The Intertwined Roles of Transcription and Repair Proteins

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Abstract

Transcription is apparently risky business. Its intrinsic mutagenic potential must be kept in check by networks of DNA repair factors that monitor the transcription process to repair DNA lesions that could otherwise compromise transcriptional fidelity and genome integrity. Intriguingly, recent studies point to an even more direct function of DNA repair complexes as co-activators of transcription and the unexpected role of "scheduled" DNA damage/repair at gene promoters. Paradoxically, spontaneous DNA double-strand breaks also induce ectopic transcription that is essential for repair. Thus, transcription, DNA damage and repair may be more physically and functionally intertwined than previously appreciated.

Introduction

Accurate processing of genetic information by transcription is vital for development and survival of the organism. Execution of these gene expression programs in a stage- and cell type-specific manner requires the coordinated assembly of the transcription apparatus at select gene promoters (Lemon and Tjian, 2000). Transcriptional activation involves the initial recognition of key regulatory DNA elements at promoters by sequence-specific DNA-binding activators and the core transcription machinery, along with the recruitment of essential cofactors (Fong et al., 2012; Lemon and Tjian, 2000; Naar et al., 2001; Roeder, 2005). Within this large protein ensemble termed the preinitiation complex (PIC), a series of enzymatic reactions and extensive remodeling of protein-DNA and protein-protein transactions must occur before transcription commences (He et al., 2013).

The highly choreographed cascade of events leading to gene activation provides numerous points of regulation and fine tuning. This remarkable flexibility, a necessary property to ensure target specificity and transcriptional fidelity, also makes transcription particularly sensitive to perturbations in the genome including DNA damage. Chromosomal DNA is under relentless attack from both endogenous byproducts of cellular metabolism (e.g. reactive oxygen species) and exogenous sources of environmental stress (e.g. ultraviolet light). These genotoxic agents create DNA breaks and adducts that, if left unresolved, can be detrimental to both DNA replication and transcription, and, ultimately, cell function and survival (Hoeijmakers, 2001). These genomic insults may be especially pertinent to stem

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cells where the consequences of unrepaired DNA damage can be profound. Mutations acquired by stem cells become amplified through self-renewal and, at the same time, propagated to progenitor cells that can differentiate to form a substantial part of a tissue, or in the case of embryonic stem cells, an entire organism (Mandal et al., 2011). In fact, proliferation of these damaged cells often manifests itself in diseases such as premature aging, developmental disorders and cancer (Diderich et al., 2011; Iyama and Wilson, 2013).

Considering the frequency at which DNA damage occurs ($\sim10^4$ events per day (Lindahl and Barnes, 2000)) and the broad spectrum of damage incurred by a cell, it is remarkable that the overwhelming majority of these offending DNA lesions are repaired with impressive accuracy and efficiency. Indeed, cells have evolved multiple, often overlapping, mechanisms to sense and demarcate the DNA lesions, while their repair is carried out by one (or more) of the four major pathways: base excision repair (BER), nucleotide excision repair (NER), mismatch repair (MMR) and recombinational repair (Figure 1 and see reviews (Iyama and Wilson, 2013; Sancar et al., 2004)).

Given that transcription and DNA repair both involve intimate transactions with DNA, it is perhaps not surprising that these two processes are often coupled and, as we shall discuss in this review, perhaps also interdependent and cross functional. It is well known that there is preferential repair of the transcribed DNA strand in expressed genes by transcription-coupled repair (TCR), a sub-pathway of NER (Hanawalt and Spivak, 2008; Mellon et al., 1987). Central to this repair process is the recruitment of Cockayne Syndrome B (CSB), TFIIH, and Xeroderma pigmentosum G (XPG) to RNA polymerase II arrested at damaged site (Saxowsky and Doetsch, 2006; Svejstrup, 2002). In addition, all three factors have well-defined roles in transcription (Citterio et al., 2000; Ito et al., 2007; Schaeffer et al., 1993) while XPG and TFIIH are also key players in global genome repair (GGR), the other branch of NER, thus highlighting the interconnected nature of transcription and DNA repair (Kamileri et al., 2012a). In this review, we discuss recent findings suggesting a more complex, unanticipated interplay between transcription and DNA repair beyond TCR. A growing list of proteins and protein complexes that were long thought to function exclusively in DNA repair are revealing themselves to be involved in transcription as well. In catastrophic events like double strand breaks (DSBs), damage-induced ectopic transcription at such lesion appears to be an essential initiating event of the repair process. On the other hand, there is also accumulating evidence pointing to a role of eliciting DNA damage at gene promoters that can influence transcriptional activation. This extensive two-way crosstalk between transcription and DNA repair may arise in part from the fact that transcription is an inherently mutagenic process.

DNA repair factors that double as transcription factors

Mutations in NER factors CSB, XPG, and the helicase subunits of TFIIH (XPB and XPD) generate complex multi-symptom phenotypes that cannot be readily explained by defects in DNA repair alone. The realization that the same set of proteins also participates in transcriptional control resolved this long standing puzzle by pointing to a transcriptional component in disease etiology (Compe and Egly, 2012; Kamileri et al., 2012a). Since then, more factors in the NER and other repair pathways have also been implicated in transcriptional activation (Bradsher et al., 2002; Fong et al., 2012; Iben et al., 2002; Le May et al., 2010a). By taking full advantage of their DNA nuclease, glycosylase, or helicase and related ATPase activities, repair proteins facilitate the remodeling of the epigenetic landscape and topology of chromatin at gene promoters. Perhaps more surprisingly, they can also recruit cofactors and/or interface with the transcription apparatus by acting as classical transcriptional activators and co-activators (Table 1 and Figure 2).

