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Base excision repair in chromatin: Insights from reconstituted systems

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ABSTRACT

The process of base excision repair has been completely reconstituted *in vitro* and structural and biochemical properties of the component enzymes thoroughly studied on naked DNA templates. More recent work in this field aims to understand how BER operates on the natural substrate, chromatin [1,2]. Toward this end, a number of researchers, including the Smerdon group, have focused attention to understand how individual enzymes and reconstituted BER operate on nucleosome substrates. While nucleosomes were once thought to completely restrict access of DNA-dependent factors, the surprising finding from these studies suggests that at least some BER components can utilize target DNA bound within nucleosomes as substrates for their enzymatic processes. This data correlates well with both structural studies of these enzymes and our developing understanding of nucleosome conformation and dynamics. While more needs to be learned, these studies highlight the utility of reconstituted BER and chromatin systems to inform our understanding of *in vivo* biological processes.

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1. Prologue

I first met Mick Smerdon in the mid 1990's when he and Ray Reeves invited me for a seminar, not long after I began my independent position at Rochester. On the long trip to Pullman I re-read through my large file of Smerdon papers and was reminded of the breadth and quality of Mick's work, both *in vitro* and *in vivo*, from studies of chromatin repair nucleosome dynamics in UV irradiated cells to investigations of how the wrapping of DNA around nucleosomes influenced the formation of specific types of damage. Mick's exacting quantitative and physical approach in biological systems has provided a seminal roadmap for many young scientists focusing on factors and mechanisms in the DNA damage and repair field. Since then, it has always been a great pleasure to discuss science

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with Mick, whether in Pullman, where I have been fortunate to visit numerous times, in Rochester, where Mick generously provided the Plenary Lecture at our Epigenetics and Genome Stability retreat, at many meetings, or by phone. In addition, I have especially appreciated Mick's friendship, valuable advice, and support through the years—and to hope to continue to enjoy these for many years to come!

2. Introduction

DNA in the cell is constantly damaged from both exogenous and endogenous sources, which can result in chemical modification of DNA, leading to stable mutations [3–5]. The cell employs a variety of mechanisms to repair DNA damage or moderate its mutagenic effects and maintain genomic integrity. Base excision repair (BER), a front-line defense in the repair of damaged bases, excises chemically modified DNA bases that generally do not cause large distortions to the DNA helix [6,7]. BER corrects an estimated 10,000 lesions/cell/day and can be reconstituted in simplest form by just four enzymes [2,8]. BER typically is initiated by a DNA glycosylase that recognizes a specific type of damaged, misincorporated, or missing (depurinated) base (Table 1). For example, Family 1 uracil DNA glycosylases (UDGs) specifically remove uracil residues that were misincorporated during DNA replication leading to A–U matches, or generated by deamination of cytosine leading to G–U mismatches. Aberrant uracil in DNA is estimated to occur hundreds of times per cell per day and can result in G:C to A:T transitions, cytotoxic/mutagenic abasic (AP) sites, or changes to transcription due to inhibition of DNA methylation [9,10]. UDGs have very low activity on uracil in RNA, or cytosine or thymine in DNA [11,12]. In addition, UDGs play a critical role in generating DNA strand breaks during immunoglobulin gene rearrangement and maturation after enzymatic cystosine deamination to produce G:U mismatches by activation-induced deaminases in B cells [13,14].

After recognition of a chemically altered or aberrant base, the specific glycosylase, catalyzes a nucleophilic attack by a water molecule at the glycosidic bond, resulting in cleavage of the base—sugar bond, creating an apurinic/apyrimidinic (AP) site in the DNA and releasing the damaged/aberrant base [7,15,16]. The backbone at the AP site is either then cleaved by a separate AP endonuclease (APE) activity, or is first cleaved by a glycosylase possessing bifunctional activity, followed by APE cleavage to remove the resulting aldehyde residue. Cleavage results in a 3′-hydroxyl end and a 5′-deoxyribose phosphate (dRP), which can then undergo either short-patch (SP-), or long-patch base excision repair (LP-BER). In short-patch repair, a DNA polymerase with an associated dRP lyase activity inserts a single base, while in long-patch repair a DNA polymerase adds 2–10 nucleotides and displaces a ssDNA flap containing the 5′-dRP, which is subsequently cleaved off by flap endonuclease 1 (FEN1). Both pathways result in a nick that is sealed by DNA ligase [7,8]. The BER pathway has been entirely reconstituted with purified components *in vitro* on free DNA substrates. In this review we will consider characterizations of the activity of the major enzymes involved in BER on reconstituted chromatin substrates *in vitro*.

