Nucleus 5:3, 1–8; May/June 2014; © 2014 Landes Bioscience

Gearing up chromatin

A role for chromatin remodeling during the transcriptional restart upon DNA damage

Imke K Mandemaker, Wim Vermeulen, and Jurgen A Marteijn*
Department of Genetics; Erasmus Medical Centre; Rotterdam, the Netherlands

During transcription, RNA polymerase may encounter DNA lesions, which causes stalling of transcription. To overcome the RNA polymerase blocking lesions, the transcribed strand is repaired by a dedicated repair mechanism, called transcription coupled nucleotide excision repair (TC-NER). After repair is completed, it is essential that transcription restarts. So far, the regulation and exact molecular mechanism of this transcriptional restart upon genotoxic damage has remained elusive. Recently, three different chromatin remodeling factors, HIRA, FACT, and Dot1L, were identified to stimulate transcription restart after DNA damage. These factors either incorporate new histones or establish specific chromatin marks that will gear up the chromatin to subsequently promote transcription recovery. This adds a new layer to the current model of chromatin remodeling necessary for repair and indicates that this specific form of transcription, i.e., the transcriptional restart upon DNA damage, needs specific chromatin remodeling events.

Keywords: transcription coupled nucleotide excision repair (TC-NER), transcription restart, chromatin remodeling, HIRA, DOT1l, FACT, SPT16

*Correspondence to: Jurgen A Marteijn;
Email: J.Marteijn@erasmusmc.nl

Submitted: 03/29/2014; Revised: 04/28/2014;
Accepted: 04/30/2014;
Published Online: 05/08/2014

http://dx.doi.org/10.4161/nucl.29085

Extra View to: Dinant C, Ampatziadis-Michailidis G, Lans H, Tresini M, Lagarou A, Grosbart M, Theil AF, van Cappellen WA, Kimura H, Bartek J, et al. Enhanced chromatin dynamics by FACT promotes transcriptional restart after UV-induced DNA damage. Mol Cell 2013; 51:469-79; PMID:23973375; http://dx.doi.org/10.1016/j.molcel.2013.08.007

Introduction

Gene transcription is an essential process for proper cellular function. However, translocating RNA polymerases may encounter lesions in the DNA template that impede the transcription machinery and cause slowing down or even stalling of these polymerases, thereby depriving cells from essential RNA molecules or triggering a signaling response leading to apoptosis.[1] In some cases RNA Polymerase II (RNAPII) might bypass lesions, an activity most likely depending on two flexible regions of the Rpb1 subunit of RNAPII.[2] However, this translesion synthesis might result in aberrant transcripts.[3] Therefore, more preferably, these RNA polymerase blocking DNA lesions are repaired to restore transcription and thereby proper cellular functioning.[4] An example of transcription blocking lesions are cyclobutane-pyrimidine dimers (CPD) and 6-4 pyrimindinepyrimidone photo products (64PP), which are the most abundant occurring DNA lesions upon UV irradiation. Transcription coupled nucleotide excision repair (TC-NER) is a dedicated branch of the nucleotide excision repair (NER) pathway, which removes transcription-blocking lesions specifically from transcribed strands.[5] RNAPII constantly interacts with TC-NER proteins, so that upon stalling, the lesions can rapidly be recognized and repaired. For example, CSB transiently interacts with RNAPII. Upon lesion stalling of RNAPII, CSB gets more tightly associated,[6] resulting in recruitment of CSA to the damage.[7] Recently, also two other crucial TC-NER factors, UVSSA and USP7, were shown to interact with RNAPII in unperturbed conditions.[8] Together these proteins are involved in the damage recognition step and play a crucial role in the formation and function of the complete TC-NER complex (**Fig. 1**, left side). Lesions located throughout the genome are targeted by the global genome repair (GG-NER) pathway. GG-NER is initiated by the

concerted action of the XPC and DDB2 DNA damage sensing protein complexes. TC-NER and GG-NER only differ in the DNA damage recognition after which TFIIH is recruited, which partially unwinds the damaged DNA and plays an important role, together with XPA and RPA, in the damage verification. Next the endonucleases XPF/ERCC1 and XPG incise the damaged DNA strand and remove about 30 nucleotides. DNA polymerases synthesize new DNA over the single-stranded gap followed by the sealing of the nick by DNA ligases I or III to complete the NER reaction.[9] Importantly, in the specific case of TC-NER, an essential step for proper cellular functioning, is that transcription needs to resume after repair is completed.[4,5] This recovery of RNA synthesis (RRS) can be measured by pulse chase labeling with uridine analogs and is commonly used to determine TC-NER efficiency.[10] The biological relevance of a failure to restart transcription upon DNA damage, due to an impaired TC-NER, is illustrated by patients with inborn TC-NER defects. Inactivating mutations in UVSSA result for example in the UV sensitive syndrome characterized by a hypersensitivity to UV-irradiation. While patients with nonfunctional CSA or CSB exhibit additional severe clinical features such as growth and development failure and premature segmental aging.[4,11]

Importantly, when RNAPII is stalled at a lesion the damaged bases are enclosed in the active polymerase complex, thereby shielding the damage from repair factors.[12] Therefore, to make the lesion accessible for repair, the stalled RNAPII has to be removed or backtracked. Currently, there are two, not mutually exclusive, models describing the displacement of the polymerase from the DNA lesion. RNAPII might reverse translocate along the DNA resulting in the initiation of TC-NER[4,13,14] (**Fig. 1**, left side). If the RNAPII blocking lesion cannot be resolved efficiently by the above process, the stalled RNAPII may be ubiquitylated resulting in the dissociation and subsequent degradation of the large subunit of the RNA polymerase complex, RPB1,[15] thereby making the lesion accessible for other repair systems (**Fig. 1**, right side).