Nucleotide excision repair

XPC is a member of the Xeroderma pigmentosum family that is dispensable for TCR but essential for initiating GGR (Venema et al., 1990). If XPC functions purely as a sensor for bulky DNA lesions, scanning for damage by XPC ought to be transient (Camenisch et al., 2009), and its interaction with DNA should display neither sequence nor positional preference. Instead, in HeLa cells and human fibroblasts, XPC was found to bind hormone-inducible gene promoters (e.g. *RARβ2*) and nucleate the assembly of an entire NER complex in a transcription-dependent but DNA damage-independent manner, thus arguing for an expanded role of NER factors in gene regulation (Le May et al., 2010b) (Figure 2A). Indeed, depletion of XPC disrupted NER complex assembly at *RARβ2* and significantly attenuated its transcriptional response to retinoic acid induction. Optimal expression of *RARβ2* requires DNA breaks elicited by endonucleases XPG and XPF, which, by a yet unknown mechanism, enable CTCF recruitment and gene looping (Le May et al., 2012). These coordinated events are accompanied by active DNA demethylation that could depend on the DNA damage-inducible protein GADD45A, although its involvement remains controversial (Barreto et al., 2007; Jin et al., 2008; Schmitz et al., 2009). Consistent with these observations, the ERCC1-XPF complex has also been shown to regulate transcription initiation of genes associated with growth in mice (Kamileri et al., 2012b).

It is worth noting that the absence of NER factors blunted but did not abolish transcriptional activation of only a subset of genes (Le May et al., 2010b). The intrinsic affinity of XPC for DNA (Krasikova et al., 2013) alone cannot explain how XPC is directed to this subset of hormone-inducible and transcriptionally-poised gene promoters. Instead, we favor a model where XPC is recruited to promoters by gene-specific activators, or potentially through recognition of DNA bends and distortions induced by binding of nuclear hormone receptors to their cognate response elements (Nardulli and Shapiro, 1993; Robinson et al., 1998; Sugasawa et al., 2001). Assembly of the NER complex at gene promoters can be further stabilized by interactions with core components of the PIC (Kamileri et al., 2012b; Yokoi et al., 2000). Therefore, DNA repair factors may act as "facilitators" of gene activation by creating a favorable structural and epigenetic configuration for transcription in a gene- and possibly cell type-specific manner.

Additional evidence of cell type-specific transcriptional regulation by NER factors came from a recent study using an unbiased in vitro reconstituted system to biochemically screen for factors that are required for transcription of the stem cell pluripotency gene *Nanog* (Chambers et al., 2003; Mitsui et al., 2003). The use of a highly integrated in vitro transcription assay uncovered an activity enriched in an embryonic stem (ES) cell nuclear extract that is essential for the key stem cell-specific activators OCT4 and SOX2 to activate *Nanog* (Fong et al., 2011). This newly detected activity turned out surprisingly to be the XPC-RAD23B-Centrin 2 complex (referred to as XPC hereafter) (Araki et al., 2001) (Figure 2B). Although this result mirrors to some extent what is known about the role of XPC and other NER factors in general transcription, how XPC mediates OCT4/SOX2-specific activated transcription in ES cells seemed to differ fundamentally from its action in differentiated cell types in several respects. First, XPC can directly potentiate the transcription of *Nanog* in the absence of other XP proteins (except TFIIH that is required to form an active prototypic PIC), suggesting that the assembly of a NER complex initiated by XPC and the subsequent DNA breaks induced by XPF and XPG are dispensable for *Nanog* activation at least in vitro. Furthermore, XPC occupies distal enhancers of a remarkably high number of OCT4/SOX2-target genes in ES cells (~70%) (Fong et al., 2011). It also became clear that XPC is not merely a passive partner of OCT4 and SOX2 because disruption of the XPC complex compromises ES cell transcriptional responses, self-renewal and somatic cell reprogramming. Instead, a wealth of evidence suggests that XPC acts as a functionally

important stem cell selective co-activator for OCT4 and SOX2 wherein XPC is recruited to enhancers likely via a direct interaction with the activators (Fong et al., 2011). Coopting a DNA repair factor by activators to drive stem cell-specific transcription may also provide the added benefits of protecting the integrity of genes essential for self-renewal and pluripotency from DNA damage (Etchegaray and Mostoslavsky, 2011).

Base excision repair

Active DNA demethylation is thought to play a key role in transcriptional control and resetting of epigenetic memory during embryonic development and cellular reprogramming (Wu and Zhang, 2010). Although there are multiple mechanisms by which 5-methylcytosine (5mC) is removed in mammals, a common end point to some of these processes appears to be excision of the deaminated and/or oxidized derivatives of 5mC by the BER enzyme thymine DNA glycosylase (TDG) (Cortazar et al., 2007; Franchini et al., 2012). However, how prevalent and where TDG-mediated active DNA demethylation occurs in the mammalian genome is not well understood. Four recent reports suggest that it is extensive (Cortazar et al., 2011; Cortellino et al., 2011; Shen et al., 2013; Song et al., 2013). Dynamic DNA demethylation occurs preferentially at promoters of silent and developmentally poised genes, and distal enhancers of active genes in mouse ES cells (Shen et al., 2013; Song et al., 2013). Given the pervasive nature of DNA methylation/demethylation in the genome, it was surprising that TDG knockdown in mouse ES cells only affected the expression of a handful of genes. Apparently, active DNA demethylation at distal enhancers is dispensable for ongoing transcription. Presumably, the binding of regulatory factors (i.e. activators) is not particularly perturbed by the loss of regulated 5mC/C turnover at enhancers (Shen et al., 2013). On the other hand, the ability to dynamically control DNA methylation homeostasis at promoters of developmentally- and transcriptionally-poised genes by TDG appears to be essential for the rapid and complete transcriptional response to inducers like retinoic acid (Cortazar et al., 2011; Cortellino et al., 2011) (Figure 2C). Therefore, TDG depletion evidently compromised primarily the reactivation of signal-dependent and developmentally-poised genes during ES cell differentiation and embryogenesis. This may also explain why TDG inactivation is embryonically lethal, a rather unusual phenotype given that many related DNA glycosylases are dispensable for embryogenesis (Cortazar et al., 2007).

