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Nucleotide Excision Repair in *S. cerevisiae*

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1. Introduction

Each day organisms are faced with a barrage of genomic insults which damage and jeopardize the integrity of DNA (Lindahl and Wood 1999). DNA damage stems from both endogenous sources such as water and reactive oxygen species generated by regular cellular metabolism and exogenous sources such as sunlight, chemicals, and tobacco smoke. These DNA damaging agents can cause various types of genomic damage including base losses and modifications, strand breaks, crosslinks, bulky chemical adducts, and other DNA alterations. These genomic insults alter the chemistry of DNA and can accumulate and become mutagenic and/or cytotoxic. At the cellular level, DNA damage that is undetected or left unrepaired can result in genomic instability, apoptosis, or senescence, which can greatly affect the aging and development processes. At the level of the organism, genetic instability can predispose the organism to immunodeficiency, neurological disorders, and cancer, illustrating the need to understand the molecular basis of mutagenesis and the mechanisms of DNA repair.

In an effort to maintain the integrity of the genome, evolution has led cells to develop an elaborate DNA damage response system to counteract potentially mutagenic and cytotoxic genomic insults. This highly evolutionarily conserved system is made up of multiple DNA repair pathways, each focusing on a specific category of lesion, as well as multiple checkpoint, signal transduction, and effector systems which crosstalk with replication, transcription, recombination, and chromatin remodeling in order to control DNA damage (Harper and Elledge 2007; Hoeijmakers 2009). The complexity of and the energetic expense dedicated by cells to this process underscores the importance of preserving genomic integrity (Hoeijmakers 2009).

One of the various DNA repair pathways cells have at their employ is the highly conserved nucleotide excision repair (NER), which is the most versatile repair mechanism in terms of lesion recognition [for a recent review, see (Nouspikel 2009)]. NER deals with a wide class of bulky, helix-distorting lesions that generally obstruct transcription and normal replication, such as UV-induced cyclobutane pyrimidine dimers (CPDs) and 6-4 photoproducts [(6-4)PP], as well as adducts and crosslinks induced by chemical agents (e.g. benzo[a]pyrene and cisplatin). NER was first discovered in bacteria in the mid-1960s by Philip Hanawalt and David Pettijohn with the observation of non-semiconservative DNA synthesis during the excision of CPDs (Pettijohn and Hanawalt 1964). Almost simultaneously, excision repair of UV-induced DNA damage was identified in mammalian cells (Rasmussen and Painter 1964).

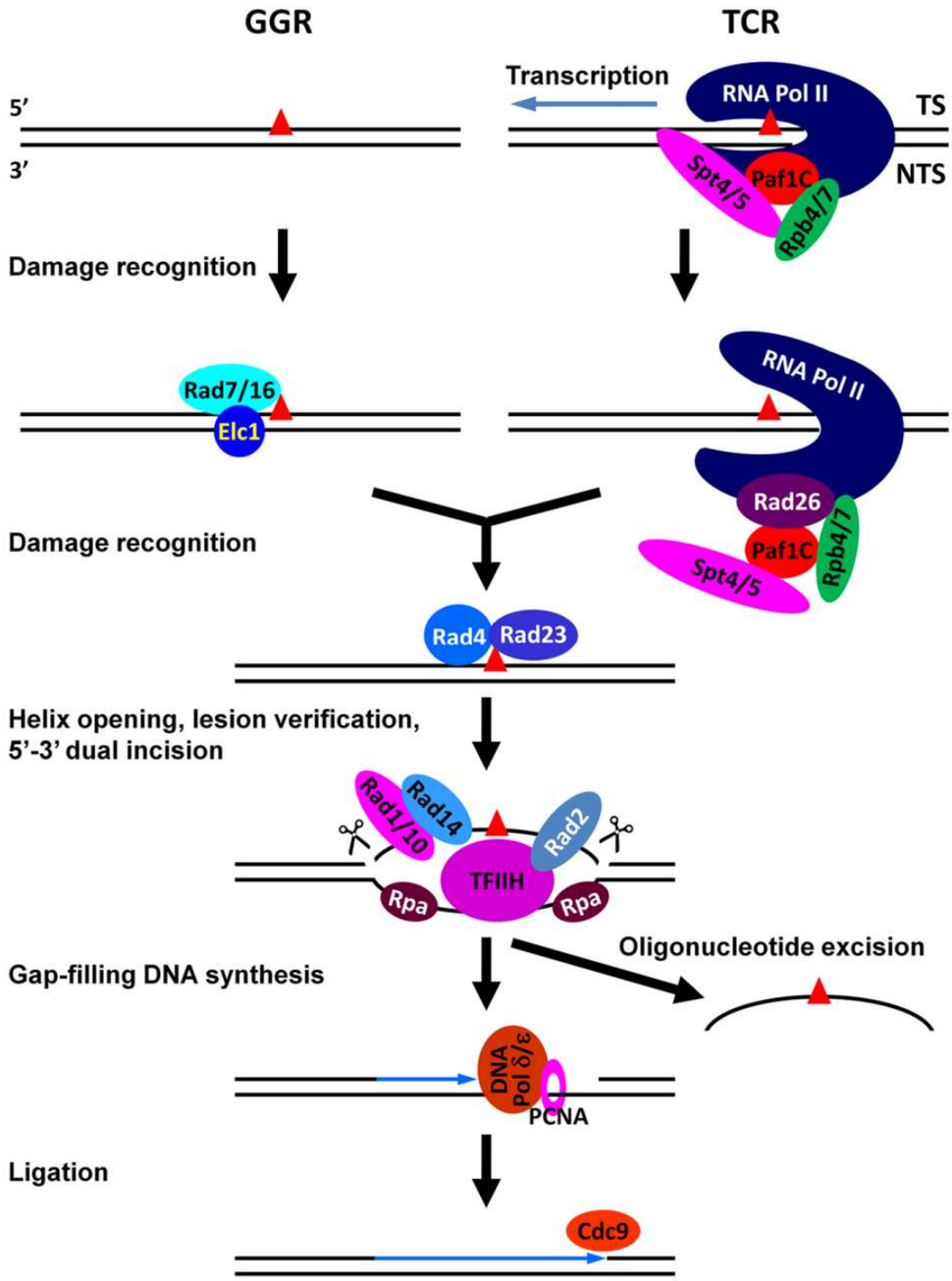


Fig. 1. The process of NER in *S. cerevisiae*. Red triangle denotes a DNA lesion. TS, transcribed strand; NTS, nontranscribed strand.

NER is a multistep reaction which includes damage recognition, helix opening, lesion verification, dual incision of the damaged strand bracketing the lesion, excision of an oligonucleotide containing the lesion, gap-filling DNA synthesis and ligation (Figure 1) (Prakash and Prakash 2000). The distinguishing characteristic of NER is that the damaged bases are enzymatically excised from the genome as an oligonucleotide fragment of about 24-32 nucleotides in length in mammalian cells and 24-27 nucleotides in length in yeast

(Prakash and Prakash 2000). The biological importance of NER for human health is illustrated by the existence of rare autosomal recessive human disorders which result from defects of genes involved in NER, namely xeroderma pigmentosum (XP), Cockayne syndrome (CS), and trichothiodystrophy (TTD), all of which are associated with increased sensitivity to sunlight (Cleaver, Lam, and Revet 2009).

Although the process of NER in eukaryotes and prokaryotes share many similar features, such as damage recognition, excision, repair synthesis and ligation, the molecular mechanisms in eukaryotic cells seem much more complicated. In both prokaryotes and eukaryotes, the core NER factors, defined as the proteins which are necessary and sufficient to carry out the NER reaction, have been identified. The NER process in *Escherichia coli* is relatively well understood and requires only six proteins, whereas the NER process in eukaryotes displays a considerably higher degree of genetic complexity, requiring more than 30 proteins to reconstitute the reaction *in vitro* (Aboussekhra et al. 1995; Guzder et al. 1995).

NER is a heterogeneous process which repairs lesions in the transcribed strands of transcriptionally active genes faster than it repairs lesions in the nontranscribed strands or transcriptionally silent regions. Based on this heterogeneity, NER is traditionally divided into two pathways: global genomic repair (GGR) and transcription coupled repair (TCR). Damage in transcriptionally silent regions and in the nontranscribed strand (NTS) of active genes is repaired by GGR, while TCR is dedicated to repairing lesions in the transcribed strand (TS) of active genes. Though TCR and GGR are generally differentiated as distinct pathways of NER, they only differ in the initial steps of DNA damage recognition. Therefore, after lesion recognition and verification, a general outline of the GGR process becomes applicable to TCR as well. A defining characteristic of NER substrates is that they cause local distortion of the DNA double helix, and in GGR, this local distortion appears to be the first structure recognized.

2. NER in *S. cerevisiae*

Studies using the budding yeast *Saccharomyces cerevisiae* as a model organism have made major contributions in elucidating the core NER mechanism in eukaryotes and have yielded important insights into the functions of a multitude of NER proteins (Prakash and Prakash 2000). Many cellular processes such as replication, repair, cell division, and recombination are highly conserved from lower to higher eukaryotes. Indeed, most NER factors are conserved proteins and have orthologs in humans, yeast, and other eukaryotes (Table 1). In addition to having many homologs to humans, yeast offers many advantageous features to researchers, such as having a well-defined genetic system, the ease of growth and maintenance in the laboratory, and the ability to be maintained in either a haploid or diploid state. Taken together, these features provide researchers with a powerful genetic tool to study NER.

2.1 GGR in *S. cerevisiae*

In *S. cerevisiae*, Rad7, Rad16, and Elc1 are specifically required for GGR (Lejeune et al. 2009; Verhage et al. 1994). Rad4 is the homolog of human XPC (Legerski and Peterson 1992). However, unlike XPC which is specifically required for GGR but dispensable for TCR in human cells, Rad4 is essential for both TCR and GGR in yeast (Prakash and Prakash 2000).

S. cerevisiae	**Human homolog or counterpart**	**Function(s)**	**Reference**
Rad4	XPC	DNA damage recognition and binding	(Guzder et al. 1998)
Rad23	hRAD23B	Interacts with and stimulates Rad4	(Guzder et al. 1998)
TFIIH	TFIIH	DNA helicase activity mediates helix opening	(Egly and Coin 2011)
Mms19	MMS19L	Stabilizes XPD subunit of TFIIH	(Kou et al. 2008)
Rad14	XPA	Stabilizes preincision complex; lesion recognition	(Guzder et al. 2006)
Rpa	RPA	Stabilizes open single stranded DNA; damage recognition	(Guzder et al. 1995)
Rad2	XPG	Catalyzes 3′ incision; stabilizes open complex	(Habraken et al. 1993)
Rad10	ERCC1	Catalyzes 5′ incision; forms complex with Rad1	(Sung et al. 1993; Tomkinson et al. 1994)
Rad1	XPF	Catalyzes 5′ incision	(Sung et al. 1993; Tomkinson et al. 1994)
Rad26	CSB	TCR-specific factor; DNA-dependent ATPase	(van Gool et al. 1994)
Rpb9	Rpb9	TCR-specific factor; subunit of RNA polymerase II	(Li and Smerdon 2002)
Rad7-Rad16	DDB1-DDB2	GGR-specific factor; damage recognition; ubiquitinates Rad4	(Gillette et al. 2006; Ramsey et al. 2004; Reed 2005; Verhage et al. 1994)
Elc1	Elongin C	GGR-specific factor; forms complex with Rad7-Rad16	(Lejeune et al. 2009; Ramsey et al. 2004)
DNA polymerase δ	DNA polymerase δ	Gap-filling repair synthesis	(Wu et al. 2001)
DNA polymerase ε	DNA polymerase ε	Gap-filling repair synthesis	(Wu et al. 2001)
PCNA	PCNA	Sliding clamp for DNA polymerase δ	(Huang et al. 1998)
Cdc9	DNA ligase I	Ligation	(Wu, Braithwaite, and Wang 1999)

Table 1. Core yeast NER factors, their human homologs or counterparts, and their functions in NER.