The RNA polymerase is likely not the only obstacle that may impede accessibility of lesions by repair factors. Another group of proteins that can restrict protein access are the building blocks of the nucleosomes, the histones, which define the chromatin organization and compaction. Therefore, like transcription and replication, it is expected that efficient DNA repair also requires chromatin remodeling. The access-repair-restore model, already described by Smerdon over two decades ago, proposes that the nucleosomes need to be removed or remodeled to permit access of the repair proteins to a DNA lesion.[16] Additionally, upon completion of the repair reaction the chromatin structure needs to be restored to its pre-damaged state to maintain specific chromatin organization, as defined by the presence of specific histone variants, and preserve epigenetic information like histone modifications, which are necessary for proper cellular functioning.[16,17] Histone chaperones and ATP-dependent chromatin remodelers are responsible for histone sliding, eviction, and insertion to remodel the chromatin to facilitate different DNA transacting processes. Different ATP-dependent chromatin remodelers were identified to function in DNA repair, including the UV DNA damage response.[18] For example, BRG1, part of the SWI/SNF family, associates with the GG-NER recognition factors XPC and DDB2, thereby stimulating CPD removal in mammalian cells.[19,20] Also, loss of INO80, an ATP dependent chromatin remodeler, was shown to abolish the assembly of the NER complex, suggesting that chromatin remodeling is needed for repair.[21] In addition, chromatin restoration after repair was shown to be dependent on CAF1, a histone chaperone that incorporates new histone H3.1 variants after completion of NER.[22]

Since transcription requires a more open chromatin structure, it was expected that TC-NER is less dependent on chromatin remodeling in comparison to GG-NER. However, several chromatin remodelers, specifically involved in TC-NER, are identified. The key TC-NER protein CSB, contains a SWI2/SNF2 ATPase domain and in vitro it has been shown that CSB can remodel chromatin, an activity stimulated by the histone chaperone NAP1.[23-25] HMGN1, a nucleosomal binding protein, and p300, a histone acetyl transferase, are other examples of proteins that are part of the TC-NER complex and involved in reducing chromatin compaction.[5,7]

Transcription Restart after UV Damage Requires Chromatin Remodeling

After completion of DNA repair in the transcribed strand of an expressed gene the transcription machinery needs to be restarted in order to restore normal expression levels of the affected gene. Whether specific proteins, next to the essential repair proteins, are involved in the transcriptional restart after repair has so far remained elusive. Specific histone variants and histone PTMs play an important role in gene expression regulation, and therefore it is expected that chromatin organization is also important for RRS. Recently three different papers identified chromatin modulating factors implicated in the transcription restart after DNA repair.[26-28] Together these papers show that repair of the transcribed strand alone is not sufficient for the cell to restore expression levels of the affected genes, but that specific chromatin modifications and remodeling events are crucial as well.

Two of these papers[26,27] identified the involvement of key histone chaperones during the TC-NER reaction directly resulting in nucleosome remodeling. Previously it was shown that the histone chaperone "Histone regulator A" (HIRA) is involved in chromatin remodeling in the response to double strand breaks (DSBs) and is also recruited to UV-A-induced lesions.[29] The HIRA chaperone deposits the histone variant H3.3 in transcriptionally active chromatin.[30] Knockdown of HIRA does not affect recruitment of NER factors nor the repair synthesis, indicating that HIRA is not involved in GG-NER.[26] However, downregulation of HIRA resulted in an impaired RRS after UVC damage