Independent of its catalytic activity, TDG can also potentiate transcription by acting as a scaffold to bridge the transcriptional co-activator CBP/p300 to transcription factors like c-JUN (Chevray and Nathans, 1992), RAR/RXR (Cortellino et al., 2011; Um et al., 1998) and estrogen receptor α (ERα) (Chen et al., 2003) to facilitate histone modification (Figure 2C). Interestingly, the NER factor XPC has been shown to stimulate the DNA glycosylase activity of TDG via a direct interaction in vitro (Shimizu et al., 2010). This raises the intriguing possibility that XPC could also regulate transcription by coordinating with TDG and BER in active DNA demethylation at gene regulatory regions (Figure 2B). Recently, it has been shown that the most dynamically regulated and differentially methylated regions (DMRs) across different cell types are overrepresented by enhancers and transcription factor binding sites that display tissue or cell type-specific regulation (Ziller et al., 2013). In light of this finding, it is tempting to speculate that NER and BER factors could contribute to this process by cooperating with cell type-specific transcription factors.

Recombinational repair

Fanconi anemia (FA) is an autosomal recessive cancer susceptibility syndrome characterized by developmental abnormalities, bone marrow failure and increased predisposition to squamous cell carcinoma of the skin (D'Andrea and Grompe, 1997). In response to DNA damage that creates inter-strand cross-links, mono-ubiquitination of Fanconi anemia (FA) protein FANCD2 by the FA core complex facilitates the binding of FANCD2 to chromatin

(Montes de Oca et al., 2005) and initiates the repair process by recruiting SLX4/FANCP and other downstream FA proteins (Yamamoto et al., 2011). However, a recent study uncovered an unanticipated role for the mono-ubiquitinated form of FANCD2 (FANCD2-Ub) in the transcriptional activation of the tumor suppressor gene *TAp63* (Park et al., 2013). It is unclear how FANCD2-Ub regulates transcription, but its recruitment to the regulatory region of the *TAp63* promoter is dependent on SLX4, presumably through the recognition of the ubiquitin moiety on FANCD2 by the ubiquitin binding domains in SLX4. Genome-wide analysis of FANCD2-Ub binding sites identified many DNA damage-dependent or -enhanced targets including BRCA2, a key player in homologous recombination repair (Roy et al., 2011). This suggests that FANCD2-Ub and SLX4 may cooperate to generate a coordinated response to DNA damage by repairing the lesion and perhaps simultaneously acting as transcription factors to establish a gene expression program that promotes cellular senescence of damaged and potentially tumorigenic cells (Figure 2D). In fact, using the same DNA damage-induced posttranslational modification (i.e. mono-ubiquitination) on FANCD2 as a trigger for both transcriptional activation and DNA repair provides a potentially elegant solution to synchronizing the two processes. It is worth noting that XPC is both ubiquitinated and sumoylated in response to UV-induced DNA damage (Sugasawa et al., 2005; Wang et al., 2005). It would therefore be of interest to examine the functional consequences of posttranslational modifications on XPC in transcription and DNA repair and the putative crosstalk between the two processes.

Transcription facilitates DNA damage

As vital as transcription is, it is not entirely an innocuous process. Transcription inevitably exposes the DNA template to attacks by genotoxic agents and generates potentially harmful DNA structures that are prone to mutagenesis and recombination (Figure 3A). Oddly enough, gene activation sometimes also requires transient and localized DNA damage at promoters that must be repaired (Figure 3B). Therefore, involvement of DNA repair factors in transcriptional control might originate as an adaptive measure by cells to preserve genetic information that evolved to take on additional roles in transcriptional regulation, thus further blurring the line between transcription and DNA repair factors, and, to a certain extent, the processes they mediate.

Transcription-associated mutagenesis and recombination

The very act of transcription requires the separation of the two strands of a DNA double helix by RNA polymerase II (RNAPII). As RNAPII transverses the DNA, negatively supercoiled DNA accumulated behind the advancing polymerase can cause strand opening and expose single-stranded (ss) regions to chemical modifications and genotoxic agents (Rahmouni and Wells, 1992). For example, deamination of cytosine by spontaneous hydrolysis or by activation-induced cytidine deaminase (AID)/APOBEC: a family of deaminases that preferentially target ssDNA regions that, if left unresolved by BER, could lead to C-to-T substitutions (Conticello, 2008; Frederico et al., 1990). In addition to nucleotide substitutions, ssDNA regions are prone to recombination as well as expansion and contraction of tri-nucleotide repeats. These likely arise as a result of attempts by transcription and replication enzymes to negotiate local secondary structures (e.g. hairpins and loops) formed at DNA sequence repeats (see review (Kim and Jinks-Robertson, 2012)). It therefore comes as no surprise that high levels of transcription are often associated with increased spontaneous rates of mutagenesis and recombination events in phenomena known as transcriptional-associated mutation (TAM) and transcription-associated recombination (TAR) (Aguilera, 2002; Lippert et al., 2011; Mischo et al., 2011; Takahashi et al., 2011).

The good and bad of R loops

R loops are three-stranded nucleic acid structures containing an RNA-DNA duplex and a displaced, single stranded DNA (Reaban et al., 1994). They occur naturally during lagging strand DNA synthesis when DNA primase synthesizes a short RNA oligomer on ssDNA, and during transcription in the form of a transient hybrid between the newly synthesized messenger RNA (mRNA) and the template DNA at the catalytic center of RNAPII, where the length of the RNA-DNA hybrid can dictate stability and processivity of the transcription complex (Bochkareva et al., 2012; Nudler, 2012). The recombinogenic potential of R loop is highlighted in immunoglobulin (Ig) class switch recombination (CSR) at the Ig heavy chain locus, a process that is critical to diversifying the antibody repertoire during an immune response (Lieber, 2010). Transcription through the G-rich switch (S) regions generates R loops that can exceed 1 kilobase in size (Yu et al., 2003). This enables AID-dependent modification of the displaced ssDNA. Deamination of cytosine to uracil by AID is thought to provoke base excision by the BER factor uracil DNA glycosylase, followed by recruitment of endonucleases to create single- and double-strand breaks within the S region. Class switching as a result of forming new S-S junctions is then achieved by the non-homologous end joining (NHEJ) recombinational repair mechanism. R loops are therefore functionally important regulatory structures that appear to impact a wide array of cellular processes (Aguilera and Garcia-Muse, 2012).

