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Histone modification and chromatin remodeling during NER

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ABSTRACT

Here we review our developments of and results with high resolution studies on global genome nucleotide excision repair (GG-NER) in *Saccharomyces cerevisiae*. Technologies were developed to examine NER at nucleotide resolution in yeast sequences of choice and to determine how these related to local changes in chromatin. We focused on how GG-NER relates to histone acetylation for its functioning and we identified the histone acetyltransferase Gcn5 and acetylation at lysines 9/14 of histone H3 as a major factor in enabling efficient repair. Factors influencing this Gcn5-mediated event are considered which include Rad16, a GG-NER specific SWI/SNF factor and the yeast histone variant of H2AZ (Htz1). We describe results employing primarily *MFA2* as a model gene, but also those with *URA3* located at subtelomeric sequences. In the latter case we also see a role for acetylation at histone H4. We then consider the development of a high resolution genome-wide approach that enables one to examine correlations between histone modifications and the NER of UV-induced cyclobutane pyrimidine dimers throughout entire yeast genome. This is an approach that will enable rapid advances in understanding the complexities of how compacted chromatin in chromosomes is processed to access DNA damage before it is returned to its pre-damaged status to maintain epigenetic codes.

Contents

1. Introduction

The seminal research of Smerdon [1–3] and Thoma [3,4], and the fact that in vitro core nucleotide excision repair (NER) factors could not repair DNA damage in chromatin [5][reviewed in 5], showed that chromatin influences how NER operates in eukaryotic cells. Results from Ramanathan and Smerdon showed that in mammalian cells there was a role for histone acetylation in NER [1,2] and those from the Thoma group, primarily working with yeast, indicated that linker DNA between nucleosomes was repaired more rapidly than the core DNA residing within nucleosomes [3,4]. These seminal experiments showed that chromatin structure impinged on the efficiency of NER.

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In this review we will focus on work from our own groups, as other reviews in this edition are covering various additional aspects of how chromatin impinges on NER and the review by House et al. [5] elegantly covers the broader aspects of this topic. We have had a long standing interest in yeast global genome NER [7–24].

NER has two sub-pathways, transcription coupled NER (TC-NER) and global genome NER (GG-NER) [6][reviewed in 6]. TC-NER uniquely operates on the transcribed strand of transcriptionally active genes and is triggered when the RNA polymerase during transcription is stalled by DNA damage. GG-NER operates on the transcribed strand of genes where the region is not being actively transcribed by RNA polymerase, as well as on the non-transcribed strand of genes, plus on all transcriptionally silent regions of the genome. These sub-pathways differ only in the means of detecting DNA damage. GG-NER in *Saccharomyces cerevisiae* relies on a GG-NER-specific complex that is composed of Rad16/Rad7 and the autonomously replicating sequence binding factor I (Abf1) [21,22]. Following damage recognition the subsequent steps in NER appear to be the same between TC- and GG-NER.

As stated above, our research has focussed on GG-NER [6]. Rad16 is a member of the SWI/SNF super-family of chromatin remodelling factors [25]. This superfamily of proteins exhibits ATPase activity that is stimulated by DNA or chromatin [26,27,28], and all SWI/SNF-like proteins generate superhelical tension in linear DNA fragments via a DNA translocase activity associated with their ATPase function [28,29]. The generation of superhelicity in DNA is a common mechanism of SWI/SNF-like chromatin remodelling complexes for altering chromatin structure [28]. We reported that the Rad7 and Rad16 containing GG-NER protein complex also has DNA translocase activity. However, unlike many SWI/SNF superfamily complexes, it is unable to slide nucleosomes [23]. Rad7 and Rad16 form a stoichiometric complex [21,30] that binds damaged DNA in an ATP-dependent manner [31]. Rad7 is part of an E3 ligase complex that ubiquitinates Rad4, a core yeast NER protein that binds to damaged DNA [24]. This ubiquitination of Rad4 in response to UV specifically regulates NER via a pathway that requires de novo protein synthesis and it directly influences NER and UV survival [24]. Abf1 was originally identified for its ability to bind DNA at a variety of origins of DNA replication, as well as the silencing loci *HML* and *HMR* [32]. A plethora of literature subsequently identified the ability of Abf1 to bind within the upstream activating sequences (UASs) of a large array of gene promoters. It is well established that Abf1 is an abundant, essential, global site-specific DNA binding protein [33]. A role for Abf1 in GG-NER came from the observation that the protein co-purifies with Rad7. In the absence of UV damage, Abf1 forms a stable heterotrimeric complex with Rad7 and Rad16, termed the GG-NER complex. Approximately one third of cellular Abf1 is predicted to be in a complex with Rad7 and Rad16 [21].