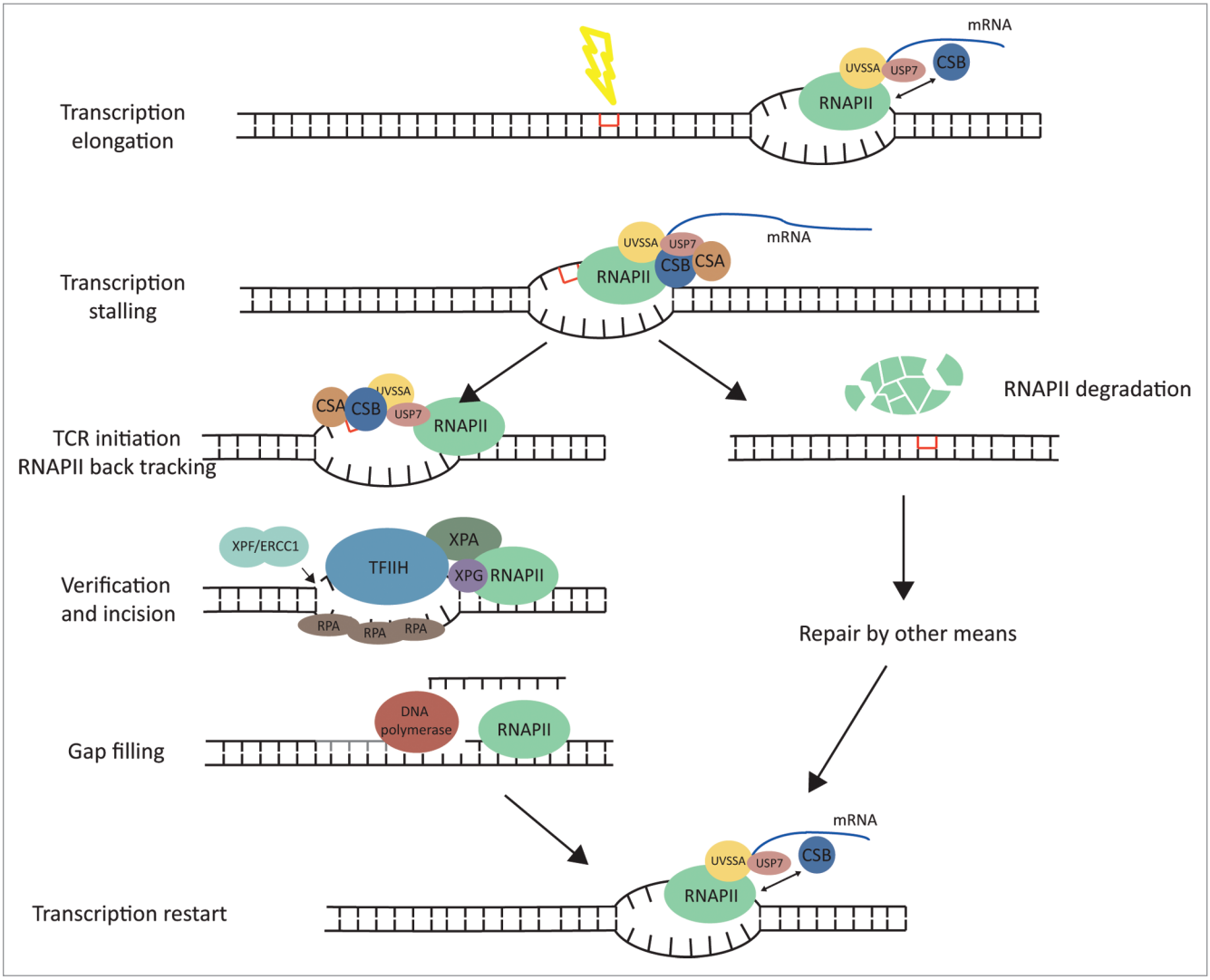

Figure 1. Model of mammalian transcription coupled repair. During transcription UVSSA, USP7, and CSB transiently interacts with RNAPII. When the polymerase encounters a lesion in the DNA, the stalled complex will block access of the repair proteins to the lesion. Therefore, RNAPII has to be either moved back along the DNA to enable TC-NER initiation by CSB, UVSSA, USP7, and CSA (left side) or degraded to allow repair by other repair mechanisms (right side). In TC-NER, the DNA is unwound by TFIIH that together with XPA is responsible for the damage verification. The endonucleases XPF/ERCC1 and XPG are positioned by RPA and cleave the damaged DNA strand and remove about 30 bases around the damage. DNA polymerases fill the single-stranded gap followed by ligation of the nick by DNA ligases I or III to complete the NER reaction. Upon damage removal the transcription need to restart for proper cellular functioning.

to an extent comparable to TC-NER-deficient cells, implying an important role in transcription recovery.[26] The HIRA function at transcription blocking DNA lesions was elegantly shown using SNAP-tagged histones, enabling to specifically identify novel incorporated H3.3 at the damaged site.[26] As H3.3 is normally present in transcriptional active chromatin and carries specific modifications, which either promote transcription or exclude transcription inhibiting factors from the chromatin,[31] it is very likely that H3.3 incorporation at sites of UV damage plays an essential role during transcription recovery upon DNA damage.

In addition it was shown that the histone chaperone FACT (Facilitating Chromatin Transcription) is also involved in transcription recovery upon UV damage. FACT is a heterodimer, consisting of the SPT16 and SSRP1 subunits, and is a known H2A/H2B chaperone.[32] FACT was already known to function in the UV damage response where it acts in p53 signaling together with casein kinase 2.[33] Both subunits of FACT are recruited to the site of local UV damage; however, only SPT16 depletion results in an impaired recovery of RNA synthesis after UV damage. These data suggest a specific role for SPT16 in the transcription recovery after UV damage, besides its other functions together with SSRP1.[27,32] In line with this, SPT16 downregulation results in a UV-hypersensitivity whereas knock down of SSRP1 does not affect the UV-sensitivity. The combination of fluorescence recovery after photobleaching (FRAP) and local UV damage induction uncovered a SPT16-dependent enhanced removal and incorporation of new H2A/

H2B dimers at the site of local UVC damage.[27] The postulated function of the FACT-dependent H2A/H2B exchange, during normal transcription, is to enable translocation of RNAPII along the chromatin by destabilizing the nucleosomes.[32] After DNA damage the SPT16 activity might result in a prolonged plasticity of the chromatin, as shown by the increased H2A/H2B turnover at UV-damage.[33] A possible function of this SPT16-mediated chromatin plasticity is to facilitate backtracking of stalled RNAPII, a crucial step in TC-NER.[4,15,34] When RNAPII is not efficiently pulled back from the lesion to allow access of repair factors, the polymerase might get polyubiquitylated and degraded, referred to as the "last resort." Turnover of RNAPII may lead to a delay in transcription recovery after UV damage repair since new RNAPII has to be loaded onto this transcribed gene.[15] Instead of playing a role in backtracking, it is also conceivable that the SPT16 mediated H2A/H2B exchange is necessary for forward movement of the RNAPII. This can either be necessary for the restart of transcription by the stalled polymerase after the blocking lesion has been successfully repaired, or it might be involved in lesion bypass synthesis. This latter, more remote scenario is unfavorable as it might result in aberrant transcripts.[35] Another possibility is that the chromatin remodeling stimulates the recruitment of other (unknown) factors involved in repair or specifically involved in transcription restart after UVC damage.

Strikingly, while both histone chaperones are suggested to play an important role specifically for transcription restart after repair, both factors are recruited to sites of UV-damage with comparable accumulation kinetics as early NER-factors. This early response was further corroborated as both factors are still recruited in XPA- or XPG-deficient patient cells in which both GG-NER, and more importantly, TC-NER cannot take place.[26,27] In line with this it was shown that repair is also not a pre-requisite for both histone H3.3 deposition or H2A/H2B exchange.[26,27] HIRA is shown to only remain transiently enriched at DNA lesions as its recruitment decreased already 1 h after damage infliction, while it takes much longer to fully restore expression levels to pre-damage levels (20–24 h).[26] However the maximum levels of H3.3 are reached after 1 h and remain stable until transcription restarts. These results indicate that HIRA functions early during TC-NER in order to prepare the damaged chromatin for transcription recovery. In contrast to HIRA accumulation, SPT16 remains present for longer periods at sites of damage. This difference between HIRA and SPT16 colocalization with DNA lesions is in line with their chromatin remodeling functions. HIRA causes the incorporation of the histone variant H3.3, which remains stably incorporated in the chromatin, thereby most likely replacing other H3 histone variants. However, UV stimulates a continuous exchange of H2A/H2B at sites of damage, which most logically needs long lasting histone chaperone activity of SPT16 and therefore explains the persistent enrichment of SPT16 at damaged sites.