R loops are also sources of TAM and TAR (Figure 3A). In addition to an increased vulnerability of ssDNA regions to attacks by genotoxic agents and nucleases, the RNA-DNA hybrid in an R loop can potentially prime error-prone DNA synthesis (Aguilera and Garcia-Muse, 2012). As in the case of CSR, ectopic R loops can generate DNA strand breaks by nucleases and promote downstream recombination events (Li and Manley, 2006). Indeed, defects in the molecular pathways limiting R loop genesis and clearance have been linked to human diseases (Lin and Wilson, 2012; Yuce and West, 2013). Fortunately, redundant mechanisms exist to suppress ectopic R loop structures induced by transcription. DNA strand opening due to torsional strain generated by transcription (or replication) is counteracted by Topoisomerase I (TopoI) that removes DNA supercoils. In fact, it has recently been shown that transcription of exceptionally long genes (>200 kilobases) is particularly sensitive to TopoI inactivation (King et al., 2013). A key step in R loop formation is the invasion and hybridization of the nascent mRNA to the template strand. It has been shown that coating of mRNA by splicing factor ASF/SF2 precludes RNA-DNA hybridization by packaging nascent mRNA into ribonucleoprotein complexes (Li and Manley, 2005). RNA-DNA hybrids can also be removed through unwinding by helicases or digestion of the RNA moiety by RNase H (Aguilera and Garcia-Muse, 2012). Co-transcriptional R loops are therefore rare but highly disruptive structures that pose major threats to genome stability when one of these fail-safe mechanisms is compromised (Gan et al., 2011; Wahba et al., 2013).

Transcriptional activation may require "scheduled" DNA damage

Cells have developed multiple mechanisms to minimize the mutagenic potential of DNA damage. In the case of DSBs in gene bodies, Polycomb repressive complexes (PRCs) accumulate at break sites and are thought to mediate transcriptional silencing as an adaptive response to reduce interference between repair and the transcription apparatus (Vissers et al., 2012). Transcriptional inhibition has also been observed in BER of oxidative DNA damage sites that do not arrest transcribing RNAPII (and therefore do not evoke TCR), likely as a preventive measure against miscoding by RNAPII (Khobta and Epe, 2012). While global damage on DNA is generally associated with gene silencing, targeted DSB formation and localized DNA base damage have been implicated in transcriptional activation by nuclear hormone receptors (Ju et al., 2006; Lin et al., 2009; Perillo et al., 2008). Eliciting a

"scheduled" DNA base damage appears to be a common first step in hormone-dependent activation. Liganded androgen receptor recruits AID to deaminate cytosine (Lin et al., 2009) while estrogen receptor activates resident lysine-specific demethylase 1 (LSD1) to demethylate histone 3 lysine 9, an oxidative process that releases hydrogen peroxide and converts nearby guanines into 8-oxo-guanines (Perillo et al., 2008). Their ensuing repair by DNA glycosylases is thought to generate transient DNA nicks that act as entry points for DNA endonucleases like Topoisomerase IIβ (TopoIIβ) (Ju et al., 2006; Perillo et al., 2008) or LINE-1 repeat encoded ORF2 endonuclease (Lin et al., 2009). The resulting DSBs are thought to relax DNA strands and facilitate the recruitment of other damage sensing and DNA repair enzymes (e.g. PARP-1 and DNA-PK) to collectively induce a permissive chromatin architecture for transcriptional activation (Ju et al., 2006). These observations are reminiscent of the proposed role of DNA breaks induced by NER factors XPG and XPF in chromatin looping (Le May et al., 2012). Perhaps relaxation of DNA strands via DSBs or nicks permits chromosome bending thereby facilitating enhancer-promoter communication and dynamic gene transcription (Figure 3B).

It is thus evident that DNA damage can have opposite effects on transcription. We presume that there must exist a mechanism that enables cells to distinguish between these "scheduled" DNA damage events linked to gene activation from those that arise spontaneously and cause undesirable consequences. After all, both processes could generate damage-induced assembly of DNA repair complexes that are, for all intents and purposes, highly similar if not identical to each other. Perhaps the transient nature of transcription-induced lesions like DSBs by TopoIIβ prevents them from eliciting a persistent DNA damage response (DDR) (Ciccia and Elledge, 2010). It is also likely that transcription can limit the amplification of a DDR by suppressing the accumulation and spreading of the phosphorylated form of histone variant H2AX (γ-H2AX) that would normally mark DSB sites and promote the retention of repair factors (Iacovoni et al., 2010).

DNA damage induces "unscheduled" transcription

Efficient repair of DSBs by recombinational mechanisms (i.e. homologous recombination (HR) or NHEJ) requires the coordinated action of factors that sense, mark and process the lesions before the repair machinery can be recruited to the damage sites. Although there is a wealth of information on protein factors involved in the initial phase of DSB repair, two recent studies point to an unanticipated role of small non-coding RNAs (ncRNAs) in repair that is conserved from plants to humans (Francia et al., 2012; Wei et al., 2012). These ~21 nucleotide short ncRNAs, termed DSB-induced small RNAs (diRNAs), are derived from ectopic transcription induced at DSB sites and processed by machinery in the microRNA biogenesis pathway. They are proposed to guide downstream DSB repair proteins to damaged sites to facilitate the repair process (Francia et al., 2012). It would be of interest to examine whether diRNAs are also involved in the repair of physiological DSBs created in V(D)J recombination and CSR during an immune response (Lieber, 2010).

