Chromatin modifiers and remodellers in DNA repair and signalling

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Following the seminal unravelling of the double helical structure of DNA by Watson, Crick and colleagues in 1952, work of equal significance and similarly recognised by a Nobel Prize, led to the appreciation that DNA is an unstable structure subject to damage from chemical attack by agents arising endogenously or exogenously, and from metabolic transactions, such as replication and transcription [1, 2]. The past fifty years has seen mounting recognition of the enormous significance of DNA damage response (DDR) pathways in protecting against the harmful effects of this damage, and particularly our understanding of the DNA repair processes [1]. Indeed, we now understand the importance these pathways play in cancer avoidance, protection against ageing and in ensuring normal development [3, 4]. We now have a good understanding of the basic DNA repair processes, at least when considering their action on naked DNA. However, in a cellular setting, our DNA is organised within a chromatin environment, which can represent a diverse range from open to closed conformations of distinct types. Our DNA sequences can be unique or repetitive. And there are ongoing DNA transactions, which can profoundly influence the DNA repair processes. Thus, a current focus of research is to understand how chromatin is modified and reorganised to allow optimal DNA repair and interplay between the DDR and metabolic processes such as transcription and replication.

Our goal in this theme issue is to review our current understanding of the epigenetic changes that arise in the vicinity of DNA double strand breaks (DSBs) and the chromatin remodelling complexes employed to reorganise chromatin. While the focus lies on DSBs, we include a consideration of how DNA damage influences transcription/replication as well as how chromatin is remodelled to allow replication since an evaluation of these interfacing processes is integral to our understanding of the processes arising following DNA damage. This area of research is still at an early stage. It is highly dynamic and, like all current research, confusion and conflicting data sometimes precedes clarity – and the underlying mechanisms remain poorly defined. In this introductory report, we summarise the goals of this theme issue and consider the current questions, insights and apparent contradictions.

The ataxia telangiectasia mutated kinase (ATM) is the central orchestrator of the DDR to DSBs [5]. ATM has long been recognised as a central regulator of processes such as cell cycle checkpoint arrest, that enhance the opportunity for optimal DSB repair [6]. Recent studies have extended this notion to include roles in inhibiting transcription specifically in the DSB vicinity [7, 8]. Critically, however,

more recent studies have unearthed the central role that ATM plays in orchestrating chromatin changes at a DSB. Indeed, whilst ataxia telangiectasia (A-T), the disorder caused by mutations in ATM, was originally considered to be a DNA repair disorder and later a checkpoint disorder, it could now be argued to be a disorder that fails to appropriately orchestrate DSB-induced chromatin changes, helping to explain its more significant role in higher compared to lower organisms [9-15]. In our opening article, Aaron Goodarzi sets the scene by reviewing the complex nature of the chromatin changes regulated by ATM at a DSB. The route by which ATM effects epigenetic changes at a DSB has been emerging for several years. The process starts by ATM-dependent phosphorylation of H2AX with this signal being read and transduced via MDC1 binding to promote or expose additional histone modifications including ubiquitination, SUMOylation and methylation [16, 17]. Importantly, these histone modifications exert two somewhat distinct end points; firstly, histone modifications can directly effect the recruitment of DDR proteins, such as BRCA1 and 53BP1 and secondly, coupled with direct ATM-dependent phosphorylation of DDR proteins, they can lead to the recruitment or modification of chromatin remodelling complexes. Aaron Goodarzi and colleagues review insight into the mechanism of the ATM-dependent processes regulating chromatin reorganisation where detailed knowledge is available. Marcus Wilson and Dan Durocher, in our second review, discuss how the distinct histone modifications can be read to influence recruitment of DDR proteins. They discuss the characterised domains at a mechanistic and structural level revealing important insight into the "reading" signatures and the downstream consequences. Such "reading" encompasses roles for BRCT, Tudor and Ubiquitin binding (UBD) domains in binding to phosphorylation, methylation and ubiquitin modifications but their interplay with readers of, for example, acetylation, provides a network of balances. Extending this theme, Kyle Miller and colleagues focus on bromodomain proteins (BRD), discussing how they "read" histone acetylation and the route by which they promote chromatin remodelling. Indeed, BRD containing proteins are central to several chromatin remodelling complexes, providing an interface with the reviews that focus more on chromatin remodelling.