The question remains how these factors are specifically recruited to sites of stalled RNAPII as the recruitment of both HIRA and SPT16 is independent of repair. The recruitment of FACT is transcription independent, indicating it is either a very early step in the NER pathway or a parallel process that happens independent of NER and transcription.[27] During transcription the recruitment of FACT is mediated by the histone methyltransferase SETD2.[36] It is thus possible that, also in the UV damage response, specific histone modifications will mediate the FACT recruitment. The HIRA recruitment was shown to depend on the ubiquitylation activity of Cul4/DDB1.[26] However, thus far there is no indication that HIRA is ubiquitylated itself or that it can interact directly with ubiquitylated proteins. Since HIRA is able to bind to naked DNA,[37] it is possible that the DDB1-CUL4 ubiquitin ligase activity toward nucleosomes might result in the generation of nucleosome sparse DNA, making it available for HIRA binding.[38]

Post Translational Modifications

Next to regulating the recruitment of histone chaperones to sites of DNA damage, post translational histone modifications are well established to regulate the promoter activity. Transcription normally requires specifically marked chromatin in order to open up the chromatin and stimulate transcription initiation. These marks consist, among others, of high levels of H4K16 and H4K20 acetylation and methylation of lysine 4, 36, and 79 on histone H3, compared with heterochromatin.[32,39] A large group of proteins is responsible for creating, maintaining or removing these modifications. For instance, the yeast lysine methyltransferase DOT1, which specifically methylates H3K79, is an important transcription regulator.[40,41]

The mammalian homolog of DOT1, DOT1 like protein (DOT1L), is part of a complex, containing several myeloid or mix-lineage leukemia fusion partners. Next to regulating H3K79 di- and tri-methylation in transcription,[42] DOT1L also stimulates recruitment of 53BP1 to DNA DSBs and has a thus far unknown function in the UV damage response.[43,44] Recent findings show that knock out of DOT1L, in mouse embryonic fibroblasts, leads to increased UV sensitivity.[28] Interestingly, DOT1L depletion does not influence GG-NER or TC-NER, but does result in a strong impairment of transcription recovery after UV damage.[28] As loss of DOT1L and the subsequent reduction of H3K79 methylation normally results in the repression of transcription, the authors tested if general chromatin relaxation could overcome the effect of DOT1L absence by using Trichostatin A. Treatment with this class I Histone deacetylase inhibitor resulted in a rescue of the DOT1L phenotype, both in transcription recovery and UV-survival, suggesting that DOT1L normally results in an open chromatin structure around the promoter of UV-repressed genes to allow transcription re-initiation.[28] These data suggest a transacting effect of DOT1L at the promoters, in contrast to HIRA and FACT that most likely act in the vicinity of the lesions. In line with this, Oksenych and colleagues show that in absence of DOT1L, the transcription initiation machinery is not assembled at promoters after UV irradiation, while in wild type cells the machinery reassembles 6–10 h after irradiation.[28]

Specific histone modifications, associated with either active or repressive chromatin regions were tested in the promoter region of the *DFHR* gene. Over time the WT cells show a gradual increase in H4 acetylation and 6 h after irradiation there is a large increase in DOT1L mediated H3K79 dimethylation in these cells.[28] However no signs of these active chromatin marks are found after irradiation of DOT1L depleted cells.[28] In addition, the heterochromatin H3K9me2 mark is upregulated after UV in DOT1L depleted cells in comparison to WT MEFs.[28] DOT1L mediated H3K79 trimethylation plays a role in transcription restart after UV by creating an active open chromatin state at promoters to allow reassembly of the pre-initiation complex. Dot1L trimethylates H3K79, a lysine in the core region of the histone, which is remarkable since most modifications occur on histone tails.[45] H3K79 methylation might serve as a first landmark for chromatin remodeling, preceding the recruitment of other modifiers and histone modifications.[28] Most likely, Dot1L functions in transcription recovery by limiting the spreading of heterochromatin marks at promoters immediately after irradiation, which allows RNAPII to re-accumulate at the promoters to re-activate transcription as soon as the UV damage is repaired.[28]

Specific Chromatin Remodeling for Transcriptional Restart upon DNA Damage

An interesting question remains whether most chromatin modifying enzymes are implicated or whether only specific factors have acquired or adapted a specific role in the DDR. In addition, it is currently unknown whether these factors are specifically involved in transcription resumption upon transcription blocking DNA damage or are also involved in the recovery of other transcription pausing events. Methyltransferases, like DOT1L, MYST2, and G9a, are involved in facilitating an open chromatin environment and might therefore play a role in general transcription initiation. Interestingly, only the chromatin modifier DOT1L was found to be specifically required for transcription restart after UV damage, as MYST2 and G9a do not play a role during RRS.[28] The effect of DOT1L on transcription restart is specific for transcription inhibition upon genotoxic insults, since after incubation with the transcription inhibitor DRB, transcription can be restarted in a DOT1L independent manner.[28] A similar, specific involvement in transcription restart upon DNA damage holds for FACT en HIRA, as experiments show that another H3.3 chaperone DAXX did not have an effect on the UV-induced H3.3 incorporation.[26] In line with this, the SPT16 subunit of the H2A/H2B chaperone FACT is essential for H2A/H2B exchange after UV, while the SPT16 binding partner SSRP1, required for the canonical FACT function or other H2A/H2B chaperones, like NAP1L1, seem not to be involved.[27] The activities HIRA, FACT and DOT1L are thought to play a role in the priming of the chromatin for proper transcription recovery. Just like the methyltransferase activity of DOT1L, most likely the incorporation of H3.3 and eviction of the nucleosomal H2A/H2B histones results in a temporarily open chromatin state. This indicates that transcription activation upon DNA damage needs extra chromatin plasticity compared with normal transcription, as for example HIRA and DOT1L are not needed for transcription restart upon DRB inhibition.[26,28] Together these findings suggest that there is a specific form of transcription: namely the recovery of RNA synthesis upon genotoxic insults, which needs specific chromatin changes to be fully functional.