It is worth noting that in *Arabidopsis thaliana*, plant-specific RNA polymerases IV and V are able to generate transcripts at and near DSB sites in both sense and antisense strands. In human cells, RNAPII can also initiate transcription from both strands but is conspicuously excluded from regions immediately surrounding the DSB sites (Wei et al., 2012). This is likely due to the mutual antagonism between γ-H2AX accumulation at DSB sites and RNAPII transcription (Iacovoni et al., 2010). This also underscores a fundamental difference in how RNAPIV/V and RNAPII can or cannot navigate DSB sites. Since the experimentally-induced DSBs were created at a single defined position within the protein coding region of an integrated reporter (Wei et al., 2012), a more immediate question is what drives ectopic initiation when regulatory sequences critical for the assembly of a

typical PIC are likely absent at DSB sites. Therefore, an alternative PIC recruitment mechanism must exist. Whether or not this DSB-induced PIC is identical to the "canonical" PIC assembled at gene promoter remains to be elucidated. However, given the recent appreciation that the make-up of a functional PIC is more flexible than previously thought (D'Alessio et al., 2009; Goodrich and Tjian, 2010; Muller et al., 2010), and that many DNA repair factors can function as transcription factors, it is conceivable that in this context DNA repair factors could directly recruit RNA polymerase to the damaged sites and initiate transcription. Thus, damage-induced PICs could be compositionally and perhaps functionally distinct from prototypical PICs.

Conclusion

In this review, we have examined various ways in which DNA repair factors can impact gene activation. But they all share one common thread: DNA repair factors are often required for transcription of developmentally-regulated and activator-dependent genes, but less so for constitutive housekeeping transcription. Based on these observations, DNA repair factors likely facilitate de novo PIC assembly at gene promoters, a rate-limiting step for gene activation (Lemon and Tjian, 2000; Michel and Cramer, 2013), by facilitating chromatin remodeling and enhancer-promoter communication.

Development of a multicellular organism from a zygote is critically dependent on a highly controlled process of cellular proliferation and differentiation. Proper execution of these processes is largely regulated at the transcriptional level (Levine and Tjian, 2003). The reverse is also true; reprogramming of cells with restricted developmental potential back to a pluripotent state requires dynamic changes in chromatin organization, histone/DNA modifications and reactivation of a complex transcriptome (Apostolou et al., 2013; Phillips-Cremins et al., 2013; Polo et al., 2012; Wei et al., 2013; Zhang et al., 2013). The expanded roles of DNA repair factors in transcriptional control may explain why inactivation of factors in the NER, HR, NHEJ and Fanconi anemia (FA) repair pathways poses such strong barriers to somatic cell reprogramming by OCT4, SOX2, KLF4 and c-MYC (Fong et al., 2011; Gonzalez et al., 2013; Molina-Estevez et al., 2013; Muller et al., 2012; Takahashi and Yamanaka, 2006). On the other hand, rapid ectopic induction of transcription of a large number of genes by these factors during the initial phase of reprogramming could promote TAM and TAR due to a surge in transcriptional load and replication stress (Helmrich et al., 2012), thus activating a DNA damage response that is prohibitive to the conversion process (Hong et al., 2009; Kawamura et al., 2009; Li et al., 2009; Marion et al., 2009; Utikal et al., 2009). This is consistent with the observation that there is a dramatic increase in frequency of DSBs, as marked by accumulation of γ-H2AX and FANCD2 foci, in partially reprogrammed cells (Gonzalez et al., 2013; Muller et al., 2012). Taken together, these finding suggest that a major threat to the intrinsic ability of somatic cells to reprogram could indeed come from within.

Requirement of DNA repair factors in stem cell pluripotency and in development may not be just a preventive mechanism to safeguard genome integrity but also a proactive mechanism to ensure that their respective transcriptional programs are robust yet dynamic enough to change in response to developmental cues. The "symbiotic" relationship between transcription and DNA repair may originate from the fact that integrity of one is dependent on the other.

