, they would destroy the integrity of genomic information. Biologically, they are necessary to preserving genome integrity and life itself.

How are these nucleases regulated? What is the basis for their exquisite specificity? Nuclease cutting is a committed step and thus tightly regulated. Structural biology provides key knowledge to address specificity questions and to contribute to a more complete and detailed understanding of their activities and biological functions. Particularly for these nucleases, structures furthermore provide detailed and rigorous information with which all other data should be reconciled and that often allows the integration of biochemical and genetic results. Examining the existing structures provides a basis to design mutants and inhibitors for separation of functions as seen for Mre11 [11,12]. Yet, structures provide key knowledge not only to design mutations and inhibitors but also to interpret the impact of disease-causing mutations, as seen for XPD helicase [13], and the likelihood that polymorphisms may impact risks. As we come to understand DNA repair networks as more accurate than classical linear pathway concepts, we wish to control pathway choice and network crosstalk and interactions for biology and medicine. A detailed structural and mechanistic understanding of structure-specific nucleases, which is the focus of this review, is key to this goal. Increasingly we are finding that repair nuclease function requires changes in protein and DNA architecture that impacts binding, activity, and partner recruitment. Furthermore, flexible components (intrinsically unstructured regions) reshape or fold themselves in the presence of target DNA, as shown for FEN1 and its family members such as XPG [14–17]. In essence these nucleases behave like molecular level transformers that can rebuild themselves by sometimes altering their protein conformations and typically sculpting the DNA to control both their specificity and efficiency functions. This knowledge suggests we need to re-think our understanding and the classic lock and key concept of how interactions, specificity, and activity are regulated with implications for inhibitor design.

2. Cell biology of DNA repair nucleases and increasing role as therapeutic targets

DNA repair nucleases permeate every DNA repair and processing pathway and are essential to the cell (Fig. 1). Damaged DNA can form spontaneously from endogenous metabolic sources, exogenously by DNA damaging agents (chemicals, radiation), or are intermediates from other repair or DNA processing enzymes. Damaged DNA must be incised from the DNA strand to prevent errors in coding or regulatory regions, to prevent mutations during replication, and to maintain genomic stability. Additionally, damaged DNA can often arrest RNA polymerase, setting the cell on a path towards apoptosis [18]. Thus, nucleases play a crucial role in removing the damaged DNA.

Many DNA repair nucleases are essential for the cell. Homozygous null mutations are often cellular or embryonic lethal. Single site mutations are associated with increased risk for cancer, ageing, and neurological diseases. Once cancer has occurred, these enzymes may become upregulated and provide cancer cells resistance to DNA damaging treatments such as chemotherapy and radiation treatments. Thus, many of these nucleases have become targets for developing inhibitors that can lead to sensitizing cancer cells to DNA damaging treatments. Three DNA repair nucleases, APE1, TDP2, and FEN1, which have been particularly well-studied, will be reviewed as typifying examples.

Apurinic/apyrimidinic endonuclease 1 (APE1) acts on abasic sites that form spontaneously or are repair intermediates from BER glycosylases [19–24]. It is estimated that as many as 10,000 abasic sites are formed in one cell, each day in humans [25]. APE1 null mice are embroynic lethal [22,26,27], and heterozygous mice showed increased tumor susceptibility [28]. In humans, some APE1 mutations have been associated with amyotrophic lateral sclerosis (ALS) [29,30] and endometrial cancers [31]. On the flip side, APE1 activity gives cells increased survival after radiation, oxidative stress, and chemotherapy, making it a drug target; down-regulation of APE1 can lead to increased sensitivity of tumor cells to various cancer treatments, reviewed in [19,23,32–34].

The duality of nucleases both preventing cancer, but also sustaining cancer once it has started is also true for FEN1. FEN1 incises