Outlook

It should be noted that at this moment it cannot be excluded that HIRA and FACT chromatin remodeling activities facilitate TC-NER, and therefore these proteins are crucial factors for transcription restart in an indirect manner. This might also explain their presence early in the TC-NER reaction. Crucial experiments to specifically measure TC-NER repair capacity, like, for example, strand specific repair assays, should point out if HIRA and FACT activity affects repair or specifically transcription recovery as was shown for DOT1L.

It is not known whether the activities of HIRA, FACT, and DOT1L are always needed together to stimulate RRS, or that subsets of these chromatin remodelers are needed under specific conditions. As discussed before, lesion stalled RNAPII is either degraded or reverse translocated to allow repair and finally transcription restart. Different chromatin remodeling activities could be needed for these different events and might explain the involvement of different chromatin remodelers in the transcription restart. For example, the RNAPII backtracking followed by damage removal by TC-NER (**Figs. 1 and 2**) could be stimulated through enhancing the chromatin plasticity by FACT, which is in line with the early FACT recruitment to the damaged site. In case of degradation of the RNAPII upon stalling at the lesion, either an already active transcription complex, that did not encounter this lesion yet will continue transcription, or a new transcription complex needs to be built up at the promoter. In this latter event, DOT1L activity, which promotes re-accumulation of the transcription machinery at promoters,[28] might be specifically important (**Fig. 2**). Further studies will have to point out when exactly specific remodeling activities are needed and how the interplay between histone chaperones and modifiers is defined.

The incorporation of new histones will result in a loss of pre-damage PTMs as new histones can carry specific modifications different from the old nucleosomal histones. So, incorporation of these histones upon damage will result in a defined chromatin region with specific post translational modifications. These new modifications might either stimulate transcription restart itself or recruit other factors needed for transcription recovery. Interestingly, the recently identified UV-specific interaction partner of TFIIH, ELL is not involved in repair but knock down results in an impaired recovery of RNA synthesis.[46] FRAP data shows that, in absence of ELL, upon UV irradiation a larger immobile fraction of RNAPII was observed, suggesting that without ELL RNAPII remains stalled upon repair. ELL

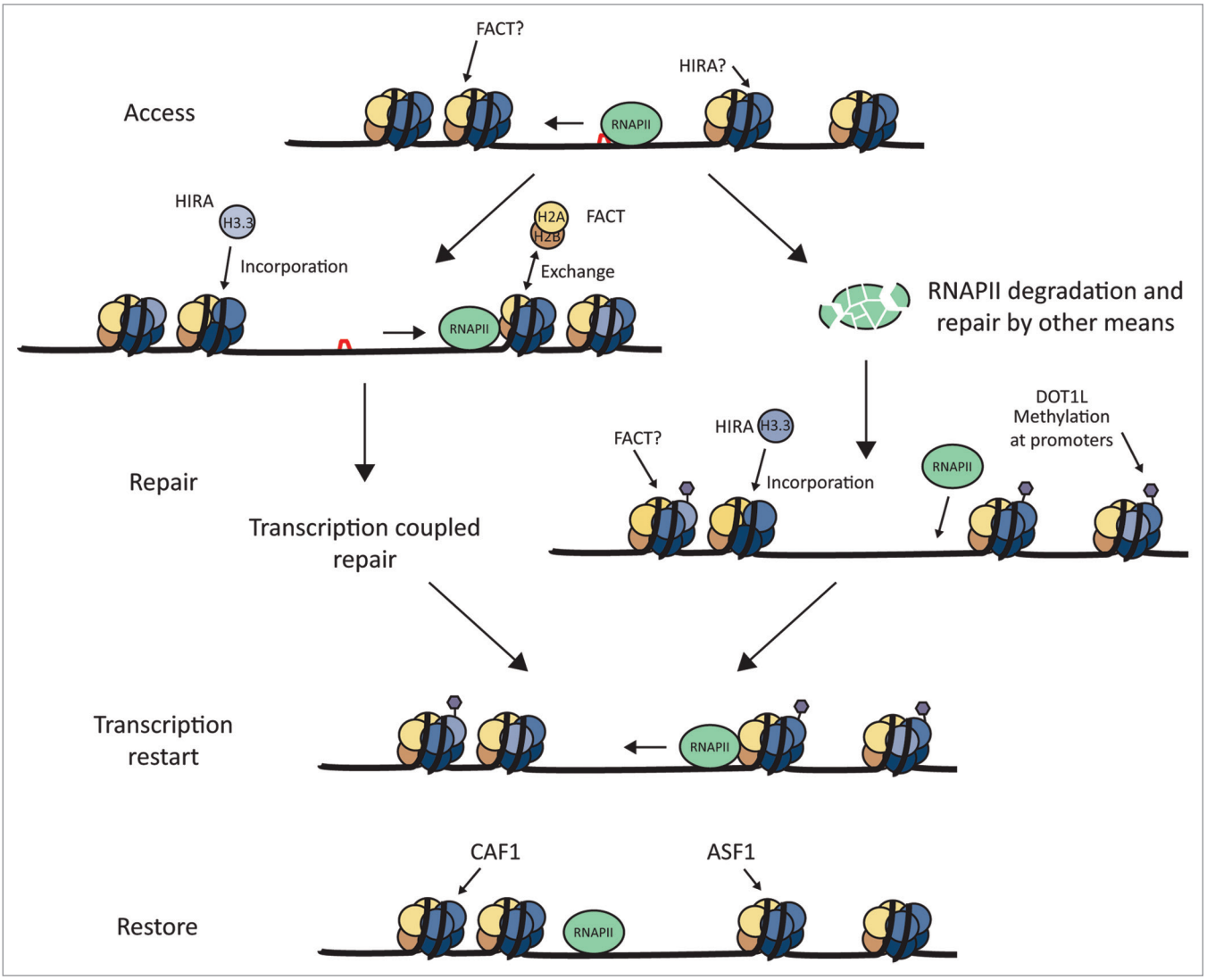

Figure 2. Chromatin remodeling during the transcriptional restart upon DNA damage. This model shows an overview of chromatin remodeling necessary for transcriptional restart after UV damage. When RNAPII is stalled by a lesion, the damaged site has to be made accessible for repair. This is done by chromatin remodelers like NAP1L1, p300, and HMGN1 (not shown), which are most likely involved in the reverse translocation of RNAPII. FACT mediates H2A/H2B exchange which might give the chromatin extra plasticity needed for this backtracking. In another scenario, if RNAPII is degraded, other repair mechanisms will remove the damage and in order for transcription to restart new RNAPII has to be loaded onto the DNA. As DOT1L is a histone methyltransferase that prevents heterochromatin spreading and might therefore stimulates loading of new RNAPII after it has been degraded upon DNA damage. Both these pathways could be stimulated by the incorporation of new histone H3.3 by HIRA. DOT1L and FACT might also play a role in either of these events. This chromatin remodeling provides an specific environment stimulating the transcription restart after DNA damage. When transcription rates are recovered, the DNA can be remodeled back to its predamaged state to maintain epigenetic information by for instance CAF1 and ASF1.