Acknowledgments

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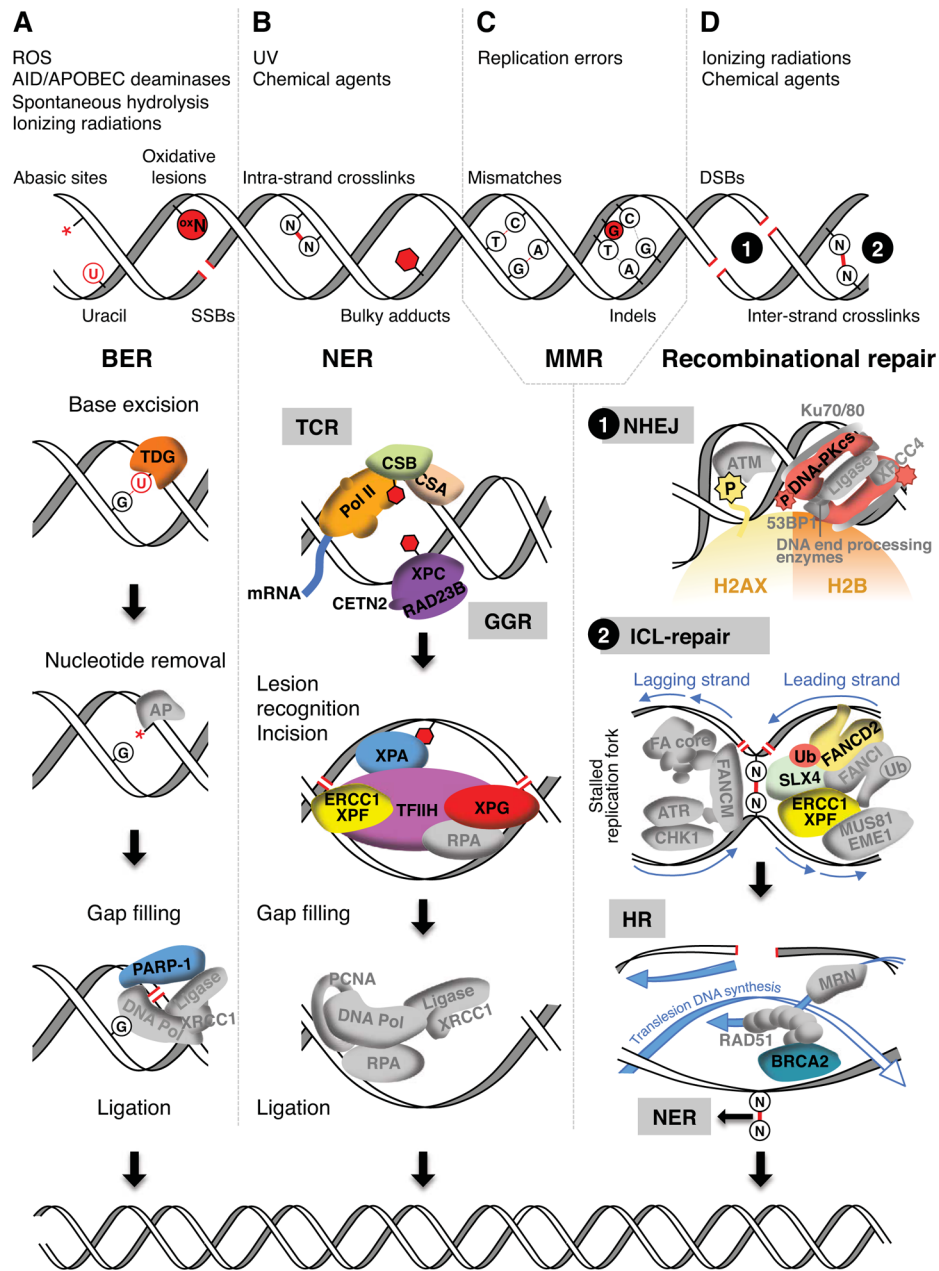
Figure 1. Major DNA repair pathways in mammals

Exogenous and endogenous genotoxic agents (top) generate a variety of DNA damage, such as single and double strand breaks (SSBs, DSBs), insertions and deletions (indels). Lesions are detected and repaired by four major DNA repair pathways: base excision repair (BER, A), nucleotide excision repair (NER, B), mismatch repair (MMR, C) and recombinational repair (D). Mechanisms of BER, NER and recombinational repair are depicted. For each pathway, factors discussed in this review are in colors.

A. Removal of uracil from DNA by BER. Thymine DNA glycosylase (TDG) removes the nitrogenous base and generates an abasic site (*). Apurinic/apyrimidinic (AP) endonuclease finalizes the nucleotide removal and creates a nick in the sugar-phosphate backbone. Poly ADP-ribose polymerase 1 (PARP-1) senses the SSB and recruits DNA polymerase and ligase to fill in the gap.

B. *Removal of large bulky adducts by NER*. Transcribing RNA polymerase II (Pol II) stalls at DNA lesions and triggers transcription-coupled NER (TCR). TCR is initiated by the recruitment of Cockayne syndrome proteins A and B (CSA, CSB) to the arrested polymerase. DNA damage on the non-transcribed regions of the genome is repaired by global genome NER (GGR) instead. DNA lesion is recognized by the repair complex comprising Xeroderma pigmentosum C (XPC), RAD23B and Centrin 2 (CETN2). Completion of TCR and GGR requires the recruitment of downstream NER factors (XPA, RPA, TFIIH, ERCC1-XPF, XPG). ERCC1-XPF and XPG endonucleases incise the damaged strand a few bases 5′ and 3′ to the DNA lesion, respectively. The gap is filled in by DNA polymerase and sealed by ligase.