The exact roles of Rad7, Rad16, and Elc1 in GGR are not yet clear. Rad7 and Rad16 form a complex that binds specifically to UV-damaged DNA in an ATP-dependent manner (Guzder et al. 1997). Although no structural homologs of Rad7 and Rad16 have been identified in human cells, some striking functional similarities exist between Rad7-Rad16 and DDB1-DDB2 (XPE) complexes [for a review see, (Reed 2005)]. Mutations in both the

yeast and human genes result in defective GGR. Both exhibit physical interactions and can bind damaged DNA. Both form components of a class of cullin based E3 ubiquitin ligase whose substrate includes Rad4/XPC, that are homologues of the same repair factor in yeast and humans, respectively. The ATP dependence of the Rad7-Rad16 complex for damage binding distinguishes this complex from Rad14, RPA, and the Rad4–Rad23 complex, which do not exhibit such dependence on ATP for damage binding (Prakash and Prakash 2000). Rad16 shares marked homology with Snf2, the catalytic subunit of the SWI/SNF chromatin remodeling complex (Bang et al. 1992), and Snf2 contains conserved motifs found in a superfamily of ATPases thought to be involved in chromatin remodeling activities (Eisen, Sweder, and Hanawalt 1995). Accordingly, the Rad7–Rad16 complex displays a DNA-dependent ATPase activity. It has been shown that this ATPase activity is inhibited when the complex comes across DNA damage (Guzder et al. 1998). This finding led to the formation of a model which suggested that the Rad7-Rad16 complex may act as an ATP-dependent motor which translocates along the DNA in search of damage, and upon encountering a lesion, the complex is stalled, which may remodel and open damaged chromatin, thereby facilitating recruitment of other NER factors (Guzder et al. 1998) According to this model, the Rad7–Rad16 complex would arrive first on the scene of a damage site in nontranscribed regions of the genome and serve as the nucleation site for the recruitment of the other NER factors. It was also previously suggested that the ATPase activity of Rad16 generates superhelical torsion in DNA that has an altered structure due to UV-induced damage, and that this torsion is necessary for the excision of damaged bases in GGR (Yu et al. 2004), suggesting a role for Rad16-Rad7 in the later steps of GGR.

Yeast Elc1 is a homolog of mammalian elongin C which forms a heterotrimeric complex with elongins A and B (Aso et al. 1995; Bradsher et al. 1993). The *elc1*Δ mutation was shown to be epistatic to *rad7*Δ and *rad16*Δ mutations, but resulted in a synergistic enhancement of UV sensitivity when combined with *rad26*Δ (Ribar, Prakash, and Prakash 2006). A study utilizing a technique which measures NER at the nucleotide level revealed that Elc1 plays an important role in GGR, as *elc1*Δ cells showed no detectable repair of CPDs in the NTS of the constitutively expressed *RPB2* gene, but no role in TCR (Lejeune et al. 2009). The role of Elc1 is not via stabilizing Rad7 or Rad16, as levels of either do not change in *elc1*Δ cells. Furthermore, the role of Elc1 does not seem to be subsidiary to that of Rad7 or Rad16, as overexpression of either or both in the absence of Elc1 did not restore GGR (Lejeune et al. 2009). The precise nature of the role of Elc1 in GGR remains unknown. Genetic studies have revealed multiple roles for this gene in separate cellular processes (Ribar, Prakash, and Prakash 2006). In one of these, Elc1 is a component of a ubiquitin ligase (E3) that contains Rad7 and Rad16 and is responsible for regulating the levels of Rad4 protein in response to UV damage (Gillette et al. 2006; Ramsey et al. 2004). It has also been suggested that Elc1 is a component of another ubiquitin ligase complex, which contains Ela1, Cul3, and Roc1 and is responsible for the polyubiquitylation and subsequent degradation of RNA polymerase II (Pol II) in response to DNA damage (Ribar, Prakash, and Prakash 2006, 2007).

The TFIIH multiprotein complex, which is organized into a 7-subunit core associated with a 3-subunit CDK-activating kinase module (CAK), is involved in both Pol II-mediated transcription and NER (Egly and Coin 2011). Tfb5, the homolog of human TTD-A, is a subunit of the core TFIIH. Unlike other subunits of the core TFIIH which are required for both GGR and TCR, Tfb5 has been shown to be essential for GGR but not absolutely required for TCR, as no apparent repair can be detected in the NTS, but a certain extent of

repair can be seen in the TS of either the *RPB2* or *GAL1* genes (Ding et al. 2007). The effect is unlikely due to changes in the steady state levels of other TFIIH subunits, as Tfb5 does not seem to affect the stability of other TFIIH components (Ranish et al. 2004). This may be different from human cells, where TTD-A (the homolog of yeast Tfb5) has been shown to stabilize other subunits of TFIIH (Vermeulen et al. 2000). Yeast Tfb5 interacts with Tfb2, another subunit of the TFIIH core (Zhou, Kou, and Wang 2007), and it was proposed that yeast Tfb5 acts as an architechtural stabilizer giving structural rigidity to the core TFIIH so that the complex is maintained in its functional architecture (Zhou, Kou, and Wang 2007). Another possibility is that the Rad25 ATPase activity of TFIIH needs to be stimulated by Tfb5 (Coin et al. 2006) in order to efficiently unwind the double helix around a lesion in the chromatin environment *in vivo* (Ding et al. 2007).

2.1.1 GGR in the context of chromatin

Although the core biochemical mechanism of NER is known, much remains unanswered. One of the looming questions currently being addressed is the issue of NER, especially GGR, in chromatin. As with all DNA-related processes, the NER machinery must deal with the presence of organized chromatin and the physical obstacles that it presents. How cells detect and repair lesions in diverse chromatin environments is a question that remains unanswered. Rearrangement of chromatin structure during NER was discovered more than two decades ago, however the molecular basis of chromatin dynamics during NER in eukaryotic cells is still not well understood (Gong, Kwon, and Smerdon 2005; Nag and Smerdon 2009; Waters et al. 2009).

The basic repeating unit of chromatin is the nucleosome, which is comprised of 146 base pairs of DNA wrapped around an octamer of the four core histone proteins H2A, H2B, H3, and H4 (Luger et al. 1997). Most of this DNA is tightly wrapped in about 1.6 left-handed superhelical turns around the histone octamer, with linker DNA (ranging from 20 to 90 base pairs in length) separating nucleosome cores, and giving the "beads-on-a-string" appearance familiar from electron microscopy. DNA is then further compacted by the organization of nucleosomes into higher order structures, such as 30 nm fibers and the highly condensed state of chromosomes (Wolffe 1999). Adjacent nucleosomes can be arranged in various configurations which affect the accessibility of DNA, thus the DNA-nucleosome polymer must be flexible in order to allow various cellular processes such as replication, transcription, recombination, and repair (Zhang, Jones, and Gong 2009). The two primary mechanisms which are involved in this flexibility are histone modifications and chromatin remodeling (Palomera-Sanchez and Zurita 2011). Below we summarize new findings regarding NER and the roles of histone modifications and chromatin remodeling activities. The results of previous studies focused on elucidating these mechanisms have been summarized in several very good recent reviews (Altaf, Saksouk, and Cote 2007; Ataian and Krebs 2006; Palomera-Sanchez and Zurita 2011; Zhang, Jones, and Gong 2009).

2.1.1.1 Chromatin remodeling and GGR

The complexity of NER and the size of the repair machinery can make it difficult to imagine how DNA distorting lesions can be recognized and processed without temporary rearrangement of chromatin (Thoma 1999). Instead of utilizing specific enzymes that each recognize a specific type of lesion, NER's damage recognition factors recognize helix distortion and bind to DNA to test its local conformation (which explains the wide versatility of NER). The excision step in NER requires considerable space. About 25-30 bp of

DNA are unwound in the open complex during NER (Evans et al. 1997), and the human excision complex requires about 100 bp of DNA to excise the lesion *in vitro* (Huang and Sancar 1994). Such a complex is incompatible with the structure of the nucleosome, and the linker DNA between nucleosomes is too short to accommodate a repair complex (Thoma 1999). Thus it is obvious that *in vivo* alterations of chromatin either by the lesion itself, by the action of chromatin remodelers or histone modifications, or by DNA metabolizing processes such as transcription, are required to facilitate damage recognition and repair (Fousteri et al. 2005).

Although chromatin structures can restrict the NER machinery from accessing sites of DNA damage, limited pieces of evidence have emerged recently that chromatin metabolism may also play an active role in the repair process (Waters et al. 2009). The SWI/SNF superfamily of ATP-dependent chromatin remodeling enzymes all possess an ATPase subunit which can disrupt or alter DNA-histone associations. SWI/SNF is the prototypical chromatin remodeling factor and is present in all eukaryotes (Martens and Winston 2003). Previous studies have demonstrated that these chromatin remodeling enzymes play an important role in enabling access of the NER machinery to nucleosomal DNA [for a review, see (Osley, Tsukuda, and Nickoloff 2007)]. As transcription disturbs chromatin structure, only the GGR pathway of NER is modulated by chromatin. As DNA damage recognition is a slow and rate-limiting step in NER (Mone et al. 2004) and it has been thought that this step required chromatin remodeling activities, a *in vivo* study examined the possible association between SWI/SNF and the DNA damage recognition complex Rad4-Rad23 in yeast (Gong, Fahy, and Smerdon 2006). Using His-tag pulldown and coimmunoprecipitation assays, this study provided evidence linking a chromatin remodeling complex with NER by demonstrating that Snf6 and Snf5, two subunits of the SWI/SNF complex in yeast, co-purify with the Rad4-Rad23 heterodimer (Gong, Fahy, and Smerdon 2006). It was further shown that this association was stimulated by UV irradiation, indicating that SWI/SNF facilitates chromatin remodeling during NER and that it has a role in facilitating GGR. Based on these findings, it was postulated that Rad4-Rad23 may recruit the SWI/SNF complex to facilitate NER at damage sites *in vivo*, or that SWI/SNF may recognize and bind to another feature of damaged chromatin and aid in recruiting Rad4-Rad23 (Gong, Fahy, and Smerdon 2006). The GGR-specific factor Rad16 is also a member of the SWI/SNF family of DNA-dependent ATPases and is thought to have a role in DNA damage recognition (Prakash and Prakash 2000). Interestingly, no association was found between Snf6 and Rad16, which is surprising given that Rad16 is required for GGR. This suggests that SWI/SNF and Rad16 may operate at different stages in the repair process (Gong, Fahy, and Smerdon 2006).