might be a promoting factor necessary for transcription restart itself, or may serve as a binding site for other factors that are needed to stimulate RNAPII to initiate transcription after repair is finished.[46] Recently, different NER factors, including XPC and ERCC1, were suggested to be involved in transcriptional control as well. These proteins might regulate changes in the epigenetic landscape or are suggested to induce chromatin looping and CTCF recruitment to facilitate transcription initiation. Even though these effects were found to be gene and most likely cell type specific, it might be interesting to study whether this activity is involved in transcription recovery upon UV-damage.[47-49]

According to the access-repair-restore model, chromatin remodeling may be implicated before and after DNA repair, thereby maintaining proper cellular functioning.[16] These three recent studies show that in addition to the in this model suggested chromatin modifications, specific chromatin changes are needed for proper transcription restart upon UV-damage.[26-28] This adds an important novel layer to the access-repair-restore model in which the chromatin is geared up for proper transcription restart already before the actual damage is repaired. Histone H3.3 is normally present in

actively transcribed chromatin regions and therefore incorporation of H3.3 at damaged sites by HIRA will provide a transcription stimulating environment. FACT mediated H2A/H2B exchange will most likely give the chromatin extra plasticity needed for RNAPII movement or recruitment of other involved factors. In addition, DOT1L is preventing a heterochromatin structure that would block transcription recovery after repair. All together these factors create a specific chromatin environment of newly incorporated histones and their chromatin marks so that, after the lesion is repaired, RNAPII can restart transcription to restore mRNA expression levels. After repair and transcription restart have taken place, more remodeling might be needed to restore the chromatin to pre-damaged conditions, thereby preserving the epigenetic information of the damage region.[17,22] The assays used to monitor chromatin plasticity upon DNA damage, including live-cell histone exchange assays and visualization of newly incorporated histone at sites of damage, will enable us to also study the involvement of specific histone variants during TC-NER and transcription restart. Furthermore, recent advances in proteomics will enable us to uncover specific histone PTMs that are crucial to overcome the detrimental consequences of transcription blocking lesions. Finally, the identification of DOT1L, FACT, and HIRA will fuel further in-depth research to detect roles of the changes in the chromatin landscape involved in specific forms of transcriptional restart like the recovery of RNA synthesis upon genotoxic events.

Disclosure of Potential Conflicts of Interest

No potential conflict of interest was disclosed.

Acknowledgments

This work was funded by Netherlands Organization for Scientific Research projects (TOP ALW grant 854.11.002; ZonMW TOP 912.08.031 and 917.96.120, Horizon Zenith project 93511042), Erasmus MC fellowship, and ERC advanced grant.

