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Physiological functions of programmed DNA breaks in signal-induced transcription

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Abstract

The idea that signal-dependent transcription might involve the generation of transient DNA nicks or even breaks in the regulatory regions of genes, accompanied by activation of DNA damage repair pathways, would seem to be counterintuitive, as DNA damage is usually considered harmful to cellular integrity. However, recent studies have generated a substantial body of evidence that now argues that programmed DNA single- or double-strand breaks can, at least in specific cases, have a role in transcription regulation. Here, we discuss the emerging functions of DNA breaks in the relief of DNA torsional stress and in promoter and enhancer activation.

Chromatin undergoes dynamic changes in structure and flexibility to permit regulation of fundamental processes such as transcription, DNA replication and apoptosis. Correct separation of DNA strands during replication and transcription is necessary to allow the polymerization activity of DNA and RNA polymerases^{1,2}. However, deployment of RNA polymerase II (Pol II) on template DNA does not come without a cost, as the movement and rotation along the DNA of this powerful molecular motor during RNA synthesis results in the generation of DNA supercoils. Given that a positive and a negative supercoil is generated for every ten base pairs transcribed, with approximately seven supercoils generated by the transcribing polymerase per second, the amount of supercoiling is potentially enormous. As proposed in the ‘twin supercoiled domain model’, positive supercoils in front of the advancing polymerase and negative supercoils behind it (FIG. 1a) can propagate and affect transcription elongation by mechanically modifying DNA topology, structure and nucleosome arrangement^{3–5}. The natural bends in the DNA and higher-order chromatin

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structure may add to the viscous drag to ‘anchor’ DNA into a topological domain that, according to the twin supercoiled domain model, would support the formation of torsional stress⁶.

Although negative supercoiling can initially facilitate transcription initiation by enhancing DNA melting on promoters and helping Pol II to form an open complex during transcription initiation^{4,7,8}, it can subsequently lead to the generation of R-loops — three-stranded structures composed of a DNA–RNA hybrid and non-template, single-stranded DNA — which have been shown to impede transcription elongation⁹. Similarly, positive or overwound supercoils can potentiate transcription by destabilizing nucleosomes ahead of Pol II, but encumber transcription elongation through the build-up of positive torsional stress. Therefore, torsional stress-resolving enzymes such as topoisomerases are emerging as crucial components of active transcription.

In this Progress article, we discuss recent findings that highlight the significance of eukaryotic DNA topoisomerases in transcription regulation, placing particular emphasis on DNA topoisomerase 1 (TOP1)- and TOP2-mediated cleavage at promoters and enhancers of signal-regulated genes. We consider a model in which recruitment of catalytically engaged TOP1 and TOP2 at gene regulatory elements results in generation of single- and double-strand DNA breaks with concomitant activation of DNA damage response (DDR) pathways. Last, we discuss an emerging interplay between transcription machineries and components of the DDR.

DNA cleavage by topoisomerases

DNA topoisomerases evolved to relieve topological stress, the existence of which is rooted in the DNA double-helix structure. During strand breakage by a DNA topoisomerase, a Tyr oxygen of the enzyme nucleophilically attacks a phosphorus atom of the DNA, forming a covalent phosphotyrosine link and breaking a DNA phosphodiester bond at the same time¹⁰. The two major classes of topoisomerase are distinguished by the nature of the break. Whereas TOP1 (type 1B) relaxes the double helix by generating transient single-strand breaks (SSBs or nicks) (FIG. 1b), TOP2 (type IIA; TOP2 α and TOP2 β) induces transient double-strand breaks DNA (DSBs) (FIG. 1c). Of note, both classes of enzyme have the capacity to rejoin cleaved DNA ends through intrinsic intramolecular ligation activity following resolution of superhelical strain^{11–15}.