Another example of chromatin remodeling in NER comes from a recently published report which showed that the Ino80 chromatin remodeling complex promotes removal of UV lesions in regions with high nucleosome occupancy (Sarkar, Kiely, and McHugh 2010). More specifically, the study showed that Ino80 interacts with the early damage recognition complex of Rad4-Rad23 and was recruited to Rad4 in a UV-dependent manner. *ino80*Δ mutants were shown to be defective in both recruitment of repair factors to the damage site and restoration of nucleosome structure after repair. This suggests that Ino80 is recruited to sites of UV DNA damage through interactions with the NER machinery and is required for restoration of chromatin structure after repair (Sarkar, Kiely, and McHugh 2010). The role of Ino80 in NER appears to be conserved in eukaryotic cells. In mammalian cells deletion of two core components of the Ino80 complex, INO80 and ARP5, significantly hampered cellular removal of UV-induced photo lesions but had no significant impact on the

transcription of NER factors (Jiang et al. 2010). Loss of INO80 abolished the assembly of NER factors, suggesting that prior chromatin relaxation is important for the NER incision process.

Because transcription disturbs chromatin, only GGR is modulated by chromatin structure. Indeed, there is no correlation between the heterogeneity in NER and chromatin structure in TCR. However, chromatin remodeling activities associated with the transcription process are likely to play a role in damage recognition during TCR (Zhang, Jones, and Gong 2009). As mentioned previously, Rad26 is a DNA-dependent ATPase of the SWI/SNF superfamily (Guzder et al. 1996). CSB, the human homolog of yeast Rad26, has been shown to interact with XPG (Sarker et al. 2005) and attracts repair factors and a histone acetyltransferase to the site of a damage-stalled Pol II (Newman, Bailey, and Weiner 2006). Similar to its human homolog, Rad26 has also been found to play a role in repairing apparently transcriptionally inactive genes, a role possibly exacted through its putative chromatin remodeling activities (Bucheli, Lommel, and Sweder 2001). However, caution needs to be exercised regarding the explanation of role of Rad26 in repairing transcriptionally repressed genes, which can be through TCR initiated by noise transcription that commonly occurs in both strands of supposedly repressed genes (Li et al. 2007; Tatum and Li 2011).

2.1.1.2 Histone modifications and GGR

From recent evidence, it is clear that GGR requires different mechanisms to relax chromatin and ultimately removes lesions (Palomera-Sanchez and Zurita 2011). In addition to chromatin remodelers, histone modifications have been implicated in various mechanisms of DNA repair. Histones are subject to a multitude of post-translational modifications including acetylation, methylation, phosphorylation, sumoylation, and ubiquitination (Kouzarides 2007). Some of these modifications may modulate the NER process (Gong, Kwon, and Smerdon 2005; Nag and Smerdon 2009; Waters et al. 2008). However, the effects of histone modifications on NER in living cells documented previously are generally quite modest and are most likely due to the alteration of chromatin compaction and/or stability. Some recent studies implicating histone modifications in the facilitation of NER are discussed below.

The functional correlation between histone hyperacetylation and efficient NER has been known for some time (Ramanathan and Smerdon 1989; Smerdon et al. 1982). More recent studies have confirmed this correlation, demonstrating reduced CPD removal in yeast cells lacking the histone acetyltransferase (HAT) Gcn5, which acetylates histone H3 on lysines 9 and 14 (H3K4 and H3K19). Furthermore, the acetylation of H3K9 and H3K14 was shown to increase throughout the genome after irradiation with UV light and resulted in more efficient GGR (Teng et al. 2008; Yu et al. 2005). This modification seems to be conserved in mammalian cells, as a recent report showed that the E2F1 transcriptional factor in human cells is recruited in the chromatin at sites of UV damage and associates with GCN5 to acetylate H3K9 (Guo et al. 2011). Histone H3 in yeast has also been shown to be hyperacetylated in strains lacking the damage recognition factors Rad4 or Rad14, indicating that H3 acetylation occurs before the repair process and is not stimulated by NER (Yu et al. 2005). However, reinstating the acetylation level to a pre-UV state was shown to be dependent on NER (Yu et al. 2005). Additionally, UV-induced hyperacetylation of H3K9 and K14 was shown to be mediated by the GGR-specific factor Rad16 (Teng et al. 2008). Interestingly, it was demonstrated that pre-hyperacetylated regions could undergo efficient repair even in the absence of Rad16 (Teng et al. 2008), thus providing a direct link between

GGR and histone acetylation. However, it remains to be elucidated if the Rad16-independent repair is indeed GGR or TCR initiated by noise transcription that may not occur at normal conditions but takes place when the chromatin is pre-hyperacetylated. The noise transcripts can be hard to detect by traditional techniques as they are rapidly degraded after being produced (Struhl 2007). It has been postulated that histone hyperacetylation could regulate NER either directly through generating a suitable binding surface for repair proteins or indirectly through altering the compaction of nucleosomes (Irizar et al. 2010).

Much like the trend observed for gene expression, the effect of histone acetylation on repair varies according to chromatin status. In yeast, heavily compacted and suppressive regions of chromatin (i.e. heterochromatin) such as telomeres, silenced mating loci, and rDNA repeats, show reduced levels of histone H3 and H4 acetylation after UV. A recent study examined GGR of the *URA3* gene in subtelomeric regions (Irizar et al. 2010). These regions are hypoacetylated and bound by Sir proteins, which are involved in establishing silenced and heterochromatic regions in the genome. One particular Sir protein, Sir2, a NAD^+-dependent histone deacetylase (HDAC), has been shown to have a preference for removing the acetyl group from H3K9 and K14 as well as H4K16 (Imai et al. 2000; Landry et al. 2000). Repair of CPDs in these regions was shown to be slow, likely a result of the reduced histone acetylation. Furthermore, a significant increase in histone H3 and H4 acetylation after UV was shown in *sir*2Δ deletion mutants, indicating an important role for Sir2 in regulating histone acetylation in response to UV. This increase in histone acetylation resulted in improved NER efficiency, suggesting that the action of the different mechanisms that modify histones to facilitate NER may be influenced by the type of chromatin environment and the prevalence of specific factors like Sir2 in subtelomeric chromatin (Palomera-Sanchez and Zurita 2011).

In addition to histone acetylation, histone methylation has also been shown to play a role in the GGR process. Dot1 is a histone methyltransferase required for methylation of histone H3 lysine 79 (H3K79). *dot1*Δ mutants are sensitive to UV (Bostelman et al. 2007) and have a defect in activation of DNA damage checkpoints (Giannattasio et al. 2005). Indeed, H3K79 methylation was shown to be required for efficient NER in a silenced locus of yeast (Chaudhuri, Wyrick, and Smerdon 2009). An even more recent study demonstrated Dot1 to be a novel GGR-specific factor which mediates GGR by methylating its sole known substrate, H3K79 (Tatum and Li 2011). Using a nucleotide resolution method which uses streptavidin magnetic beads and biotinylated oligonucleotides to facilitate isolation and strand-specific end-labeling of DNA fragments of interest to measure GGR, the study showed that Dot1 and H3K79 methylation are required for GGR in both nucleosomal core regions and inter-nucleosomal linker regions, but play no role in TCR (Tatum and Li 2011). It was previously suggested that the role of H3K79 methylation in GGR may be via affecting expression of repair factors, such as Rad16 (Chaudhuri, Wyrick, and Smerdon 2009). However, it was shown that overexpression of Rad16 in cells whose genomic H3 genes (*HHT1* and *HHT*2) were deleted and complemented with a plasmid encoding the K79A mutant histone H3 (H3K79A) cells did not affect GGR, suggesting that the effect of H3K79 methylation on GGR is not through regulating the expression of Rad16 (Tatum and Li 2011). It was proposed that the addition of methyl moieties to H3K79 may serve as a docking site for repair factors on the chromatin. In the absence of the methyl groups, the repair machinery may be excluded from the chromatin, including the vicinities of inter-nucleosomal linker regions (Tatum and Li 2011).

We have recently found additional evidence for the involvement of histone modifications in GGR in studies involving the yeast Pol II-associated factor 1 complex (Paf1C). Paf1C is comprised of 5 subunits, namely Paf1, Rtf1, Cdc73, Leo1, and Ctr9 and interacts with Pol II and chromatin at both promoters and throughout the coding regions of genes [for a recent review, see (Jaehning 2010)]. Loss of Rtf1 or Cdc73 causes the dissociation of Paf1C from Pol II and chromatin. Paf1C has been shown to be required for monoubiquitination of histone H2B at lysine 123 (H2BK123) by Bre1 (an E3 ubiquitin ligase) in complex with Rad6 (an E2 ubiquitin conjugase) (Krogan et al. 2003; Ng, Dole, and Struhl 2003; Wood et al. 2003). The Bre1-Rad6-mediated monoubiquitination of H2BK123 is, in turn, partially required for dimethylation and fully required for trimethylation of H3K79 by Dot1 (Levesque et al. 2010; Nakanishi et al. 2009; Shahbazian, Zhang, and Grunstein 2005). Dot1 can add one methyl group to H3K79 by itself, meaning that Paf1C indirectly enables di- and trimethylation of H3K79. Although it can be associated with Pol II, Paf1C may function in enabling these histone modifications independent of Pol II, as both monoubiquitination of H2BK123 (Schulze et al. 2009) and methylation of H3K79 (Ng et al. 2003; Pokholok et al. 2005; van Leeuwen, Gafken, and Gottschling 2002) do not seem to be correlated with the transcriptional activity of a gene. Elimination of one of the PAF components (Rtf1) resulted in significantly compromised GGR, especially in inter-nucleosomal linker regions (Tatum et al. 2011). Genetic analysis revealed an epistatic relationship between *RTF1* and *BRE1* and *DOT1*, indicating that these proteins function in the same pathway in response to UV damage. It was further demonstrated that elimination of Rtf1 in *bre1*Δ or *dot1*Δ cells did not affect GGR speed, confirming the presence of an epistatic relationship as well as indicating that the function of Paf1C in GGR is accomplished through enabling monoubiquitination of H2BK123 by Bre1, which in turn permits di- and tri-methylation of H3K79 by Dot1 (Tatum et al. 2011).

In addition to acetylation and methylation, studies have also provided evidence for multiple roles of histone ubiquitination in NER (Nouspikel 2011). Nucleosome stability is controlled mainly by acetylation, but also to some degree by ubiquitination. Histone H2A is constitutively ubiquitinated even in the absence of DNA damage, especially in condensed chromatin. This ubiquitination was shown to disappear rapidly after UV-induced DNA damage and reappear within 30 minutes to 2 hours (Kapetanaki et al. 2006). Histones H2B, H3, and H4 are also constitutively ubiquitinated but to a much lower level (Nouspikel 2011). It was shown that ubiquitination of H3 and H4 increased within 1 hour of UV irradiation, decreased by 4 hours, and returned to original levels at 8 hours (Wang et al. 2006). It was postulated that this may act as a means of destabilizing nucleosomes, permitting better access of the repair machinery to the site of the lesion. However, there is a lack of experimental support for this idea. In fact, *in vitro* experiments showed that ubiquitination of H3 and H4 does not cause dissociation from DNA, and *in vivo*, only about half of H3 ubiquitinated after UV-induced damage dissociated from chromatin (Bergink et al. 2006; Wang et al. 2006).