In vitro characterizations of the activities of the component enzymes involved in BER on well-defined chromatin complexes reconstituted from purified components has been critical in understanding how BER occurs on chromatin substrates *in vivo*. The basic repeating subunit of chromatin is the nucleosome, consisting of ∼147 bp of DNA wrapped ∼1.7 times around a protein spool consisting of two copies each of the four core histones H2A, H2B, H3, and H4 [17,18]. The nucleosome unit also includes 10–80 bps of linker DNA that links cores together to form chromosome-sized oligonucleosome arrays that fold and condense into chromatin fibers and higher order chromatin structures. Typically each nucleosome repeat is bound by one linker histone (*e.g.* H1) and non-histone chromosomal proteins (NHCPs) that modulate the folding and condensation of nucleosomes into higher order structures present in the cell nucleus [17].

Nuclease digestion studies indicate that the core region is relatively resistant to cleavage due to tight association with the core histones, while the linker DNA is relatively accessible [17]. Likewise, accessibility of nucleosome core DNA is highly restrictive to most DNA-binding factors, including those involved in DNA repair. For example, the activities of factors involved in nucleotide excision repair (NER) are greatly inhibited by chromatin, and thus, ATP-dependent chromatin remodeling activities are required for efficient NER [19–23]. However, remodeling processes impose energetic costs to the cell and must be targeted to specific damage locations or at specific times within the cell cycle when access to the DNA is required [19,24]. Conversely, some BER-associated enzymes appear to exhibit significant activity on nucleosome substrates *in vitro*, suggesting that some BER events in cells do not require chromatin remodeling. This review will focus on investigations of the activities of the components of the BER pathway on model chromatin substrates.

3. DNA glycosylases

3.1. Nucleotide excision by DNA glycosylases

The recognition and excision of damaged bases by DNA glycosylases is a critical first step in the base excision repair pathway. Perhaps the best characterized is Uracil DNA glycosylase (UDG), which is conserved from bacteria to humans and serves as a prototypical example of this type of enzyme [15]. UDG has been extensively characterized by X-ray crystallographic studies and biochemical analyses [15,25–29]. Early UDG crystal structures showed a conically shaped basic channel along one surface with a pocket at the end, suggested to be the damaged duplex DNA-binding site and the active site, respectively [25,26]. Since uracil was observed to specifically bind within the pocket, substrate recognition was suggested to occur via a base "flipping out" mechanism, similar to that observed for DNA methylases, requiring disruption of canonical base-pairing in order for the uracil to adopt an extrahelical orientation [25,26]. Later crystal structure studies with UDG in complex with DNA substrates and products substantiated this mechanism, showing that UDG undergoes an open-to-closed structural transition upon binding its target. It was also shown that conserved residues within UDG probe for uracil by binding to primarily the damaged DNA strand and pinching the DNA backbone at successive phosphates, causing disruption of intra-helical uracil base-pairing [15,28]. In addition, the insertion of a leucine side chain that helps push the uracil base toward the uracil-binding pocket and intercalates into the void, along with the attraction of the highly specific uracil binding pocket, leads to the "flipped out" conformation and hydrolysis of the glycosidic bond. This mechanism appears to be conserved in other DNA glycosylases that use 'helix-invading' residues to distort the DNA helix, destabilize damaged bases, and flip them into extra-helical orientations for excision [30,31].

In most cases, binding of DNA glycosylases to target DNA causes a bending of the DNA sequence away from the body of the enzyme. For example, bacterial UDG bends DNA in a 45° angle tangentially away from the enzyme, while the enzyme hOGG1, a glycosylase that initiates the repair of 7,8-dihydro-8-oxoguanine (oxoG) in DNA, causes a sharp, 70° bend in the target DNA directly away from the enzyme [28,32]. Considering this feature, and the fact that DNA in eukaryotic cells is highly bent around nucleosomes, researchers

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