Topoisomerases have an active role in maintaining chromatin structure. For example, TOP2 α decatenates newly replicated sister chromatids by a process that involves the ATP-dependent helicase BRM/SWI2-related gene 1 (BRG1; also known as SMARCA4), which is a component of the BAF (Brg1 or HBRM-associated factors) chromatin-remodelling complex, to ensure proper chromosome segregation during mitosis¹⁶. Studies examining topoisomerase activity in nucleosomal DNA substrates have revealed that TOP2 works much more quickly than TOP1 to dissipate torsional stress at chromatin fibres. By efficiently relaxing DNA supercoiling in the chromatin fibre, where strand crossing may be frequent, TOP2 may modulate chromatin structure more effectively than TOP1 (REF. 17).

In *Saccharomyces cerevisiae*, TOP1 and TOP2 are required to maintain promoters in a superhelical state that is permissive for proper activation. The enzymes regulate the expression of a range of inducible genes — in particular, those characterized by high transcriptional plasticity¹⁸. Interestingly, in human Burkitt's lymphoma cells, the requirement for TOP1 and TOP2 to resolve dynamic supercoiling suggests a model in which the activity of promoters that support low levels of transcription are managed mainly by TOP1, distributed over a broad upstream region, whereas high transcription activity recruits TOP2 to the vicinity of transcription start sites (TSSs)⁵. Furthermore, at least a subset of highly active transcription units (defined as the gene coding sequences together with their regulatory elements, such as promoters and terminators) might require the combined activity of TOP1 and TOP2. As functional enhancers are generally transcription units themselves, which generate capped, usually non-polyadenylated, long non-coding RNAs known as enhancer RNAs (eRNAs)¹⁹, it is not unreasonable to assume that accumulated torsional stress that is generated over these relatively short (500–1500 bp) transcription units would also have to be appropriately resolved. In addition, because enhancer cores are characterized as nucleosome-depleted regions, another potential source of topological stress relates to the accumulation of negative supercoils by chromatin remodelling. During this process, ATP-dependent chromatin remodelling complexes eject or reposition nucleosomes along the DNA. The resulting unconstrained negative supercoiling, manifested as a change in DNA twist and/or writhe, can potentially aid or hinder the recruitment of transcription factors to gene regulatory sequences²⁰.

The necessity to relieve topological stress for transcription was first appreciated in prokaryotes. For example, earlier studies that examined the expression of bacteriophage T4 late expression genes showed that a nick in the DNA strand that is located a considerable distance either upstream or downstream from the promoter can promote transcription once bound by transcription factors and RNA polymerase²¹. However, cleaving DNA to regulate transcription should be weighed against the potential impact of unrepaired SSBs on genome stability. A nick in the DNA can serve as a strong R-loop initiation zone and thus block transcription^{22,23}. In addition, replication fork collapse at a nick can lead to the formation of DSBs and instigate genomic instability^{24,25}. One way to cope with an unrepaired nick would be to deploy DNA repair enzymes as a precautionary measure to ensure genomic stability during active transcription (FIG. 2a, b).

DNA breaks during transcription

Recent studies have begun to reveal an involvement of TOP1 and TOP2 in the resolution of torsional stress at gene regulatory elements such as promoters and enhancers, and in the generation of long-lived DNA breaks at transcription units. Here, we briefly discuss how DNA breaks can facilitate transcription.

DNA breaks at promoters

The requirement for TOP2 β cleavage activity at promoters was demonstrated by its role in the expression of oestrogen receptor- α (ER α) target genes²⁶. Induction of DSBs at ER α target promoters by TOP2 β results in the recruitment of components of the non-homologous

end joining (NHEJ) and homologous repair (HR) DSB repair machineries. In particular, exposure of breast cancer cells to oestrogen results in the formation of DSBs, which is dependent on ER α binding to hormone response elements at target promoters and on the catalytic activity of TOP2 β ²⁷ (FIG. 2a). Further examination of endogenous promoters from other signal-regulated genes revealed that transient DSBs could be detected at 30 min on the *PSA*, *RARb*, *DIO1* and *MMP12* promoters, suggesting that DSBs in those regulatory regions persist for long periods of time²⁶. However, in another study that focused on androgen-induced DNA breaks in prostate cancer cells, hormone stimulation of starved cells resulted in phosphorylated histone H2AX (γ H2AX) foci formation. TOP2 β -mediated DSBs could be detected in promoters and enhancers of signal-induced genes, even at 6 h after stimulation, and the DSBs were recognized by the repair machinery²⁸. The longer-lived breaks may be needed to allow timely mobilization of a number of regulators, including chromatin-remodelling proteins, to the activated transcription unit, and there may be other mechanisms that stimulate the release of catalytically engaged topoisomerases before the religation step, to allow longer-lived, transient DNA breaks to persist^{29,30} (see REF. 14 for a discussion of long-lived topoisomerase-mediated DNA breaks).