Evidence for UV-induced ubiquitination came from a study which used cells expressing GFP-tagged ubiquitin (Bergink et al. 2006). These cells were UV-irradiated through a micropore filter to induce localized spots of DNA damage in the nucleus. Interestingly, after induction of DNA damage by UV, ubiquitinated histone H2A was found to accumulate at damage sites. This ubiquitination of histone H2A was shown to be dependent on NER and occurred after incision of the damaged strand, indicating a role in the later steps of NER. Indeed, a subsequent study demonstrated that UV-induced accumulation of ubiquitinated H2A at damage sites is a part of the chromatin restoration process (Zhou et al. 2008).

2.2 TCR in *S. cerevisiae*

Lesions that arrest or stall transcription by Pol II on the transcribed strand (TS) are repaired 5-10 times faster than the nontranscribed strand (NTS) by TCR (Hanawalt 1994). TCR has been shown to function in *E. coli* (Mellon and Hanawalt 1989), *S. cerevisiae* (Smerdon and Thoma 1990), and mammalian cells (Mellon, Spivak, and Hanawalt 1987). While the mechanistic details of TCR in *E. coli* are relatively well understood, the mechanisms of TCR in eukaryotes appear to be extremely complicated [for reviews, see (Fousteri and Mullenders 2008; Hanawalt and Spivak 2008)].

TCR in eukaryotic cells is believed to be triggered by stalled Pol II at a lesion in the transcribed strand of a gene (Fousteri and Mullenders 2008; Hanawalt and Spivak 2008). Rad26, the yeast homolog of human CSB and a putative transcription repair coupling factor, is important for TCR but dispensable for GGR (van Gool et al. 1994). However, TCR in yeast is not solely dependent on Rad26, as a significant amount of repair still occurs in cells lacking Rad26 (Li and Smerdon 2002, 2004; Verhage et al. 1996). Rpb9, a nonessential subunit of Pol II, has also been shown to play a role in mediating TCR (Li and Smerdon 2004, 2002; Li et al. 2006; Li et al. 2007).

Rad26- and Rpb9- mediated TCR subpathways have been shown to have different efficiencies in different regions of a gene (Li et al. 2006). Rpb9-mediated TCR operates more effectively in the coding region than in the region upstream of the transcription start site; whereas the Rad26-mediated subpathway operates equally well in both regions (Li and Smerdon 2002, 2004). Additionally, in log phase wild type cells, the relative contributions of these two subpathways of TCR may be different from gene to gene. For the *URA3* gene, Rad26 seems to be absolutely required, except for a short region close to the transcription start site (Tijsterman et al. 1997), indicating that TCR is accomplished primarily by the Rad26 subpathway. Rad26 is partially required for TCR in the *RPB2* gene (Bhatia et al. 1996; Gregory and Sweder 2001; Li and Smerdon 2002; Verhage et al. 1996), indicating that both subpathways contribute to TCR in this gene. For the *GAL1* gene, Rad26 is almost dispensable, especially in the coding region, indicating that TCR in this gene of log-phase cultures is fulfilled primarily by the Rpb9 subpathway. The different contributions of the two subpathways of TCR in different genes may be caused by different levels of transcription. Rad26- and Rpb9-mediated repair are also differently modulated by different promoter elements (Li et al. 2006). In the yeast *GAL1* gene, the efficiency of TCR mediated by Rad26 is determined by the upstream activating sequence (UAS), but not by the TATA or local sequences. However, both the UAS and TATA are necessary to confine Rad26-mediated repair to the transcribed strand of the gene. Abrogating or abolishing transcription by mutation or deletion of the TATA sequence or mutation of the UAS results in Rad26-mediated repair in both the TS and NTS of the *GAL1* gene (Li et al. 2006). This suggests that Rad26-mediated repair can be either transcription-coupled, provided that a substantial level of transcription is present, or transcription-independent, if transcription is too low or absent. However, as mentioned above, noise transcription, which cannot be easily detected by traditional techniques, may occur in both strands upon the mutation or deletion of the UAS or TATA. This unexpected noise transcription may cause Rad26-mediated repair (which is TCR) to occur in both strands. Conversely, Rpb9-mediated TCR only occurs in the transcribed strand and is efficient only if the TATA and UAS sequences are present, suggesting that TCR mediated by Rpb9 is strictly transcription coupled and is only efficient when the level of transcription is high (Li et al. 2006).

Rpb9 also plays an important role in promoting ubiquitylation and degradation of Rpb1, the largest subunit of Pol II, in response to UV damage (Chen, Ruggiero, and Li 2007). Rpb9 is

composed of three distinct domains: the N-terminal Zn1, the C-terminal Zn2, and the central linker. The Zn1 and linker domains are essential for both transcription elongation and TCR functions, but the Zn2 domain is almost dispensable (Li et al. 2006). However, the Zn2 domain is essential for Rpb9 to promote degradation of Rpb1, whereas the Zn1 and linker domains play a subsidiary role in the degradation. This function of Rpb9 seems to be unrelated to any pathways of NER, including both subpathways of TCR, and it remains to be determined how Rpb9 promotes ubiquitination and degradation of Rpb1 (Chen, Ruggiero, and Li 2007).

2.2.1 The role of Rad26 in TCR

Like its human homolog CSB, Rad26 is a DNA-stimulated ATPase and functions in transcription elongation (Lee et al. 2001; Selby and Sancar 1997). Due to its ATPase activity, Rad26 is the most promising yeast transcription repair coupling factor (Svejstrup 2002). However, how Rad26 functions in TCR remains to be elucidated. Several models have been proposed based on its DNA-dependent ATPase activity (Svejstrup 2002). Because other members of the Swi/Snf family are able to alter contacts between DNA and DNA-binding proteins, one possibility is that Rad26, through its Swi/Snf-like activity, may displace a stalled Pol II complex at a damage site (Svejstrup 2002). This is the case in *E. coli* where the transcription repair coupling factor Mfd, an ATP-dependent translocase, moves stalled Pol II forward from the damage site, allowing it to continue transcription (Selby and Sancar 1994; Park, Marr, and Roberts 2002). However, other than ATPase domains, there is little structural homology between Rad26 and Mfd. Furthermore, an *in vitro* study demonstrated that CSB cannot displace Pol II stalled at a damage site (Selby and Sancar 1997).

A second model postulates that a Pol II complex stalled at a lesion may be pushed back by the general transcription factor TFIIS, which facilitates Pol II elongation through transcriptional arrest sites and stimulates transcript cleavage, allowing resumed forward translocation during normal transcription elongation (Kettenberger, Armache, and Cramer 2003; Saeki and Svejstrup 2009). Yeast strains lacking Rad26 exhibit a synergistic increase in sensitivity to the DNA-damaging agent methyl methanesulfonate (MMS) when combined with inactivating mutations in NER, suggesting a role for Rad26 in promoting Pol II transcription elongation through damage sites in DNA (Lee et al. 2002). However, TFIIS does not seem to play any role in TCR in both yeast (Verhage et al. 1997) and mammalian cells (Jensen and Mullenders 2010).

Alternative models addressing the fate of a damage-stalled Pol II, such as accessory-factor-mediated lesion bypass and keeping Pol II at a distance through damage-binding factors, might also be relevant in certain situations (Svejstrup 2002). The finding that Rpb1, the largest subunit of Pol II, is ubiquitinated and subsequently degraded in the CSA- and CSB-dependent manner in response to DNA damage that blocks transcription prompted researchers to propose a model whereby Pol II degradation facilitates lesion access and repair (Bregman et al. 1996; Ratner et al. 1998). However, a more recent report showed that CSA and CSB are not directly involved in Rpb1 ubiquitylation. The defects in Rpb1 ubiquitylation observed in CS cells are caused by an indirect mechanism: these cells shut down transcription in response to DNA damage, effectively depleting the substrate for ubiquitylation, namely elongating Pol II (Anindya, Aygun, and Svejstrup 2007). Also, evidence has shown that the ubiquitination and degradation of Rpb1 do not seem to be necessary for TCR in yeast. Rsp5, the only yeast ubiquitin-protein ligase that modifies Pol II,

is not required for TCR (Lommel, Bucheli, and Sweder 2000). Def1, which forms a complex with Rad26 in chromatin, is required for Pol II degradation in response to DNA damage but is not required for TCR (Woudstra et al. 2002). Furthermore, as mentioned above, Rpb9 plays an important role in ubiquitination and degradation of Rpb1 (Chen, Ruggiero, and Li 2007). However, this function of Rpb9 is unrelated to TCR mediated by Rpb9 itself and that mediated by Rad26 (Chen, Ruggiero, and Li 2007).

As will be discussed below, recent evidence indicates that the role of Rad26 in TCR may be entirely through indirect mechanisms, by antagonizing the actions of TCR suppressors (Figure 1).

2.2.2 Suppressors of Rad26-independent TCR

Recently, a number of TCR suppressors have been identified. Interestingly, in each case, the release of suppression (i.e. reinstatement of TCR) is present only in cells lacking Rad26. Below is a discussion of each of the known suppressors of Rad26-independent TCR and their possible interactions.

Yeast Spt4 and Spt5 form a complex which has been shown to physically interact with Pol II (Hartzog et al. 1998). The *SPT4* gene is dispensable (Malone, Fassler, and Winston 1993), whereas *SPT5* is essential (Swanson, Malone, and Winston 1991), for cell viability. These proteins are conserved transcription elongation factors and are generally required for normal development and viral gene expression in multicellular eukaryotes (Winston 2001). It was previously shown that deletion of *SPT4* negates the requirement of Rad26 for TCR in yeast, suggesting that Spt4 suppresses Rad26-independent TCR (Jansen et al. 2000). It has been further demonstrated that the suppression effect of Spt4 is indirect via protecting its interacting partner, Spt5, from degradation and by stabilizing the interaction of Spt5 with Pol II (Ding, LeJeune, and Li 2010). Indeed, overexpression of Spt5 in the absence of Spt4 suppresses Rad26-independent TCR (Ding, LeJeune, and Li 2010), supporting the notion that Spt4 plays an indirect role in this suppression.

Spt5 possesses a C-terminal repeat (CTR) domain, which is dispensable for cell viability and is not involved in interactions with Spt4 and Pol II (Ding, LeJeune, and Li 2010). Repair analysis of *rad26Δ* cells whose genomic *SPT5* gene had been deleted and complemented with a plasmid encoding either the full length or CTR-deleted Spt5 revealed that TCR in these cells expressing the CTR-deleted Spt5 was significantly faster than in those expressing full length Spt5, indicating that the Spt5 CTR is involved in suppressing Rad26-independent TCR (Ding, LeJeune, and Li 2010). Additional evidence for the role of the CTR in this suppression came from analyzing the phosphorylation state of the CTR. The CTR domain contains 15 6-amino acid repeats with the consensus sequence S(A/T)WGG(A/Q) (Swanson, Malone, and Winston 1991), with the serine and threonine residues being potential phosphorylation sites. It has been shown that the Spt5 CTR is phosphorylated by the Bur kinase (Ding, LeJeune, and Li 2010; Liu et al. 2009; Zhou et al. 2009). The kinase activity of Bur1 is dependent upon its cyclin partner Bur2. Deletion of Bur1 is lethal to cells, but deletion of Bur2 is not. Additionally, *bur1*Δ and *bur2*Δ mutations result in nearly identical phenotypes (Yao, Neiman, and Prelich 2000). Interestingly, it was shown that deletion of Bur2 also partially alleviates the necessity of Rad26 for TCR, suggesting that the phosphorylation of the Spt5 CTR may be partially responsible for suppressing TCR in the absence of Rad26 (Ding, LeJeune, and Li 2010).