References

1. Ljungman M. The transcription stress response. Cell Cycle 2007; 6:2252-7; PMID:17700065; http://dx.doi.org/10.4161/cc.6.18.4751
2. Walmacq C, Cheung ACM, Kireeva ML, Lubkowska L, Ye C, Gotte D, Strathern JN, Carell T, Cramer P, Kashlev M. Mechanism of translesion transcription by RNA polymerase II and its role in cellular resistance to DNA damage. Mol Cell 2012; 46:18-29; PMID:22405652; http://dx.doi.org/10.1016/j.molcel.2012.02.006
3. Marietta C, Brooks PJ. Transcriptional bypass of bulky DNA lesions causes new mutant RNA transcripts in human cells. EMBO Rep 2007; 8:388-93; PMID:17363972; http://dx.doi.org/10.1038/sj.embor.7400932
4. Vermeulen W, Fousteri M. Mammalian transcription-coupled excision repair. Cold Spring Harb Perspect Biol 2013; 5:a012625; PMID:23906714; http://dx.doi.org/10.1101/cshperspect.a012625
5. Hanawalt PC, Spivak G. Transcription-coupled DNA repair: two decades of progress and surprises. Nat Rev Mol Cell Biol 2008; 9:958-70; PMID:19023283; http://dx.doi.org/10.1038/nrm2549
6. van den Boom V, Citterio E, Hoogstraten D, Zotter A, Egly JM, van Cappellen WA, Hoeijmakers JH, Houtsmuller AB, Vermeulen W. DNA damage stabilizes interaction of CSB with the transcription elongation machinery. J Cell Biol 2004; 166:27-36; PMID:15226310; http://dx.doi.org/10.1083/jcb.200401056
7. Fousteri M, Vermeulen W, van Zeeland AA, Mullenders LH. Cockayne syndrome A and B proteins differentially regulate recruitment of chromatin remodeling and repair factors to stalled RNA polymerase II in vivo. Mol Cell 2006; 23:471-82; PMID:16916636; http://dx.doi.org/10.1016/j.molcel.2006.06.029
8. Schwertman P, Lagarou A, Dekkers DH, Raams A, van der Hoek AC, Laffeber C, Hoeijmakers JH, Demmers JA, Fousteri M, Vermeulen W, et al. UV-sensitive syndrome protein UVSSA recruits USP7 to regulate transcription-coupled repair. Nat Genet 2012; 44:598-602; PMID:22466611; http://dx.doi.org/10.1038/ng.2230
9. Schärer OD. Nucleotide excision repair in eukaryotes. Cold Spring Harb Perspect Biol 2013; 5:a012609; PMID:24086042; http://dx.doi.org/10.1101/cshperspect.a012609
10. Mayne LV, Lehmann AR. Failure of RNA synthesis to recover after UV irradiation: an early defect in cells from individuals with Cockayne's syndrome and xeroderma pigmentosum. Cancer Res 1982; 42:1473-8; PMID:6174225
11. Laugel V. Cockayne syndrome: the expanding clinical and mutational spectrum. Mech Ageing Dev 2013; 134:161-70; PMID:23428416; http://dx.doi.org/10.1016/j.mad.2013.02.006
12. Tornaletti S, Reines D, Hanawalt PC. Structural characterization of RNA polymerase II complexes arrested by a cyclobutane pyrimidine dimer in the transcribed strand of template DNA. J Biol Chem 1999; 274:24124-30; PMID:10446184; http://dx.doi.org/10.1074/jbc.274.34.24124
13. Saeki H, Svejstrup JQ. Stability, flexibility, and dynamic interactions of colliding RNA polymerase II elongation complexes. Mol Cell 2009; 35:191-205; PMID:19647516; http://dx.doi.org/10.1016/j.molcel.2009.06.009
14. Lagerwerf S, Vrouwe MG, Overmeer RM, Fousteri MI, Mullenders LHF. DNA damage response and transcription. DNA Repair (Amst) 2011; 10:743-50; PMID:21622031; http://dx.doi.org/10.1016/j.dnarep.2011.04.024
15. Wilson MD, Harreman M, Svejstrup JQ. Ubiquitylation and degradation of elongating RNA polymerase II: the last resort. Biochim Biophys Acta 2013; 1829:151-7; PMID:22960598; http://dx.doi.org/10.1016/j.bbagrm.2012.08.002
16. Smerdon MJ. DNA repair and the role of chromatin structure. Curr Opin Cell Biol 1991; 3:422-8; PMID:1892653; http://dx.doi.org/10.1016/0955-0674(91)90069-B
17. Soria G, Polo SE, Almouzni G. Prime, repair, restore: the active role of chromatin in the DNA damage response. Mol Cell 2012; 46:722-34; PMID:22749398; http://dx.doi.org/10.1016/j.molcel.2012.06.002
18. Lans H, Marteijn JA, Vermeulen W. ATP-dependent chromatin remodeling in the DNA-damage response. Epigenetics & Chromatin 2012; 5.
19. Zhang L, Zhang Q, Jones K, Patel M, Gong F. The chromatin remodeling factor BRG1 stimulates nucleotide excision repair by facilitating recruitment of XPC to sites of DNA damage. Cell Cycle 2009; 8:3953-9; PMID:19901545; http://dx.doi.org/10.4161/cc.8.23.10115
20. Zhao Q, Wang QE, Ray A, Wani G, Han C, Milum K, Wani AA. Modulation of nucleotide excision repair by mammalian SWI/SNF chromatin-remodeling complex. J Biol Chem 2009; 284:30424-32; PMID:19740755; http://dx.doi.org/10.1074/jbc.M109.044982
21. Jiang Y, Wang X, Bao S, Guo R, Johnson DG, Shen X, Li L. INO80 chromatin remodeling complex promotes the removal of UV lesions by the nucleotide excision repair pathway. Proc Natl Acad Sci U S A 2010; 107:17274-9; PMID:20855601; http://dx.doi.org/10.1073/pnas.1008388107
22. Polo SE, Roche D, Almouzni G. New histone incorporation marks sites of UV repair in human cells. Cell 2006; 127:481-93; PMID:17081972; http://dx.doi.org/10.1016/j.cell.2006.08.049
23. Citterio E, Van Den Boom V, Schnitzler G, Kanaar R, Bonte E, Kingston RE, Hoeijmakers JHJ, Vermeulen W. ATP-dependent chromatin remodeling by the Cockayne syndrome B DNA repair-transcription-coupling factor. Mol Cell Biol 2000; 20:7643-53; PMID:11003660; http://dx.doi.org/10.1128/MCB.20.20.7643-7653.2000
24. Selzer RR, Nyaga S, Tuo J, May A, Muftuoglu M, Christiansen M, Citterio E, Brosh RM Jr., Bohr VA. Differential requirement for the ATPase domain of the Cockayne syndrome group B gene in the processing of UV-induced DNA damage and 8-oxoguanine lesions in human cells. Nucleic Acids Res 2002; 30:782-93; PMID:11809892; http://dx.doi.org/10.1093/nar/30.3.782
25. Cho I, Tsai PF, Lake RJ, Basheer A, Fan HY. ATP-dependent chromatin remodeling by Cockayne syndrome protein B and NAP1-like histone chaperones is required for efficient transcription-coupled DNA repair. PLoS Genet 2013; 9:e1003407; PMID:23637612; http://dx.doi.org/10.1371/journal.pgen.1003407
26. Adam S, Polo SE, Almouzni G. Transcription recovery after DNA damage requires chromatin priming by the H3.3 histone chaperone HIRA. Cell 2013; 155:94-106; PMID:24074863; http://dx.doi.org/10.1016/j.cell.2013.08.029
27. Dinant C, Ampatziadis-Michailidis G, Lans H, Tresini M, Lagarou A, Grosbart M, Theil AF, van Cappellen WA, Kimura H, Bartek J, et al. Enhanced chromatin dynamics by FACT promotes transcriptional restart after UV-induced DNA damage. Mol Cell 2013; 51:469-79; PMID:23973375; http://dx.doi.org/10.1016/j.molcel.2013.08.007