Intriguingly, ER α activates Lys-specific demethylase 1 (LSD1; also known as KDM1A) to promote local histone H3 Lys9 demethylation (at both promoters and enhancers) by an oxidative process that releases hydrogen peroxide, which in turn modifies the surrounding DNA and results in the recruitment of *N*-glycosylase/DNA lyase (also known as OGG1) for the removal of the damaged DNA bases. Removal of the oxidized bases generates transient nicks that function as entry points for TOP2 β , so that it can relax the DNA strands and support chromatin binding by the transcription initiation complex³¹. TOP2 β , the DDR factor poly(ADP-ribose) polymerase 1 (PARP1), the NHEJ complex KU70–KU80 (also known as XRCC6–XRCC5) and BRG1 are also necessary for transcription activation from endogenous promoters in response to glucocorticoid stimulation³². There is also emerging evidence that Pol II pause–release and transcription elongation require the participation of DDR proteins. Following DSB formation by TOP2 β at promoters of stimulus-regulated genes, factors such as tripartite motif containing 28 (TRIM28; also known as TIF1 β) and the DSB repair proteins ataxia telangiectasia mutated (ATM), DNA-dependent protein kinase catalytic subunit (DNA-PKcs), KU70 and γ H2AX are recruited to the sites of damage³³. Direct binding of Pol II to TOP1 was found to increase the activity of the topoisomerase within the bodies of the transcribed genes³⁴. Full activity downstream of the TSS was only observed following pause–release, a step that is highly dependent on bromodomain-containing protein 4 (BRD4)-mediated phosphorylation of Ser2 of the carboxy-terminal domain of Pol II (FIG. 2c).

The activity of TOP2 or TOP1 is not limited to hormone-regulated systems. TOP2 β -induced DSBs at promoters were recently found to mediate the expression of synaptic activity early-response genes in neurons³⁵. Intriguingly, generation of targeted breaks within the promoters of these genes is sufficient to induce their expression, even in the absence of a stimulus. For example, the *Fos* promoter is already in the transcriptionally permissive state: it is trimethylated on histone H3 Lys4 (H3K4me3) and pre-bound by Pol II and TOP2 β , as well as by transcription factors including cyclic AMP-responsive element-binding protein and serum response factor, which themselves are regulated by synaptic activity. Furthermore,

DSBs are present near TSSs in neural stem cells and neural progenitor cells, and recurrent DSB clusters are located within the bodies of long, transcribed, late-replicating genes^{36,37}. Importantly, both TOP2 β and TOP1 are essential for transcription regulation of neuronal genes that are longer than 200 kb, and their length-dependent effect on gene expression is attributed to impaired transcription elongation³⁸. Genome-wide mapping studies have also revealed that, in stem cell-derived postmitotic neurons, TOP2 β target sites are enriched at promoters and are occupied by TOP2 β during the transition from neuronal progenitors to neurons, at a time when the cells exit the cell cycle³⁹. TOP2 β occupancy positively correlates with a gene-active chromatin modification, as the TOP2 β targets are embedded in H3K4me2-enriched chromatin. Furthermore, many of the TOP2 β target genes show transcriptional changes in the absence of TOP2 β or its catalytic activity, which leads to premature neuronal degeneration³⁹. Interestingly, a recent study has also implicated TOP1 activity in the upregulation of inflammatory genes in response to infection by various pathogens⁴⁰.