It is not yet clear how the CTR of Spt5 is acting to suppress Rad26-independent TCR. It was recently reported that the Spt5 CTR is a platform for the association of proteins that promote

both transcription elongation and histone modifications (Zhou et al. 2009). One such protein complex recruited by the Spt5 CTR is Paf1C (Zhou et al. 2009). Indeed, the Rtf1 subunit of Paf1C has been shown to have extensive physical and physical connections with Spt5 (Squazzo et al. 2002). Additionally, optimal association of Paf1C with Pol II is dependent upon Spt4 (Qiu et al. 2006) and the Spt5 CTR (Tatum et al. 2011). Furthermore, recruitment of Paf1C requires the Bur-mediated phosphorylation of the CTR of Spt5 (Liu et al. 2009). Results from our lab showed that deletion of any of Paf1C's 5 subunits in *rad26*Δ cells causes increased TCR, indicating that Paf1C too is a suppressor of Rad26-independent TCR. Furthermore, simultaneous deletion of Spt4 along with a Paf1C component in *rad26*Δ cells resulted in similar degrees of repair restoration, suggesting that these suppressors are acting through a common pathway to suppress Rad26-indepedent TCR. However, unlike Spt4, Paf1C appears to be indispensable for suppressing Rad26-independent TCR, as overexpression of Spt5 in cells lacking a Paf1C component did not affect the overall TCR rate in these cells. This suggests that both Paf1C and Spt5 are required for suppressing TCR in the absence of Rad26 and that the role of Paf1C in this suppression is not subsidiary to that of Spt5.

Rpb4 is another nonessential subunit of Pol II (Woychik and Young 1989) and forms a subcomplex with Rpb7 (Armache, Kettenberger, and Cramer 2003; Bushnell and Kornberg 2003), a small but essential subunit of Pol II. This subcomplex can dissociate from Pol II, and deletion of Rpb4 abolishes the association of Rpb7 with Pol II. Interestingly, it was shown that, like Spt4/Spt5, deletion of Rpb4 reinstates TCR in *rad26*Δ cells, indicating that Rpb4 is also a suppressor of Rad26-independent TCR (Li and Smerdon 2002).

Pol II is a globular protein with a deep central cleft (Armache, Kettenberger, and Cramer 2003; Bushnell and Kornberg 2003). The DNA template enters and travels along this cleft to the active site. On one side of the cleft is a flexible clamp structure, which can switch between an open or closed position. The Rpb4-Rpb7 subcomplex is located downstream of the catalytic site in the center of this cleft, and its binding to the 10-subunit core Pol II pushes the clamp to the closed position (Armache, Kettenberger, and Cramer 2003; Bushnell and Kornberg 2003).

RNA polymerases (Cramer 2002) and Spt4/Spt5 (Ponting 2002) are conserved in all three kingdoms of life: bacteria, archaea, and eukaryotes. The archaeal Spt4/Spt5 has recently been co-crystallized with the clamp domain of an archaeal polymerase (Martinez-Rucobo et al. 2011). Based on this co-crystal structure, a model of the complete yeast Pol II-Spt4/Spt5 elongation complex has been proposed. This model posits that the NGN domain of Spt5 binds to the clamp of Pol II and closes the central cleft to lock nucleic acids and render the elongation complex processive and stable. The KOW1 domain of Spt5 may contact DNA and/or exiting RNA, which could possibly contribute to stability of the elongating Pol II complex and may also involve the Rpb4/Rpb7 subcomplex. The locations of the other domains of Spt5, including the CTR, are currently unpredictable (Martinez-Rucobo et al. 2011). Spt4, which does not directly contact Pol II, binds to the other side of the Spt5 NGN domain and points away from the surface of Pol II. How Paf1C interacts with Pol II is currently unknown, but one point of contact between Paf1C and Pol II is thought to be an indirect one via the Rtf1 subunit of Paf1C and Spt5, an idea supported by the extensive interactions of Rtf1 and Spt5.

Structure-function analyses of Pol II elongation complexes containing a thymine-thymine CPD in the TS showed that the CPD slowly passes a translocation barrier and enters into the active site of Pol II. The 5′ thymine of the CPD directs misincorporation of uridine into the

elongating mRNA, which stalls the translocation of Pol II (Brueckner and Cramer 2007). All of the above findings regarding suppression of Rad26-independed repair suggest that Rpb4/Rpb7, Spt4/Spt5, and Paf1C act cooperatively and through the same pathway to exert this suppression effect. It is possible that when Rad26 is absent, a lesion becomes "locked" into the active site of a Pol II elongation complex, which is stabilized by the coordinated interactions of these suppressors with each other and with the core Pol II complex. Deletion or mutation of any of these suppressors may result in the destabilization of elongating Pol II, making it possible for TCR to occur (Tatum et al. 2011). How Rad26 affects the association of these factors with Pol II is unknown. A possible role for Rad26 in TCR may be to destabilize the Pol II elongation complex (Figure 1). This is supported by the evidence that indicates that Rad26 is dispensable for TCR in the absence of any of these suppression factors. This may explain why this 'megasuppressor' complex only suppresses TCR in the absence of Rad26.

As an interesting aside, it has been demonstrated that Rpb4 (Li and Smerdon 2002) and Paf1C (Tatum et al. 2011) have dual roles in TCR. Not only do they suppress Rad26-independent TCR, but they have also been shown to facilitate Rad26-dependent TCR to a certain extent. However, how each serves to facilitate this subpathway of TCR remains unknown. Rad26 has been shown to play a role in transcription elongation (Lee et al. 2001), leading to the possibility that Paf1C may play a positive role in TCR by cooperating with Rad26 to promote transcription elongation. The interaction of Rpb4 with other subunits of Pol II may change the conformation of the polymerase complex, and this may, in turn, improve the interactions with Rad26 (Figure 1) (Li and Smerdon 2002).

3. Concluding remarks and future direction

Although most, if not all, core NER factors have been identified and extensively characterized, new accessory factors which modulate GGR and/or TCR are continuously being identified. It is not only important to identify these factors, but also to uncover the role they play (i.e. facilitation or suppression), how they exact their functions, and the interactions they have with other repair proteins in order to gain a more holistic understanding of the repair process. Furthermore, current understanding of NER in living yeast cells is limited to either genome-overall-level or to certain very limited regions of the genome. The detailed DNA damage distribution and NER kinetics in the vast majority of the genomes are still virtually unknown. This illustrates the need for a high-resolution, genome-wide assessment of damage, repair, and repair kinetics. Only then will we be able to paint a complete picture and have full understanding of this repair mechanism that has thus far proven elusive.

In bacteria, most NER enzymes are induced by the SOS response after DNA damage (Janion 2008), but this does not seem to be the case in higher organisms. By and large, NER in eukaryotes seems to be modulated by posttranslational modifications and protein-protein interactions, not transcriptional induction of genes encoding repair factors (Nouspikel 2011). This seems intuitive, as DNA damage (such as CPDs) can impede transcription, making it a safer choice to rely on the activation of present enzymes rather than on their damage-induced synthesis. Many posttranslational modifications of histones, including ones not discussed here (as this review is not exhaustive), have been shown to have important functions in NER. These modifications operate in a concerted manner to coordinate a plethora of tasks such as damage signaling, opening/relaxing chromatin to allow repair

factors access to damage sites, activating the DNA damage cell cycle checkpoint, facilitating lesion identification, and restoring the chromatin to its original state once the repair process is complete. The discovery that H3K79 methylation is required for GGR (Tatum and Li 2011) unveiled a critical link between chromatin modification and the repair process. However, it remains to be understood as to whether the methylation indeed serves as a docking site for the NER machinery or the modification is indirectly involved in GGR.

Though progress has been made in recent years regarding chromatin dynamics in NER, many questions remain unanswered. Many studies attempting to elucidate the roles of histone modifications during NER have focused only on specific histone tail residues or single modifications, yet there may be many other modifications involved in the NER process (Palomera-Sanchez and Zurita 2011). While informative, this provides us with only a narrow glimpse into the cellular response to genomic insult and lacks the broader scope of examining the changes to histones throughout the entire genome in response to DNA damage. This underscores the need for a genome-wide analysis to monitor the responses of the DNA damage-induced histone modifications that occur in all of the chromosomes and how these different modifications crosstalk. Until then, continued efforts to decipher the encrypted code of these modifications will provide a much clearer understanding of the tightly regulated mechanisms of NER and its crosstalk with other processes such as DNA damage-induced checkpoint activation. These future findings could prove to be valuable clinically, as they may be advantageous targets for chemotherapeutics or treatment of other diseases related to genomic instability.

The TCR mechanism in eukaryotic cells remains largely mysterious. The interactions among Pol II, Rad26 and the various known and possibly unknown TCR suppressors remain to be elucidated and are the major key to unlocking this mystery. Determining the exact binding site of Rad26 on Pol II would provide valuable insight into the antagonistic effect of Rad26 on the suppression of Rad26-independent TCR. Furthermore, Rad26 does not seem to be a true transcription-repair-coupling factor and may facilitate TCR indirectly rather than by directly recruiting NER factors, as in the absence of a suppressor, Rad26 can be entirely dispensable. It is therefore likely that either Pol II itself is intrinsically proficient in mediating TCR or a true transcription-repair-coupling factor has not been discovered. These different possibilities remain to be addressed.

4. Acknowledgment

This work was supported by National Science Foundation grant MCB-0745229.

5. References

Aboussekhra, A., M. Biggerstaff, M. K. Shivji, J. A. Vilpo, V. Moncollin, V. N. Podust, M. Protic, U. Hubscher, J. M. Egly, and R. D. Wood. 1995. Mammalian DNA nucleotide excision repair reconstituted with purified protein components. *Cell* 80 (6):859-68.

Altaf, M., N. Saksouk, and J. Cote. 2007. Histone modifications in response to DNA damage. *Mutat Res* 618 (1-2):81-90.

Anindya, R., O. Aygun, and J. Q. Svejstrup. 2007. Damage-induced ubiquitylation of human RNA polymerase II by the ubiquitin ligase Nedd4, but not Cockayne syndrome proteins or BRCA1. *Mol Cell* 28 (3):386-97.

Armache, K. J., H. Kettenberger, and P. Cramer. 2003. Architecture of initiation-competent 12-subunit RNA polymerase II. *Proc Natl Acad Sci U S A* 100 (12):6964-8.

Aso, T., W. S. Lane, J. W. Conaway, and R. C. Conaway. 1995. Elongin (SIII): a multisubunit regulator of elongation by RNA polymerase II. *Science* 269 (5229):1439-43.

Ataian, Y., and J. E. Krebs. 2006. Five repair pathways in one context: chromatin modification during DNA repair. *Biochem Cell Biol* 84 (4):490-504.

Bang, D. D., R. Verhage, N. Goosen, J. Brouwer, and P. van de Putte. 1992. Molecular cloning of RAD16, a gene involved in differential repair in Saccharomyces cerevisiae. *Nucleic Acids Res* 20 (15):3925-31.

Bergink, S., F. A. Salomons, D. Hoogstraten, T. A. Groothuis, H. de Waard, J. Wu, L. Yuan, E. Citterio, A. B. Houtsmuller, J. Neefjes, J. H. Hoeijmakers, W. Vermeulen, and N. P. Dantuma. 2006. DNA damage triggers nucleotide excision repair-dependent monoubiquitylation of histone H2A. *Genes Dev* 20 (10):1343-52.