In the mid-1990s we had decided to focus our research on unravelling how histone modification and chromatin remodelling impinged on NER. Rather than attempting this in the context of complex human cells we employed the yeast *S. cerevisiae* as a model organism. This yeast is genetically tractable, it has NER genes which bear considerable homology to those in humans, and there are cells available that harbour a broad range of mutations in genes with roles in NER and chromatin modifications [6][reviewed in 6]. We used UV irradiation at 260 nm to damage DNA and we concentrated on the repair of cyclobutane pyrimidine dimers (CPDs). These are the most frequent lesions induced by this treatment, their change in frequency has been used regularly to examine how NER operates, and they can be detected by enzymes that cut DNA at the CPDs or via antibodies to precipitate DNA that contains them [6][reviewed in 6].

As chromatin structure varied within genomes it was essential to study DNA damage and repair in specific genomic regions and where we could cross reference to particular histone modifications and the chromatin remodelling of specific nucleosomes. Methods that measured damage in total cellular DNA or even in specific genes, would divulge little with respect to our goals. In the early 1990s there were two published approaches to examine DNA damage at specific nucleotides [34,35]. However, these methods were technically demanding and they were not really suitable for the regular rapid estimation of CPDs in yeast.

As a result we began by developing technologies to address specific questions related to how NER operates on chromatin in *S. cerevisiae* exposed to DNA damaging agents. Initially we developed an approach to quantify the frequency of individual CPDs at any location in any selected sequence. This method was partly based on that of Kunala and Brash [34], but it was extensively modified to expedite the consistent analysis of CPDs. A key facet of our modification entailed a novel way of enriching damaged DNA fragments for the consistent analysis and quantification of CPDs at specific dipyrimidines in a sequence of choice, and it employed DNA sequencing gels. We first developed this method with *Escherichia coli* [7] and subsequently we modified it for use with yeast [8]. The approach employed probes to separate from the rest of the yeast genome specific yeast sequences isolated as individual single strands which could then be radiolabelled for the quantification of fragments. CPDs within these sequences were detected by virtue of cutting at CPDs with a CPD-specific DNA glycosylase. This reduced the migration of the labelled strands in alkaline agarose gels. We could not only measure the frequency of the induction of individual CPDs in the sequence of choice but also the changes in their frequency during DNA repair by quantifying the amount of radiolabel in each band on these gels. Changes in band intensity due to DNA repair were measurable to quantify NER rates [7–15,17–21].

We also needed to be able to determine where specific nucleosomes were located within the yeast sequences of choice where DNA damage and repair was being estimated. We adapted the method for detecting CPDs at nucleotide resolution for the high resolution foot printing of yeast nucleosomes [16]. Here the accessibility of the DNA in chromatin to cutting by MNase was used and sequences isolated radiolabelled and analysed as for CPD detection. Thus we were able to map nucleosome positions to within a few base pairs in the selected sequences (see later). This development enabled us to use IP and RT-PCR to examine covalent modification of histones in specific placed nucleosomes in order to see if they are modified after DNA damage and repair. This would enable us to identify enzymes with roles in these modifications [12,13,17–21].

This development also meant that we could identify restriction sites within nucleosome cores to analyze their accessibility in chromatin before and after DNA damage [17,20]. This accessibility can depend on SWI/SNF factors which use ATP to remodel chromatin and which can move nucleosomes in *cis* or *trans* [36,37]. Hence we had developed a series of methods that would enable us to examine how chromatin was modified after DNA damage and how this impinged on NER.

We first discovered that UV induced histone H3 acetylation at certain repressed yeast genes occurs via the Gcn5 histone acetyltranferase (HAT) [12,17–21]. Gcn5 was first identified as a histone acetyltransferase that has a role in regulating the transcription of numerous yeast genes. We showed that Gcn5 is essential for efficient NER at some yeast genes but not at others, and that this role is independent of Gcn5s' role in transcription [17]. These experiments are described and discussed in the following section.

In summary, here we will describe our studies on the GG-NER of CPDs from specific cellular genes and subsequently throughout the entire yeast genome. We have uncovered some covalent histone modifications that are linked to efficient GG-NER, determined how these relate to SWI/SNF activity and determined that they are facilitated by the GG-NER specific complex of Rad16/Rad7 and Abf1. These observations have led us to propose a model suggesting how