D. *Removal of DSBs and inter-strand cross-links (ICL) by recombinational repair*. **1.** Repair of DSBs by non-homologous end joining (NHEJ). DSB sites are marked by Ataxia telangiectasia mutated (ATM) kinase-mediated phosphorylation of histone H2A variant X (γ-H2AX) (Burma et al., 2001). Ku proteins direct the binding of the catalytic subunits of the DNA-dependent protein kinase (DNA-PKcs) to the exposed DNA ends. Autophosphorylation of DNA-PKcs facilitates DNA-ends processing and resealing.
2. Repair of ICLs by Fanconi Anemia (FA), homologous recombination (HR) and NER pathways (see review (Deans and West, 2011)). During S-phase, converging replication forks stall at ICLs and are sensed by FANCM protein, which recruits downstream FA proteins (FA core) and initiates ATR (ATM- and Rad3-related)-CHK1 checkpoint response. The FA core complex ubiquitinates FANCD2 and FANCI. This facilitates the recruitment of endonucleases (SLX4, XPF/ERCC1, MUS81/EME1) and resection of the lesion from one of the two cross-linked strands. Translesion DNA synthesis proceeds through the uncut strand, generating the template for the homologous recombination machinery (MRN, BRCA2, RAD51) to complete DNA replication across the nicked DNA strand. NER removes the remaining adducts.
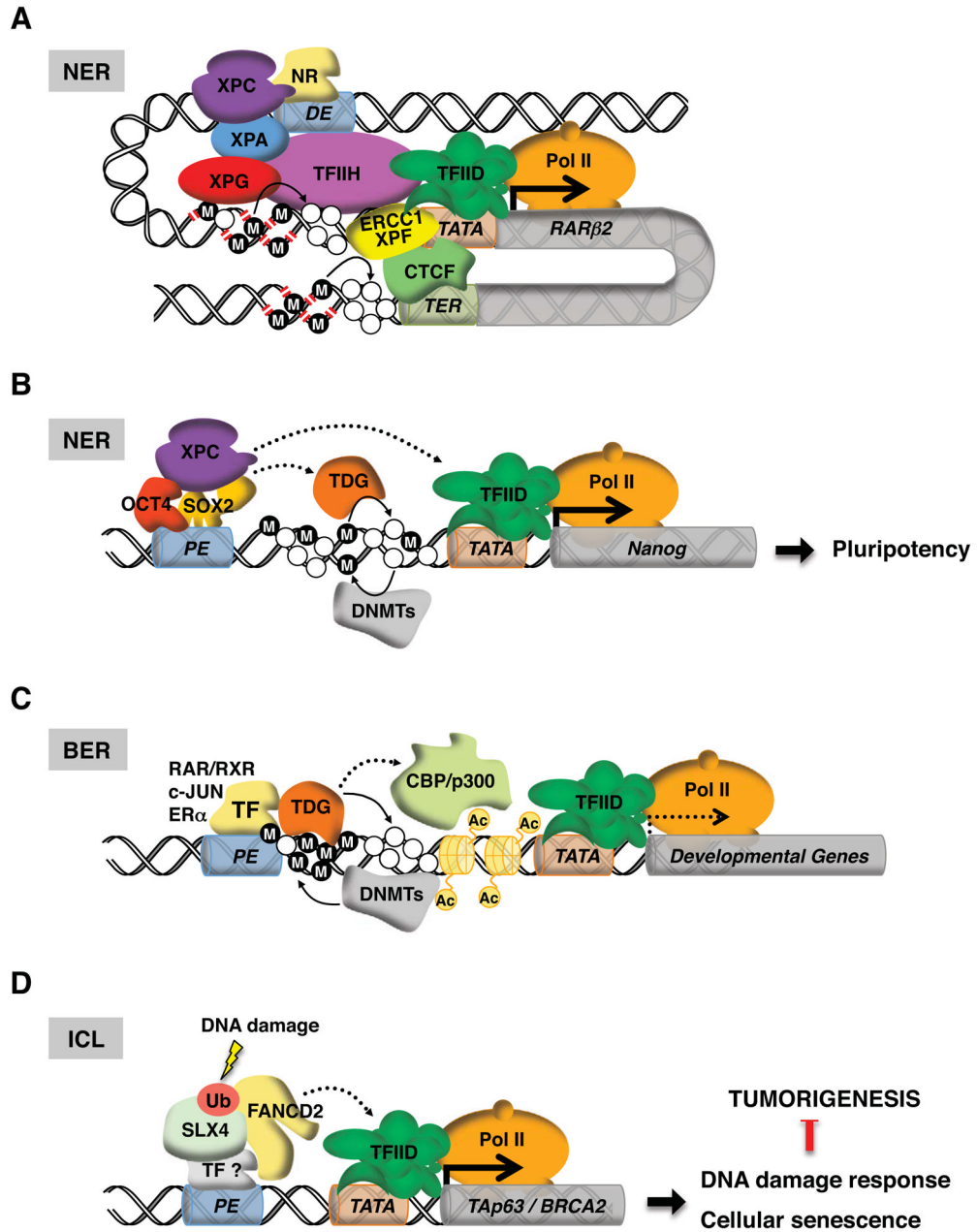
Figure 2. Proteins classically ascribed to DNA repair also participate in transcriptional control
A–B. Nucleotide excision repair (NER). **A.** XPC potentiates transcriptional activation of nuclear receptors (NR) by nucleating the assembly of the entire NER machinery at the promoter (TATA) of responsive genes (*RARβ2*, retinoic acid receptor β2 gene). XPG and ERCC1-XPF endonucleases create DNA nicks, enabling DNA demethylation (open circles represent demethylated cytosines; filled circles denote 5-methylcytosines) at gene promoter and terminator (TER), CTCF recruitment and looping between proximal and distal regulatory elements (DE, distal enhancer). **B.** In embryonic stem cells, the XPC complex functions as a transcriptional co-activator for stem cell-specific transcription factors OCT4 and SOX2 to maintain pluripotency. The mechanism by which the XPC complex stimulates transcription remains to be elucidated (dashed arrow). XPC can potentially regulate transcription by stimulating TDG-mediated DNA demethylation at gene regulatory regions.

Cytosines can be converted back to 5-methylcytosines by DNA methyltransferases (DNMTs).

B. *Base excision repair (BER).* Thymine DNA glycosylase (TDG) bridges CBP/p300 histone acetyltransferase to sequence specific transcription factors (RAR/RXR, c-JUN, ERα) and participates in active DNA demethylation at promoters (TATA) of transcriptionally-poised, developmentally-regulated genes. Re-methylation of DNA is carried out by DNMTs.

C. *Inter-strand cross-link repair (ICL).* Upon DNA damage, mono-ubiquitinated Fanconi anemia protein D2 (FANCD2) and its repair partner SLX4/FANCP bind and activate gene promoters that are implicated in tumor suppression and cellular senescence (e.g., *TAp63*, *BRCA2*). The mechanism by which FANC proteins activate transcription is unclear (dashed arrow).