Bhatia, P. K., R. A. Verhage, J. Brouwer, and E. C. Friedberg. 1996. Molecular cloning and characterization of Saccharomyces cerevisiae RAD28, the yeast homolog of the human Cockayne syndrome A (CSA) gene. *J Bacteriol* 178 (20):5977-88.

Bostelman, L. J., A. M. Keller, A. M. Albrecht, A. Arat, and J. S. Thompson. 2007. Methylation of histone H3 lysine-79 by Dot1p plays multiple roles in the response to UV damage in Saccharomyces cerevisiae. *DNA Repair (Amst)* 6 (3):383-95.

Bradsher, J. N., K. W. Jackson, R. C. Conaway, and J. W. Conaway. 1993. RNA polymerase II transcription factor SIII. I. Identification, purification, and properties. *J Biol Chem* 268 (34):25587-93.

Bregman, D. B., R. Halaban, A. J. van Gool, K. A. Henning, E. C. Friedberg, and S. L. Warren. 1996. UV-induced ubiquitination of RNA polymerase II: a novel modification deficient in Cockayne syndrome cells. *Proc Natl Acad Sci U S A* 93 (21):11586-90.

Brueckner, F., and P. Cramer. 2007. DNA photodamage recognition by RNA polymerase II. *FEBS Lett* 581 (15):2757-2760.

Bucheli, M., L. Lommel, and K. Sweder. 2001. The defect in transcription-coupled repair displayed by a Saccharomyces cerevisiae rad26 mutant is dependent on carbon source and is not associated with a lack of transcription. *Genetics* 158 (3):989-97.

Bushnell, D. A., and R. D. Kornberg. 2003. Complete, 12-subunit RNA polymerase II at 4.1-A resolution: implications for the initiation of transcription. *Proc Natl Acad Sci U S A* 100 (12):6969-73.

Chaudhuri, S., J. J. Wyrick, and M. J. Smerdon. 2009. Histone H3 Lys79 methylation is required for efficient nucleotide excision repair in a silenced locus of Saccharomyces cerevisiae. *Nucleic Acids Res* 37 (5):1690-700.

Chen, X., C. Ruggiero, and S. Li. 2007. Yeast Rpb9 plays an important role in ubiquitylation and degradation of Rpb1 in response to UV-induced DNA damage. *Mol Cell Biol* 27 (13):4617-25.

Cleaver, J. E., E. T. Lam, and I. Revet. 2009. Disorders of nucleotide excision repair: the genetic and molecular basis of heterogeneity. *Nat Rev Genet* 10 (11):756-68.

Coin, F., L. Proietti De Santis, T. Nardo, O. Zlobinskaya, M. Stefanini, and J. M. Egly. 2006. p8/TTD-A as a repair-specific TFIIH subunit. *Mol Cell* 21 (2):215-26.

Cramer, P. 2002. Multisubunit RNA polymerases. *Curr Opin Struct Biol* 12 (1):89-97.

Ding, B., D. LeJeune, and S. Li. 2010. The C-terminal repeat domain of Spt5 plays an important role in suppression of Rad26-independent transcription coupled repair. *J Biol Chem* 285 (8):5317-26.

Ding, B., C. Ruggiero, X. Chen, and S. Li. 2007. Tfb5 is partially dispensable for Rad26 mediated transcription coupled nucleotide excision repair in yeast. *DNA Repair (Amst)* 6 (11):1661-9.

Egly, J. M., and F. Coin. 2011. A history of TFIIH: Two decades of molecular biology on a pivotal transcription/repair factor. *DNA Repair (Amst)*.

Eisen, J. A., K. S. Sweder, and P. C. Hanawalt. 1995. Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucleic Acids Res* 23 (14):2715-23.

Evans, E., J. G. Moggs, J. R. Hwang, J. M. Egly, and R. D. Wood. 1997. Mechanism of open complex and dual incision formation by human nucleotide excision repair factors. *EMBO J* 16 (21):6559-73.

Fousteri, M., and L. H. Mullenders. 2008. Transcription-coupled nucleotide excision repair in mammalian cells: molecular mechanisms and biological effects. *Cell Res* 18 (1):73-84.

Fousteri, M., A. van Hoffen, H. Vargova, and L. H. Mullenders. 2005. Repair of DNA lesions in chromosomal DNA impact of chromatin structure and Cockayne syndrome proteins. *DNA Repair (Amst)* 4 (8):919-25.

Giannattasio, M., F. Lazzaro, P. Plevani, and M. Muzi-Falconi. 2005. The DNA damage checkpoint response requires histone H2B ubiquitination by Rad6-Bre1 and H3 methylation by Dot1. *J Biol Chem* 280 (11):9879-86.

Gillette, T. G., S. Yu, Z. Zhou, R. Waters, S. A. Johnston, and S. H. Reed. 2006. Distinct functions of the ubiquitin-proteasome pathway influence nucleotide excision repair. *EMBO J* 25 (11):2529-38.

Gong, F., D. Fahy, and M. J. Smerdon. 2006. Rad4-Rad23 interaction with SWI/SNF links ATP-dependent chromatin remodeling with nucleotide excision repair. *Nat Struct Mol Biol* 13 (10):902-7.

Gong, F., Y. Kwon, and M. J. Smerdon. 2005. Nucleotide excision repair in chromatin and the right of entry. *DNA Repair (Amst)* 4 (8):884-96.

Gregory, S. M., and K. S. Sweder. 2001. Deletion of the CSB homolog, RAD26, yields Spt(-) strains with proficient transcription-coupled repair. *Nucleic Acids Res* 29 (14):3080-6.

Guo, R., J. Chen, D. L. Mitchell, and D. G. Johnson. 2011. GCN5 and E2F1 stimulate nucleotide excision repair by promoting H3K9 acetylation at sites of damage. *Nucleic Acids Res* 39 (4):1390-7.

Guzder, S. N., Y. Habraken, P. Sung, L. Prakash, and S. Prakash. 1995. Reconstitution of yeast nucleotide excision repair with purified Rad proteins, replication protein A, and transcription factor TFIIH. *J Biol Chem* 270 (22):12973-6.

Guzder, S. N., Y. Habraken, P. Sung, L. Prakash, and S. Prakash. 1996. RAD26, the yeast homolog of human Cockayne's syndrome group B gene, encodes a DNA-dependent ATPase. *J Biol Chem* 271 (31):18314-7.

Guzder, S. N., C. H. Sommers, L. Prakash, and S. Prakash. 2006. Complex formation with damage recognition protein Rad14 is essential for Saccharomyces cerevisiae Rad1-

Rad10 nuclease to perform its function in nucleotide excision repair in vivo. *Mol Cell Biol* 26 (3):1135-41.

Guzder, S. N., P. Sung, L. Prakash, and S. Prakash. 1997. Yeast Rad7-Rad16 complex, specific for the nucleotide excision repair of the nontranscribed DNA strand, is an ATP-dependent DNA damage sensor. *J Biol Chem* 272 (35):21665-8.

Guzder, S. N., P. Sung, L. Prakash, and S. Prakash. 1998. Affinity of yeast nucleotide excision repair factor 2, consisting of the Rad4 and Rad23 proteins, for ultraviolet damaged DNA. *J Biol Chem* 273 (47):31541-6.

Guzder, S. N., P. Sung, L. Prakash, and S. Prakash. 1998. The DNA-dependent ATPase activity of yeast nucleotide excision repair factor 4 and its role in DNA damage recognition. *J Biol Chem* 273 (11):6292-6.

Habraken, Y., P. Sung, L. Prakash, and S. Prakash. 1993. Yeast excision repair gene RAD2 encodes a single-stranded DNA endonuclease. *Nature* 366 (6453):365-8.

Hanawalt, P. C. 1994. Transcription-coupled repair and human disease. *Science* 266 (5193):1957-8.

Hanawalt, P. C., and G. Spivak. 2008. Transcription-coupled DNA repair: two decades of progress and surprises. *Nat Rev Mol Cell Biol* 9 (12):958-70.

Harper, J. W., and S. J. Elledge. 2007. The DNA damage response: ten years after. *Mol Cell* 28 (5):739-45.

Hartzog, G. A., T. Wada, H. Handa, and F. Winston. 1998. Evidence that Spt4, Spt5, and Spt6 control transcription elongation by RNA polymerase II in Saccharomyces cerevisiae. *Genes Dev* 12 (3):357-69.

Hoeijmakers, J. H. 2009. DNA damage, aging, and cancer. *N Engl J Med* 361 (15):1475-85.

Huang, J. C., and A. Sancar. 1994. Determination of minimum substrate size for human excinuclease. *J Biol Chem* 269 (29):19034-40.

Huang, W., W. J. Feaver, A. E. Tomkinson, and E. C. Friedberg. 1998. The N-degron protein degradation strategy for investigating the function of essential genes: requirement for replication protein A and proliferating cell nuclear antigen proteins for nucleotide excision repair in yeast extracts. *Mutat Res* 408 (3):183-94.

Imai, S., C. M. Armstrong, M. Kaeberlein, and L. Guarente. 2000. Transcriptional silencing and longevity protein Sir2 is an NAD-dependent histone deacetylase. *Nature* 403 (6771):795-800.

Irizar, A., Y. Yu, S. H. Reed, E. J. Louis, and R. Waters. 2010. Silenced yeast chromatin is maintained by Sir2 in preference to permitting histone acetylations for efficient NER. *Nucleic Acids Res* 38 (14):4675-86.

Jaehning, J. A. 2010. The Paf1 complex: platform or player in RNA polymerase II transcription? *Biochim Biophys Acta* 1799 (5-6):379-88.

Janion, C. 2008. Inducible SOS response system of DNA repair and mutagenesis in Escherichia coli. *Int J Biol Sci* 4 (6):338-44.

Jansen, L. E., H. den Dulk, R. M. Brouns, M. de Ruijter, J. A. Brandsma, and J. Brouwer. 2000. Spt4 modulates Rad26 requirement in transcription-coupled nucleotide excision repair. *EMBO J* 19 (23):6498-507.

Jensen, A., and L. H. Mullenders. 2010. Transcription factor IIS impacts UV-inhibited transcription. *DNA Repair (Amst)* 9 (11):1142-50.

Jiang, Y., X. Wang, S. Bao, R. Guo, D. G. Johnson, X. Shen, and L. Li. 2010. INO80 chromatin remodeling complex promotes the removal of UV lesions by the nucleotide excision repair pathway. *Proc Natl Acad Sci U S A* 107 (40):17274-9.

Kapetanaki, M. G., J. Guerrero-Santoro, D. C. Bisi, C. L. Hsieh, V. Rapic-Otrin, and A. S. Levine. 2006. The DDB1-CUL4ADDB2 ubiquitin ligase is deficient in xeroderma pigmentosum group E and targets histone H2A at UV-damaged DNA sites. *Proc Natl Acad Sci U S A* 103 (8):2588-93.

Kettenberger, H., K. J. Armache, and P. Cramer. 2003. Architecture of the RNA polymerase II-TFIIS complex and implications for mRNA cleavage. *Cell* 114 (3):347-57.

Kou, H., Y. Zhou, R. M. Gorospe, and Z. Wang. 2008. Mms19 protein functions in nucleotide excision repair by sustaining an adequate cellular concentration of the TFIIH component Rad3. *Proc Natl Acad Sci U S A* 105 (41):15714-9.