28. Oksenych V, Zhovmer A, Ziani S, Mari PO, Eberova J, Nardo T, Stefanini M, Giglia-Mari G, Egly JM, Coin F. Histone methyltransferase DOT1L drives recovery of gene expression after a genotoxic attack. PLoS Genet 2013; 9:e1003611; PMID:23861670; http://dx.doi.org/10.1371/journal.pgen.1003611
29. Yang X, Li L, Liang J, Shi L, Yang J, Yi X, Zhang D, Han X, Yu N, Shang Y. Histone acetyltransferase 1 promotes homologous recombination in DNA repair by facilitating histone turnover. J Biol Chem 2013; 288:18271-82; PMID:23653357; http://dx.doi.org/10.1074/jbc.M113.473199
30. Skene PJ, Henikoff S. Histone variants in pluripotency and disease. Development 2013; 140:2513-24; PMID:23715545; http://dx.doi.org/10.1242/dev.091439
31. Szenker E, Lacoste N, Almouzni G. A developmental requirement for HIRA-dependent H3.3 deposition revealed at gastrulation in Xenopus. Cell Rep 2012; 1:730-40; PMID:22813747; http://dx.doi.org/10.1016/j.celrep.2012.05.006
32. Reinberg D, Sims RJ 3rd. de FACTo nucleosome dynamics. J Biol Chem 2006; 281:23297-301; PMID:16766522; http://dx.doi.org/10.1074/jbc.R600007200
33. Keller DM, Lu H. p53 serine 392 phosphorylation increases after UV through induction of the assembly of the CK2.hSPT16.SSRP1 complex. J Biol Chem 2002; 277:50206-13; PMID:12393879; http://dx.doi.org/10.1074/jbc.M209820200
34. Fousteri M, Mullenders LH. Transcription-coupled nucleotide excision repair in mammalian cells: molecular mechanisms and biological effects. Cell Res 2008; 18:73-84; PMID:18166977; http://dx.doi.org/10.1038/cr.2008.6
35. Saxowsky TT, Doetsch PW. RNA polymerase encounters with DNA damage: transcription-coupled repair or transcriptional mutagenesis? Chem Rev 2006; 106:474-88; PMID:16464015; http://dx.doi.org/10.1021/cr040466q
36. Carvalho S, Raposo AC, Martins FB, Grosso AR, Sridhara SC, Rino J, Carmo-Fonseca M, de Almeida SF. Histone methyltransferase SETD2 coordinates FACT recruitment with nucleosome dynamics during transcription. Nucleic Acids Res 2013; 41:2881-93; PMID:23325844; http://dx.doi.org/10.1093/nar/gks1472
37. Ray-Gallet D, Woolfe A, Vassias I, Pellentz C, Lacoste N, Puri A, Schultz DC, Pchelintsev NA, Adams PD, Jansen LE, et al. Dynamics of histone H3 deposition in vivo reveal a nucleosome gap-filling mechanism for H3.3 to maintain chromatin integrity. Mol Cell 2011; 44:928-41; PMID:22195966; http://dx.doi.org/10.1016/j.molcel.2011.12.006
38. Nouspikel T. Multiple roles of ubiquitination in the control of nucleotide excision repair. Mech Ageing Dev 2011; 132:355-65; PMID:21466822; http://dx.doi.org/10.1016/j.mad.2011.03.003
39. Campos EI, Reinberg D. Histones: annotating chromatin. Annu Rev Genet 2009; 43:559-99; PMID:19886812; http://dx.doi.org/10.1146/annurev.genet.032608.103928
40. Feng Q, Wang H, Ng HH, Erdjument-Bromage H, Tempst P, Struhl K, Zhang Y. Methylation of H3-lysine 79 is mediated by a new family of HMTases without a SET domain. Curr Biol 2002; 12:1052-8; PMID:12123582; http://dx.doi.org/10.1016/S0960-9822(02)00901-6
41. Lacoste N, Utley RT, Hunter JM, Poirier GG, Côte J. Disruptor of telomeric silencing-1 is a chromatin-specific histone H3 methyltransferase. J Biol Chem 2002; 277:30421-4; PMID:12097318; http://dx.doi.org/10.1074/jbc.C200366200
42. Nguyen AT, Zhang Y. The diverse functions of Dot1 and H3K79 methylation. Genes Dev 2011; 25:1345-58; PMID:21724828; http://dx.doi.org/10.1101/gad.2057811
43. Huyen Y, Zgheib O, Ditullio RA Jr., Gorgoulis VG, Zacharatos P, Petty TJ, Sheston EA, Mellert HS, Stavridi ES, Halazonetis TD. Methylated lysine 79 of histone H3 targets 53BP1 to DNA double-strand breaks. Nature 2004; 432:406-11; PMID:15525939; http://dx.doi.org/10.1038/nature03114
44. Bostelman LJ, Keller AM, Albrecht AM, Arat A, Thompson JS. Methylation of histone H3 lysine-79 by Dot1p plays multiple roles in the response to UV damage in Saccharomyces cerevisiae. DNA Repair (Amst) 2007; 6:383-95; PMID:17267293; http://dx.doi.org/10.1016/j.dnarep.2006.12.010
45. Luger K, Mäder AW, Richmond RK, Sargent DF, Richmond TJ. Crystal structure of the nucleosome core particle at 2.8 A resolution. Nature 1997; 389:251-60; PMID:9305837; http://dx.doi.org/10.1038/38444
46. Mourgues S, Gautier V, Lagarou A, Bordier C, Mourcet A, Slingerland J, Kaddoum L, Coin F, Vermeulen W, Gonzales de Peredo A, et al. ELL, a novel TFIIH partner, is involved in transcription restart after DNA repair. Proc Natl Acad Sci U S A 2013; 110:17927-32; PMID:24127601; http://dx.doi.org/10.1073/pnas.1305009110
47. Fong YW, Cattoglio C, Tjian R. The intertwined roles of transcription and repair proteins. Mol Cell 2013; 52:291-302; PMID:24207023; http://dx.doi.org/10.1016/j.molcel.2013.10.018
48. Kamileri I, Karakasilioti I, Garinis GA. Nucleotide excision repair: new tricks with old bricks. Trends Genet 2012; 28:566-73; PMID:22824526; http://dx.doi.org/10.1016/j.tig.2012.06.004
49. Le May N, Fradin D, Iltis I, Bougnères P, Egly JM. XPG and XPF endonucleases trigger chromatin looping and DNA demethylation for accurate expression of activated genes. Mol Cell 2012; 47:622-32; PMID:22771116; http://dx.doi.org/10.1016/j.molcel.2012.05.050