DNA breaks at enhancers

Nicking of DNA by TOP1 was recently shown to occur in prostate cell lines at androgen receptor-dependent enhancers that are pre-bound by the pioneer transcription factor NKX3.1 (REF. 41). NKX3.1 stimulates the formation of TOP1–DNA complexes and enhances the catalytic activity of TOP1 towards the DNA substrate⁴². Furthermore, NKX3.1 depletion attenuates the DDR in prostate cancer cell lines^{43,44}. The recruitment of TOP1 to androgen receptor-regulated enhancers peaks by 5 min and then rapidly diminishes, and is followed by the loading of a number of factors that are involved in DNA break sensing and repair⁴¹ (FIG. 2b). Interestingly, androgen receptor associates with a number of DDR proteins, such as PARP1, the subunits of the heterotrimeric DNA-PK complex KU70–KU80–DNA-PKcs, and, importantly, TOP1 and Pol II⁴⁵. In addition, the Pol II complex contains KU70, KU80 and the leading-strand replication polymerase Pol ϵ ⁴⁶, potentially enabling recruitment of the DNA repair machinery to transcription-induced DNA damage sites.

The MRN complex, which consists of meiotic recombination 11 (MRE11), RAD50 and Nijmegen breakage syndrome protein 1 (NBS1), is a central DDR factor^{47,48}. Recent evidence shows that MRE11 may function outside of its well-established role in DSB repair or meiotic DNA processing. For example, the nuclease activity of the fission yeast orthologue of MRE11, Rad32, is involved in the removal of TOP2 from DNA 5' ends, as well as TOP1 from 3' ends⁴⁹, and cleavage by MRE11 of the covalent 3' phosphotyrosyl–DNA bonds that join TOP1 to the DNA backbone generates a product with a 3' phosphate end that MRE11–RAD50 can then resect in an ATP-dependent reaction, producing a 3' hydroxyl to permit repair^{50,51}. Therefore, it is not unlikely that the highly transcribed transcription units, which navigate a higher degree of torsional strain, might also, on occasion, generate a DSB through the action of TOP2 β , and that MRE11 would be essential for the removal of the stalled covalent TOP2 β cleavage complexes and would also participate in the ensuing classical DSB repair process to prevent the persistence of single- and double-strand breaks⁵². In this regard, mobilization of androgen receptor–TOP2 β to the regulatory regions of androgen receptor-target genes is presumed to be associated with genomic rearrangements that are frequently observed in prostate cancer^{28,53,54}.

Finally, a very recent study showed that TOP1 is required for the association of certain DDR proteins (including DNA-PKcs) with the transcription factor autoimmune regulator (AIRE) to control the activity of super-enhancers in T cells. In this study, direct genomic overlap was observed between TOP1, Pol II and γ H2AX binding on stretches of chromatin (several kilobases long) in active super-enhancers⁵⁵.

Transcriptional DNA cleavage by other enzymes

Alternative mechanisms likely exist that use factors other than topoisomerases to relax chromatin at promoters or enhancers so that transcription can occur^{26,31–39}. For example, APOBEC3B, which is a member of the family of activation-induced cytidine deaminase (AID) enzymes, is co-recruited to chromatin with ER α and is required for the regulation of gene expression by ER α . The enzyme acts by mediating C-to-U changes that lead to the generation of DNA strand breaks through activation of the base excision repair (BER) pathway. DSB repair and the accompanying chromatin remodelling aid the expression of ER α target genes⁵⁶. In B cells, AID proteins are recruited to super-enhancers and clusters of regulatory elements, and they promote DSB formation at these highly transcribed units⁵⁷. In addition, non-coding RNAs recruit AID proteins to single-strand DNA at sites of antisense and divergent transcription in B cells⁵⁸. The existence of a mechanism that involves TOP1 and AID proteins could be important for transcription regulation, as decreased levels of TOP1 result in an accumulation of Pol II in transcribed genes, which leads to enhanced recruitment of AID proteins to variable genes in the immunoglobulin λ light chain locus⁵⁹.

Other DNA-cleaving enzymes have also been implicated in transcription. For example, myoblast differentiation depends on temporal activation of the protease caspase 3 and on caspase-activated DNase (CAD), as well as on deployment of the BER protein X-ray repair cross-complementing protein 1 (XRCC1) to the promoter of the gene that encodes cyclin-dependent kinase inhibitor 1 (also known as p21), in which CAD-induced strand breaks lead to the induction of p21 expression, which is crucial for differentiation of a number of cell types^{60,61}.