Kouzarides, T. 2007. Chromatin modifications and their function. *Cell* 128 (4):693-705.

Krogan, N. J., J. Dover, A. Wood, J. Schneider, J. Heidt, M. A. Boateng, K. Dean, O. W. Ryan, A. Golshani, M. Johnston, J. F. Greenblatt, and A. Shilatifard. 2003. The Paf1 complex is required for histone H3 methylation by COMPASS and Dot1p: linking transcriptional elongation to histone methylation. *Mol Cell* 11 (3):721-9.

Landry, J., A. Sutton, S. T. Tafrov, R. C. Heller, J. Stebbins, L. Pillus, and R. Sternglanz. 2000. The silencing protein SIR2 and its homologs are NAD-dependent protein deacetylases. *Proc Natl Acad Sci U S A* 97 (11):5807-11.

Lee, K. B., D. Wang, S. J. Lippard, and P. A. Sharp. 2002. Transcription-coupled and DNA damage-dependent ubiquitination of RNA polymerase II in vitro. *Proc Natl Acad Sci U S A* 99 (7):4239-44.

Lee, S. K., S. L. Yu, L. Prakash, and S. Prakash. 2001. Requirement for yeast RAD26, a homolog of the human CSB gene, in elongation by RNA polymerase II. *Mol Cell Biol* 21 (24):8651-6.

Legerski, R., and C. Peterson. 1992. Expression cloning of a human DNA repair gene involved in xeroderma pigmentosum group C. *Nature* 359 (6390):70-3.

Lejeune, D., X. Chen, C. Ruggiero, S. Berryhill, B. Ding, and S. Li. 2009. Yeast Elc1 plays an important role in global genomic repair but not in transcription coupled repair. *DNA Repair (Amst)* 8 (1):40-50.

Levesque, N., G. P. Leung, A. K. Fok, T. I. Schmidt, and M. S. Kobor. 2010. Loss of H3 K79 trimethylation leads to suppression of Rtt107-dependent DNA damage sensitivity through the translesion synthesis pathway. *J Biol Chem* 285 (45):35113-22.

Li, S., X. Chen, C. Ruggiero, B. Ding, and M. J. Smerdon. 2006. Modulation of Rad26- and Rpb9-mediated DNA repair by different promoter elements. *J Biol Chem* 281 (48):36643-51.

Li, S., B. Ding, D. Lejeune, C. Ruggiero, X. Chen, and M. J. Smerdon. 2007. The roles of Rad16 and Rad26 in repairing repressed and actively transcribed genes in yeast. *DNA Repair (Amst)* 6 (11):1596-606.

Li, S., and M. J. Smerdon. 2002. Rpb4 and Rpb9 mediate subpathways of transcription-coupled DNA repair in Saccharomyces cerevisiae. *EMBO J* 21 (21):5921-9.

Li, S., and M. J. Smerdon. 2004. Dissecting transcription-coupled and global genomic repair in the chromatin of yeast GAL1-10 genes. *J Biol Chem* 279 (14):14418-26.

Lindahl, T., and R. D. Wood. 1999. Quality control by DNA repair. *Science* 286 (5446):1897-905.

Liu, Y., L. Warfield, C. Zhang, J. Luo, J. Allen, W. H. Lang, J. Ranish, K. M. Shokat, and S. Hahn. 2009. Phosphorylation of the transcription elongation factor Spt5 by yeast Bur1 kinase stimulates recruitment of the PAF complex. *Mol Cell Biol* 29 (17):4852-63.

Lommel, L., M. E. Bucheli, and K. S. Sweder. 2000. Transcription-coupled repair in yeast is independent from ubiquitylation of RNA pol II: implications for Cockayne's syndrome. *Proc Natl Acad Sci U S A* 97 (16):9088-92.

Luger, K., A. W. Mader, R. K. Richmond, D. F. Sargent, and T. J. Richmond. 1997. Crystal structure of the nucleosome core particle at 2.8 A resolution. *Nature* 389 (6648):251-60.

Malone, E. A., J. S. Fassler, and F. Winston. 1993. Molecular and genetic characterization of SPT4, a gene important for transcription initiation in Saccharomyces cerevisiae. *Mol Gen Genet* 237 (3):449-59.

Martens, J. A., and F. Winston. 2003. Recent advances in understanding chromatin remodeling by Swi/Snf complexes. *Curr Opin Genet Dev* 13 (2):136-42.

Martinez-Rucobo, F. W., S. Sainsbury, A. C. Cheung, and P. Cramer. 2011. Architecture of the RNA polymerase-Spt4/5 complex and basis of universal transcription processivity. *EMBO J* 30 (7):1302-10.

Mellon, I., and P. C. Hanawalt. 1989. Induction of the Escherichia coli lactose operon selectively increases repair of its transcribed DNA strand. *Nature* 342 (6245): 95-8.

Mellon, I., G. Spivak, and P. C. Hanawalt. 1987. Selective removal of transcription-blocking DNA damage from the transcribed strand of the mammalian DHFR gene. *Cell* 51 (2):241-9.

Mone, M. J., T. Bernas, C. Dinant, F. A. Goedvree, E. M. Manders, M. Volker, A. B. Houtsmuller, J. H. Hoeijmakers, W. Vermeulen, and R. van Driel. 2004. In vivo dynamics of chromatin-associated complex formation in mammalian nucleotide excision repair. *Proc Natl Acad Sci U S A* 101 (45):15933-7.

Nag, R., and M. J. Smerdon. 2009. Altering the chromatin landscape for nucleotide excision repair. *Mutat Res* 682 (1):13-20.

Nakanishi, S., J. S. Lee, K. E. Gardner, J. M. Gardner, Y. H. Takahashi, M. B. Chandrasekharan, Z. W. Sun, M. A. Osley, B. D. Strahl, S. L. Jaspersen, and A. Shilatifard. 2009. Histone H2BK123 monoubiquitination is the critical determinant for H3K4 and H3K79 trimethylation by COMPASS and Dot1. *J Cell Biol* 186 (3):371-7.

Newman, J. C., A. D. Bailey, and A. M. Weiner. 2006. Cockayne syndrome group B protein (CSB) plays a general role in chromatin maintenance and remodeling. *Proc Natl Acad Sci U S A* 103 (25):9613-8.

Ng, H. H., D. N. Ciccone, K. B. Morshead, M. A. Oettinger, and K. Struhl. 2003. Lysine-79 of histone H3 is hypomethylated at silenced loci in yeast and mammalian cells: a potential mechanism for position-effect variegation. *Proc Natl Acad Sci U S A* 100 (4):1820-5.

Ng, H. H., S. Dole, and K. Struhl. 2003. The Rtf1 component of the Paf1 transcriptional elongation complex is required for ubiquitination of histone H2B. *J Biol Chem* 278 (36):33625-8.

Nouspikel, T. 2009. DNA repair in mammalian cells : Nucleotide excision repair: variations on versatility. *Cell Mol Life Sci* 66 (6):994-1009.

Nouspikel, T. 2011. Multiple roles of ubiquitination in the control of nucleotide excision repair. *Mech Ageing Dev*.

Osley, M. A., T. Tsukuda, and J. A. Nickoloff. 2007. ATP-dependent chromatin remodeling factors and DNA damage repair. *Mutat Res* 618 (1-2):65-80.

Palomera-Sanchez, Z., and M. Zurita. 2011. Open, repair and close again: chromatin dynamics and the response to UV-induced DNA damage. *DNA Repair (Amst)* 10 (2):119-25.

Park, J. S., M. T. Marr, and J. W. Roberts. 2002. E. coli Transcription repair coupling factor (Mfd protein) rescues arrested complexes by promoting forward translocation. *Cell* 109 (6):757-67.

Pettijohn, D., and P. Hanawalt. 1964. Evidence for Repair-Replication of Ultraviolet Damaged DNA in Bacteria. *J Mol Biol* 9:395-410.

Pokholok, D. K., C. T. Harbison, S. Levine, M. Cole, N. M. Hannett, T. I. Lee, G. W. Bell, K. Walker, P. A. Rolfe, E. Herbolsheimer, J. Zeitlinger, F. Lewitter, D. K. Gifford, and R. A. Young. 2005. Genome-wide map of nucleosome acetylation and methylation in yeast. *Cell* 122 (4):517-27.

Ponting, C. P. 2002. Novel domains and orthologues of eukaryotic transcription elongation factors. *Nucleic Acids Res* 30 (17):3643-52.

Prakash, S., and L. Prakash. 2000. Nucleotide excision repair in yeast. *Mutat Res* 451 (1-2):13-24.

Qiu, H., C. Hu, C. M. Wong, and A. G. Hinnebusch. 2006. The Spt4p subunit of yeast DSIF stimulates association of the Paf1 complex with elongating RNA polymerase II. *Mol Cell Biol* 26 (8):3135-48.

Ramanathan, B., and M. J. Smerdon. 1989. Enhanced DNA repair synthesis in hyperacetylated nucleosomes. *J Biol Chem* 264 (19):11026-34.

Ramsey, K. L., J. J. Smith, A. Dasgupta, N. Maqani, P. Grant, and D. T. Auble. 2004. The NEF4 complex regulates Rad4 levels and utilizes Snf2/Swi2-related ATPase activity for nucleotide excision repair. *Mol Cell Biol* 24 (14):6362-78.

Ranish, J. A., S. Hahn, Y. Lu, E. C. Yi, X. J. Li, J. Eng, and R. Aebersold. 2004. Identification of TFB5, a new component of general transcription and DNA repair factor IIH. *Nat Genet* 36 (7):707-13.

Rasmussen, R. E., and R. B. Painter. 1964. Evidence for Repair of Ultra-Violet Damaged Deoxyribonucleic Acid in Cultured Mammalian Cells. *Nature* 203:1360-2.

Ratner, J. N., B. Balasubramanian, J. Corden, S. L. Warren, and D. B. Bregman. 1998. Ultraviolet radiation-induced ubiquitination and proteasomal degradation of the large subunit of RNA polymerase II. Implications for transcription-coupled DNA repair. *J Biol Chem* 273 (9):5184-9.

Reed, S. H. 2005. Nucleotide excision repair in chromatin: the shape of things to come. *DNA Repair (Amst)* 4 (8):909-18.

Ribar, B., L. Prakash, and S. Prakash. 2006. Requirement of ELC1 for RNA polymerase II polyubiquitylation and degradation in response to DNA damage in Saccharomyces cerevisiae. *Mol Cell Biol* 26 (11):3999-4005.

Ribar, B., L. Prakash, and S. Prakash. 2007. ELA1 and CUL3 are required along with ELC1 for RNA polymerase II polyubiquitylation and degradation in DNA-damaged yeast cells. *Mol Cell Biol* 27 (8):3211-6.

Saeki, H., and J. Q. Svejstrup. 2009. Stability, flexibility, and dynamic interactions of colliding RNA polymerase II elongation complexes. *Mol Cell* 35 (2):191-205.

Sarkar, S., R. Kiely, and P. J. McHugh. 2010. The Ino80 chromatin-remodeling complex restores chromatin structure during UV DNA damage repair. *J Cell Biol* 191 (6):1061-8.

Sarker, A. H., S. E. Tsutakawa, S. Kostek, C. Ng, D. S. Shin, M. Peris, E. Campeau, J. A. Tainer, E. Nogales, and P. K. Cooper. 2005. Recognition of RNA polymerase II and transcription bubbles by XPG, CSB, and TFIIH: insights for transcription-coupled repair and Cockayne Syndrome. *Mol Cell* 20 (2):187-98.