Whilst modification of histone variant forms, such as H2AX, H2AZ and H3.3 have been recognised for some time to play critical roles in chromatin organisation after DNA damage or during transcription, the N-terminal tail of H4, which has well conserved lysine residues prime for acetylation at the amino group, is becoming

increasing recognised as a central factor regulating the DDR [18, 19]. Acetylation of the H4 tail can also directly influence chromatin organization through charge-regulated histone interactions. Moreover, as discussed in our second review (Wilson and Durocher), histone acetylation can serve to block or restrict other modifications on the same or nearby residues. Surbhi Dhar and Brendan Price provide a focused review on the N-terminal tail of H4 considering the processes influencing lysine acetylation, how it influences chromatin organisation and the downstream impact on repair.

The ubiquitin-dependent molecular unfoldase/segregase, p97, has emerged as another route by which epigenetic modifications can influence chromatin remodelling at the sites of DNA damage, as well as during transcription and repair [20]. p97 is an AAA+ATPase, which uses ATP to unfold or segregate ubiquitinated substrates, targeting them for proteasome mediated degradation and relieving their impact on chromatin structure. VCP-mediated protein degradation can directly impact upon DSB repair, such as the targeted removal of DNA-bound Ku, but can also interface and co-operate with chromatin remodelling complexes to re-organise chromatin structure after DNA damage. Somewhat distinctly, VCP can also regulate the inhibition and recovery of transcription at the sites of DNA damage via the removal of arrested RNA polymerase II. Kristijan Ramadan and colleagues provide a review of the emerging understanding of roles of VCP during DNA DSB repair.

Whilst the phosphorylation, ubiquitylation and acetylation modification machinery has been well examined, our understanding of SUMO modifiers, "reader" motifs and interacting proteins has been less well characterised, although there is clear evidence the SUMOylation occurs during the DDR and directly influences DSB repair [16]. Alexander Garvin and Jo Morris focus on these aspects of SUMOylation in their review, providing a nice addition to the reviews discussed above.

Chromatin remodelling enzymes use the energy derived from the hydrolysis of ATP to alter the structure or composition of chromatin. The enzymes can be divided into families based on their domain organisation, and most remodelling enzymes are found within multisubunit complexes. While they all share a related catalytic subunit, each remodelling enzyme (or complex) leads to different outcomes, such as nucleosome repositioning, histone eviction, or histone subunit exchange [21]. This specificity in mode of action is generated by the accessory domains and subunits attached to the motor proteins.

One remodelling complex, INO80, has been shown to play numerous important roles in the maintenance of genome stability, with many of the insights generated in work done using budding yeast as a model system. The review from Ashby Morrison focuses on the role of INO80 in mediating the checkpoint response to replicative stress, which highlights the importance of individual subunits of these complexes, since a key player in this activity is the non-catalytic Ies4 subunit of INO80. In addition, she discusses a mitotic role for INO80, which impacts on the fidelity of chromosome segregation.

In a review from Jerome Poli and colleagues the central role of INO80 in mediating the complex interplay between replication, transcription and DNA damage responses is discussed. The authors point out that this is no doubt a contributing factor to the known impact of INO80 on development and disease in higher eukaryotes. In addition, this review brings up an important concept related to the role of remodellers in DNA damage responses: that of chromosome mobility. It is perhaps intuitive that an increase in chromosome mobility might facilitate the manipulation required to carry out repair, but it was more surprising that some breaks are moved to the nuclear periphery during the repair process. The contribution of INO80 to these events is discussed.