Repair factors and transcription

There is now a substantial body of literature that collectively argues that programmed DNA nicks or breaks can, at least in specific cases, participate in transcription regulation (FIG. 2). Repair of these DNA breaks would primarily involve the NHEJ, homologous recombination or BER pathways to restore transcription units to the 'pre-break' state. The choice of the specific repair pathway would clearly be dictated by the nature of the DNA break or lesion and the phase of the cell cycle. In recent years, it has become increasingly clear that there is an interdependence and feedback between the transcriptional activity of nuclear receptors and the DDR. Androgen-induced signalling, for example, results in transcription of a number of DNA repair genes, which include those implicated in DNA damage sensing (MRE11, nibrin and ataxia telangiectasia and Rad3-related protein (ATR)), NHEJ (XRCC4 and KU80), homologous recombination (RAD54B and RAD51C), mismatch repair (MutS homologue 2 (MSH2) and MSH6), BER (PARP1 and DNA ligase 3) and DNA interstrand crosslinking repair (Fanconi anaemia group I protein (FANCI), FANCC and ubiquitin-

specific peptidase 1 (USP1))⁶². Of note, pharmacological inhibition of the androgen receptor results in decreased repair by classical NHEJ⁶². Androgens also positively regulate the expression of *PRKDC* and its protein product, DNA-PKcs, thus directly linking androgen receptor signalling to the NHEJ pathway⁶³. Furthermore, PARP1, an abundant nuclear enzyme, is recruited to the sites of androgen receptor binding to support its transcriptional function⁶⁴ (FIG. 2a) and is activated by DNA strand breaks²⁶. In oestrogen-dependent breast cancer cells, PARP1 promotes the binding of Pol II and components of the basal transcription machinery and maintains an open chromatin structure at the TSSs of positively regulated genes⁶⁵. Recently, the pioneer transcription factor forkhead box protein A1 (FOXA1; also known as HNF3 α) was reported to nucleate binding of DNA repair proteins, which would occur at most ER α -regulated enhancers⁶⁶. In the case of PARP1, the enzyme ADP-ribosylates the ER to promote its binding to oestrogen response elements. As such, it is indispensable for ER α -mediated gene expression⁶⁷.

The DNA-PK holoenzyme, which is composed of DNA-PKcs and the regulatory KU70–KU80 heterodimer, assembles in a stepwise manner in response to contact with DNA⁶⁸. DNA-PKcs becomes active following its association with KU70–KU80, which directs it to DNA ends. However, KU70 and KU80 can also function outside of the KU heterodimer. For example, KU80 deletion impairs BER at the initial lesion recognition and strand scission step, which is consistent with the idea that free KU70 and free KU80, but not the KU heterodimers, associate with the BER substrates apurinic/apyrimidinic sites^{69,70}. Furthermore, PARP1 is capable of stimulating the activity of DNA-PKcs (REF. 71), thus linking components of the BER and NHEJ pathways. As DNA-PKcs is activated by ER α ⁷² and is part of a mega transcription factor complex that is assembled at functional ER α enhancers⁷³ (FIG. 2b), we surmise that a combination therapy that targets the androgen receptor–DNA-PKcs–PARP1 or ER α –DNA-PKcs–PARP1 axis might be useful for the treatment of malignancies that are driven by these hormones.

The link between transcription and components of DNA repair machineries is exemplified by the basal transcription initiation factor IIIH (TFIIH) complex, which contains, in addition to other subunits, the helicases xeroderma pigmentosum group B-complementing protein (XPB) and XPD. These proteins have a dual role: in the initiation of transcription by Pol II and in the nucleotide excision repair (NER) pathway⁷⁴, in which the TFIIH complex participates in opening the DNA to allow the excision of damaged nucleotides. Furthermore, the nuclease XPG, which is recruited to TFIIH during NER, promotes DNA breaks and DNA demethylation of promoters and thus the expression of the retinoic acid receptor- β gene⁷⁵. In addition, the XPC–RAD23B–centrin 2 NER complex supports pluripotency in embryonic stem cells, presumably by affecting gene expression programmes⁷⁶.