Schulze, J. M., J. Jackson, S. Nakanishi, J. M. Gardner, T. Hentrich, J. Haug, M. Johnston, S. L. Jaspersen, M. S. Kobor, and A. Shilatifard. 2009. Linking cell cycle to histone modifications: SBF and H2B monoubiquitination machinery and cell-cycle regulation of H3K79 dimethylation. *Mol Cell* 35 (5):626-41.

Selby, C. P., and A. Sancar. 1994. Mechanisms of transcription-repair coupling and mutation frequency decline. *Microbiol Rev* 58 (3):317-29.

Selby, C. P., and A. Sancar. 1997. Human transcription-repair coupling factor CSB/ERCC6 is a DNA-stimulated ATPase but is not a helicase and does not disrupt the ternary transcription complex of stalled RNA polymerase II. *J Biol Chem* 272 (3):1885-90.

Shahbazian, M. D., K. Zhang, and M. Grunstein. 2005. Histone H2B ubiquitylation controls processive methylation but not monomethylation by Dot1 and Set1. *Mol Cell* 19 (2):271-7.

Smerdon, M. J., S. Y. Lan, R. E. Calza, and R. Reeves. 1982. Sodium butyrate stimulates DNA repair in UV-irradiated normal and xeroderma pigmentosum human fibroblasts. *J Biol Chem* 257 (22):13441-7.

Smerdon, M. J., and F. Thoma. 1990. Site-specific DNA repair at the nucleosome level in a yeast minichromosome. *Cell* 61 (4):675-84.

Squazzo, S. L., P. J. Costa, D. L. Lindstrom, K. E. Kumer, R. Simic, J. L. Jennings, A. J. Link, K. M. Arndt, and G. A. Hartzog. 2002. The Paf1 complex physically and functionally associates with transcription elongation factors in vivo. *EMBO J* 21 (7):1764-74.

Struhl, K. 2007. Transcriptional noise and the fidelity of initiation by RNA polymerase II. *Nat Struct Mol Biol* 14 (2):103-5.

Sung, P., P. Reynolds, L. Prakash, and S. Prakash. 1993. Purification and characterization of the Saccharomyces cerevisiae RAD1/RAD10 endonuclease. *J Biol Chem* 268 (35):26391-9.

Svejstrup, J. Q. 2002. Mechanisms of transcription-coupled DNA repair. *Nat Rev Mol Cell Biol* 3 (1):21-9.

Swanson, M. S., E. A. Malone, and F. Winston. 1991. SPT5, an essential gene important for normal transcription in Saccharomyces cerevisiae, encodes an acidic nuclear protein with a carboxy-terminal repeat. *Mol Cell Biol* 11 (6):3009-19.

Tatum, D., and S. Li. 2011. Evidence that the histone methyltransferase dot1 mediates global genomic repair by methylating histone h3 on lysine 79. *J Biol Chem* 286 (20):17530-5.

Tatum, D., Li, W., M. Placer, and S. Li. 2011. The roles of RNA polymerase II-associated factor 1 complex in different subpathways of nucleotide excision repair. *J Biol Chem.* in press.

Teng, Y., H. Liu, H. W. Gill, Y. Yu, R. Waters, and S. H. Reed. 2008. Saccharomyces cerevisiae Rad16 mediates ultraviolet-dependent histone H3 acetylation required for efficient global genome nucleotide-excision repair. *EMBO Rep* 9 (1):97-102.

Thoma, F. 1999. Light and dark in chromatin repair: repair of UV-induced DNA lesions by photolyase and nucleotide excision repair. *EMBO J* 18 (23):6585-98.

Tijsterman, M., R. A. Verhage, P. van de Putte, J. G. Tasseron-de Jong, and J. Brouwer. 1997. Transitions in the coupling of transcription and nucleotide excision repair within RNA polymerase II-transcribed genes of Saccharomyces cerevisiae. *Proc Natl Acad Sci U S A* 94 (15):8027-32.

Tomkinson, A. E., A. J. Bardwell, N. Tappe, W. Ramos, and E. C. Friedberg. 1994. Purification of Rad1 protein from Saccharomyces cerevisiae and further characterization of the Rad1/Rad10 endonuclease complex. *Biochemistry* 33 (17):5305-11.

van Gool, A. J., R. Verhage, S. M. Swagemakers, P. van de Putte, J. Brouwer, C. Troelstra, D. Bootsma, and J. H. Hoeijmakers. 1994. RAD26, the functional S. cerevisiae homolog of the Cockayne syndrome B gene ERCC6. *EMBO J* 13 (22):5361-9.

van Leeuwen, F., P. R. Gafken, and D. E. Gottschling. 2002. Dot1p modulates silencing in yeast by methylation of the nucleosome core. *Cell* 109 (6):745-56.

Verhage, R. A., J. Heyn, P. van de Putte, and J. Brouwer. 1997. Transcription elongation factor S-II is not required for transcription-coupled repair in yeast. *Mol Gen Genet* 254 (3):284-90.

Verhage, R. A., A. J. van Gool, N. de Groot, J. H. Hoeijmakers, P. van de Putte, and J. Brouwer. 1996. Double mutants of Saccharomyces cerevisiae with alterations in global genome and transcription-coupled repair. *Mol Cell Biol* 16 (2):496-502.

Verhage, R., A. M. Zeeman, N. de Groot, F. Gleig, D. D. Bang, P. van de Putte, and J. Brouwer. 1994. The RAD7 and RAD16 genes, which are essential for pyrimidine dimer removal from the silent mating type loci, are also required for repair of the nontranscribed strand of an active gene in Saccharomyces cerevisiae. *Mol Cell Biol* 14 (9):6135-42.

Vermeulen, W., E. Bergmann, J. Auriol, S. Rademakers, P. Frit, E. Appeldoorn, J. H. Hoeijmakers, and J. M. Egly. 2000. Sublimiting concentration of TFIIH transcription/DNA repair factor causes TTD-A trichothiodystrophy disorder. *Nat Genet* 26 (3):307-13.

Wang, H., L. Zhai, J. Xu, H. Y. Joo, S. Jackson, H. Erdjument-Bromage, P. Tempst, Y. Xiong, and Y. Zhang. 2006. Histone H3 and H4 ubiquitylation by the CUL4-DDB-ROC1 ubiquitin ligase facilitates cellular response to DNA damage. *Mol Cell* 22 (3):383-94.

Waters, R., S. H. Reed, Y. Yu, and Y. Teng. 2008. Chromatin modifications and nucleotide excision repair. *SEB Exp Biol Ser* 59:189-201.

Waters, R., Y. Teng, Y. Yu, S. Yu, and S. H. Reed. 2009. Tilting at windmills? The nucleotide excision repair of chromosomal DNA. *DNA Repair (Amst)* 8 (2):146-52.

Winston, F. 2001. Control of eukaryotic transcription elongation. *Genome Biol* 2 (2):REVIEWS1006.

Wolffe, A. P. 1999. *Chromatin: Structure and Function*. 3rd ed. London and New York: Academic Press.

Wood, A., N. J. Krogan, J. Dover, J. Schneider, J. Heidt, M. A. Boateng, K. Dean, A. Golshani, Y. Zhang, J. F. Greenblatt, M. Johnston, and A. Shilatifard. 2003. Bre1, an E3 ubiquitin ligase required for recruitment and substrate selection of Rad6 at a promoter. *Mol Cell* 11 (1):267-74.

Woudstra, E. C., C. Gilbert, J. Fellows, L. Jansen, J. Brouwer, H. Erdjument-Bromage, P. Tempst, and J. Q. Svejstrup. 2002. A Rad26-Def1 complex coordinates repair and RNA pol II proteolysis in response to DNA damage. *Nature* 415 (6874):929-33.

Woychik, N. A., and R. A. Young. 1989. RNA polymerase II subunit RPB4 is essential for high- and low-temperature yeast cell growth. *Mol Cell Biol* 9 (7):2854-9.

Wu, X., E. Braithwaite, and Z. Wang. 1999. DNA ligation during excision repair in yeast cell-free extracts is specifically catalyzed by the CDC9 gene product. *Biochemistry* 38 (9):2628-35.

Wu, X., D. Guo, F. Yuan, and Z. Wang. 2001. Accessibility of DNA polymerases to repair synthesis during nucleotide excision repair in yeast cell-free extracts. *Nucleic Acids Res* 29 (14):3123-30.

Yao, S., A. Neiman, and G. Prelich. 2000. BUR1 and BUR2 encode a divergent cyclin-dependent kinase-cyclin complex important for transcription in vivo. *Mol Cell Biol* 20 (19):7080-7.

Yu, S., T. Owen-Hughes, E. C. Friedberg, R. Waters, and S. H. Reed. 2004. The yeast Rad7/Rad16/Abf1 complex generates superhelical torsion in DNA that is required for nucleotide excision repair. *DNA Repair (Amst)* 3 (3):277-87.

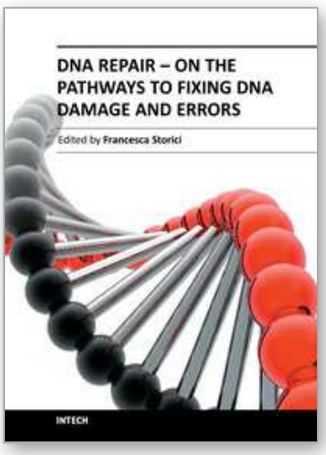
Yu, Y., Y. Teng, H. Liu, S. H. Reed, and R. Waters. 2005. UV irradiation stimulates histone acetylation and chromatin remodeling at a repressed yeast locus. *Proc Natl Acad Sci U S A* 102 (24):8650-5.

Zhang, L., K. Jones, and F. Gong. 2009. The molecular basis of chromatin dynamics during nucleotide excision repair. *Biochem Cell Biol* 87 (1):265-72.

Zhou, K., W. H. Kuo, J. Fillingham, and J. F. Greenblatt. 2009. Control of transcriptional elongation and cotranscriptional histone modification by the yeast BUR kinase substrate Spt5. *Proc Natl Acad Sci U S A* 106 (17):6956-61.

Zhou, W., P. Zhu, J. Wang, G. Pascual, K. A. Ohgi, J. Lozach, C. K. Glass, and M. G. Rosenfeld. 2008. Histone H2A monoubiquitination represses transcription by inhibiting RNA polymerase II transcriptional elongation. *Mol Cell* 29 (1):69-80.

Zhou, Y., H. Kou, and Z. Wang. 2007. Tfb5 interacts with Tfb2 and facilitates nucleotide excision repair in yeast. *Nucleic Acids Res* 35 (3):861-71.



DNA Repair - On the Pathways to Fixing DNA Damage and Errors
Edited by Dr. Francesca Storici

ISBN 978-953-307-649-2
Hard cover, 380 pages
Publisher InTech
Published online 09, September, 2011
Published in print edition September, 2011

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Danielle Tatum and Shisheng Li (2011). Nucleotide Excision Repair in S. cerevisiae, DNA Repair - On the Pathways to Fixing DNA Damage and Errors, Dr. Francesca Storici (Ed.), ISBN: 978-953-307-649-2, InTech, Available from: http://www.intechopen.com/books/dna-repair-on-the-pathways-to-fixing-dna-damage-and-errors/nucleotide-excision-repair-in-s-cerevisiae

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