A. TRANSCRIPTION INDUCES DNA DAMAGE
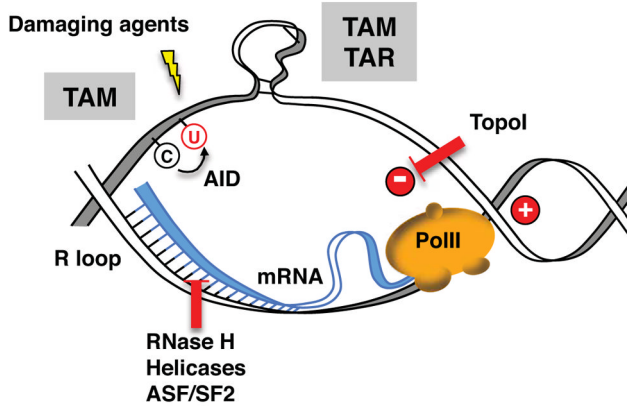
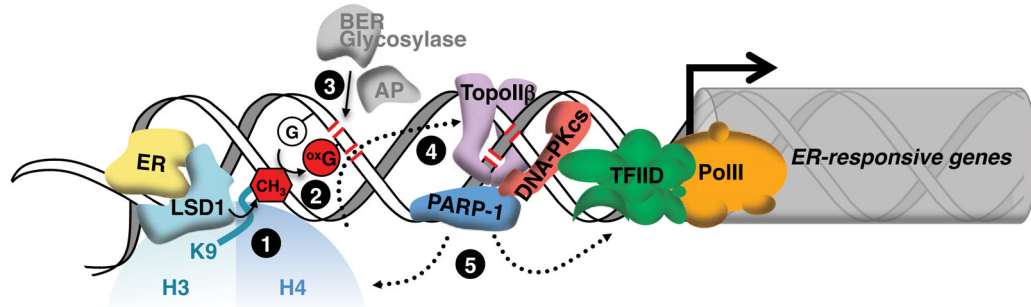## B. TRANSCRIPTION REQUIRES DNA DAMAGE

Figure 3. Transcription and DNA repair intersect

A. *Transcription is a mutagenic process.* R loops form when nascent messenger RNAs (mRNAs) hybridize back to their template. Negative (−) and positive (+) supercoiling accumulate behind and ahead of elongating RNA polymerase II (Pol II), and stabilize R loops. The displaced single-stranded DNA is highly susceptible to chemical modifications (DNA damaging agents and deamination by activation-induced cytidine deaminase (AID)), and to the formation of recombinogenic secondary structures that are prone to transcription-associated mutagenesis (TAM) and recombination (TAR). Topoisomerase I (TopoI), RNase H, helicases and splicing factors (ASF/SF2) can prevent or disrupt the formation of mutagenic R loop structures.

B. *"Scheduled" DNA damage promotes transcriptional activation.* Upon ligand binding, estrogen receptor (ER) activates histone H3 demethylase LSD1 at responsive genes ❶. The demethylation reaction releases reactive oxygen species that converts nearby guanines (G) into 8-oxo-guanines (^{ox}G) ❷. ^{ox}G removal by base excision repair (BER) creates DNA nicks ❸ that facilitate entrance of the endonuclease topoisomerase IIβ (TopoIIβ) ❹. TopoIIβ-induced double strand breaks recruit PARP-1 and DNA-PKcs repair enzymes, which induce a permissive chromatin architecture for transcription initiation ❺.

Table 1

DNA repair factors involved in repair and transcription

Protein (Official Name)	Repair Pathway	Enzymatic Activity	Function in DNA Repair	Function in Transcription
CSB (ERCC6)	TCR (NER)	DNA-dependent ATPase	Initiates TC-NER at stalled Pol II	• Functions as an ATP-dependent chromatin remodeler • Stimulates transcription by Pol I and II
DNA-PKcs	NHEJ BER	Protein kinase	• Facilitates DNA end processing and resealing in NHEJ by autophosphorylation • May stimulate BER of oxidative DNA damage	• Facilitates gene activation by chromatin remodeling • Modulates the activity of transcription factors
FANCD2	ICL	[ND]	Initiates ICL repair	Activates transcription of *TAp63* and promotes senescence of tumorigenic cells
FANCP (SLX4)	ICL	[ND]	Acts as a scaffold for multiple structure-specific endonucleases	Cooperates with FANCD2 in transcriptional activation of *TAp63*
TFIIH	TCR (NER) GGR (NER)	• ATP-dependent DNA helicase • Protein kinase	• Unwinds the DNA at damaged sites • Facilitates XPF incision	• Unwinds the DNA at gene promoters • Phosphorylates Pol II carboxy-terminal domain • Phosphorylates NRs and co-activators
PARP-1	NHEJ HR BER NER	DNA-dependent poly(ADP-ribosyl)transferase	Interacts physically and functionally with components in NHEJ, HR, BER and NER pathways	• Modulates chromatin structure • Functions as activator/co-activator or repressor • Poly(ADP-ribosyl)ates chromatin remodeling factors
TDG	BER	DNA glycosylase	Excises damaged nitrogenous bases	• Regulates DNA demethylation at gene regulatory regions • Bridges CBP/p300 to transcription factors and NRs
XPC-RAD23B-CETN2	GGR (NER) BER	[ND]	• Initiates GGR at bulky DNA lesions • Stimulates TDG-mediated BER	• Activates transcription at NR target genes • Functions as a co-activator for OCT4 and SOX2 in embryonic stem cells

Protein (Official Name)	Repair Pathway	Enzymatic Activity	Function in DNA Repair	Function in Transcription
XPF (ERCC4)	TC-NER GG-NER ICL	Structure-specific endonuclease	Incises the damaged strand 5′ to the DNA lesion	• Promotes active DNA demethylation at terminators of NR targets • Recruits CTCF and facilitates DNA looping at NR targets • Stimulates transcription initiation
XPG (ERCC5)	TCR (NER) GGR (NER)	Structure-specific endonuclease	Incises the damaged strand 3′ to the DNA lesion	• Stabilizes TFIIH • Promotes active DNA demethylation at promoters of NR targets • Recruits CTCF and facilitates DNA looping at NR targets

Definitions are as follows: BER, base excision repair; GG-NER, global genome nucleotide excision repair; HR, homologous recombination; ICL, inter-strand cross-link repair; NER, nucleotide excision repair; NHEJ, non-homologous end joining; NR, nuclear receptor; TC-NER, transcription-coupled nucleotide excision repair [ND], not determined