Conclusions and future perspectives

It is becoming increasingly clear that many DNA repair factors function directly in transcription regulation. In particular, ‘programmed’ DNA nicks and breaks seem to have a strategic role in the regulation of gene expression. DNA breaks appear to be employed not only in hormone-induced transcription, but also in the regulation of some tissue-specific genes⁶¹. Although the DNA topoisomerases TOP1 and TOP2 appear to be the most widely

used DNA cleaving enzymes, other DNA cleaving enzymes can also be used. Together, programmed DNA breaks promote not only transcription elongation by Pol II but probably also the assembly of large, multiprotein regulatory complexes and the formation of long-range interactions between transcription units.

The organization of the genome into topologically associated domains⁷⁷ suggests new possible roles for DNA breaks, which include, for example, the untangling of CCCTC-binding factor- and cohesion-mediated chromatin loops, and mediating interactions between promoters and enhancers on different loops. The next few years promise to be an exciting period in our understanding of the physiological functions of programmed DNA breaks in transcription.

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Figure 1. Transcription regulation by DNA topoisomerases 1 and 2

a | The movement and rotation of RNA polymerase II (Pol II) along the DNA template results in the generation of transcription-coupled positive (+) and negative (-) supercoils. Topoisomerases resolve this torsional strain by creating transient DNA breaks, controlling the rotation of the DNA and re-ligating it. **b** | DNA topoisomerases (TOPs) form reversible, covalent enzyme–DNA adducts through their active site Tyr residue (Y). TOP1 cleaves one DNA strand and allows the duplex to swivel around the intact phosphodiester bond. **c** | TOP2 cleaves both DNA strands and allows another duplex to pass through the break. Parts **b** and **c** are modified from REF. 15 and REF. 14, respectively, Nature Publishing Group.

Figure 2. 'Programmed' DNA damage

The generation of transient DNA nicks or breaks in sites bound by nuclear receptors (NRs) is a consequence of recruitment of specific machinery, including a DNA topoisomerase (TOP), DNA damage repair co-activators, 'pioneer' transcription factors (TFs) and the RNA polymerase II (Pol II) complex to a transcription unit. **a** | Regulated gene transcription requires the recruitment and enzymatic function of TOP2 β , which produces a transient, DNA double-strand break that is required for transcription activation by nuclear receptors. Subsequent poly(ADP-ribose) polymerase 1 (PARP1) and the heterotrimeric DNA-dependent protein kinase (DNA-PK) complex composed of KU70, KU80 and DNA-PK catalytic subunit (DNA-PKcs) participate in the ensuing classical DSB repair process to prevent the persistence of unsealed double-strand breaks at promoters. **b** | Enhancer transcription requires sequential recruitment of a nuclear receptor, TOP1, transcription co-activators, DNA-PKcs and meiotic recombination 11 (MRE11) to a 'pioneer' transcription factor-occupied enhancer. Following the generation of transient DNA nicks, the deployment of DNA damage repair proteins ensures timely repair of single- and perhaps even double-strand breaks. In the case of oestrogen receptor- α (ER α)-regulated transcription, ER α participates in the *in situ* assembly of a mega transcription factor complex at functional enhancers⁷³. **c** | Transcription-induced DNA supercoiling imposes a barrier to transcript elongation and results in Pol II promoter-proximal pausing. Bromodomain containing protein 4 (BRD4) phosphorylates (P) Pol II, which in turn activates TOP1 to assist in 'pause-release' by enhancing DNA relaxation ahead of Pol II. Independently, BRD4 also activates the transcription machinery via positive transcription elongation factor b (PTEFb). By another mechanism, tripartite motif-containing protein 28 (TRIM28) phosphorylation by DNA-PKcs or ataxia telangiectasia mutated (ATM) also regulates Pol II release. eRNA, enhancer RNA; HRE, hormone response element; TSS, transcription start site.