Highly complementary with this review is one from Irene Chiolo and colleagues, which examines the challenges associated with DSB repair in heterochromatin. This review focuses primarily on work from Drosophila, where movement of DNA breaks arising in heterochromatin is required for repair, but the authors also highlight elements of the cellular responses that are intriguingly conserved in other organisms. In addition, they also consider the distinction between expansion of heterochromatin and mobilization of the break to a new location, and discuss the dynamic nature of heterochromatin proteins in this process.

While INO80 plays a central role in DNA damage responses, many more chromatin remodelling enzymes have also been implicated. Clearly, the different enzymes contribute distinct functions to the process of repair, and understanding why so many are needed and what each one is doing is of great importance. This is the subject of the review by Maqda Rother and Haico van Attikum, who cover the current state of knowledge around nine remodellers with known functions in DNA repair. How each of these is recruited to the right place at the right time, which step in the

repair process is promoted by each, and how the complexes talk to each other are still very open questions.

As discussed above, the chromatin changes required to optimise DSB repair must be evaluated in the context of other DNA transactions, of which transcription is, arguably, the most important process. Recent findings have revealed that RNA polII-dependent transcription is arrested in the vicinity of a DSB in a manner that requires chromatin remodelling, which may itself influence the DSB repair process [7, 8, 22]. Akira Yasui and colleagues provide a review of the chromatin changes involved in that process. An emerging topic in the field of DNA repair is the contribution of transcription and RNA to the repair process [23-25]. In particular, DNA-RNA hybrids or R-loops can present a source of DNA damage but equally, can profoundly influence the repair process. Robin Sebastian and Philipp Oberdoerffer provide a timely review of the influence of RNA on genome maintenance. Although less focused on chromatin, this review is significant in overviewing the evidence for how RNA or R-loops can drive transcription-associated DNA damage as well as potentially providing a template to optimise DSB repair. Insight into this novel aspect of DSB formation and repair must be evaluated in future considerations of chromatin changes at damage sites, particularly given recent evidence that RNA can be transcribed in an end-templated manner [26].

The very early studies on A-T provided seminal evidence for a role of ATM (although the causal genetic defect was uncharacterised at that time) in arresting replication in the presence of DSBs [13]. We still have only a poor understanding of how ATM influences replication and, more significantly, the chromatin changes required to promote replication. As a step towards addressing this critical topic, James Bellush and Iestyn Whitehouse have discussed DNA replication in the context of a chromatin environment, considering origin licensing, origin firing and the replication process itself. Although somewhat distinct to our focus on DSB repair, an evaluation of replication in a chromatin environment reveals the role of factors, including chromatin remodelling complexes, which may also participate in DSB repair as well as insight into the mechanism underlying this related process.

What emerges from these reviews is the magnitude and complexity of the changes that arise in the DSB vicinity, frequently with seemingly conflicting consequences. Whilst one important contributing factor to the range of responses is the influence of transcription, replication and other transactions involving the DNA

molecule coupled with the nature of the pre-existing chromatin structure prior to DNA damage (e.g. unique or repetitive sequences, heterochromatic or euchromatic), there are also likely to be kinetic and distance related requirements for the nature of the chromatin structure at a DSB. Studies employing a site specific DSB have shown that there are temporal changes in chromatin structure with early but transient chromatin expansion followed by extensive and persistent condensation [27]. Nevertheless, others have provided evidence that these changes occur in the opposite order. Namely, there is an initial stage of recruitment of repressive complexes such as HP1, H2AZ and the NuRD complex, followed by a shift to a more open structure involving acetylation of the H4 tail compaction with subsequent chromatin relaxation [28, 29]. This apparent contradiction may be due to the different scales measured in the different approaches (for example, immunofluorescence compared with chromatin immunoprecipitation). Indeed, it seems likely that chromatin relaxation, histone sliding or eviction will be required immediately adjacent to the DSB to facilitate repair whilst a compacted environment may be required more distal to the DSB to restrict translocation formation. An important goal for future work will be to determine how the modifications and chromatin dynamics change in a temporal and location-dependent manner, as well as how they are influenced by ongoing DNA transactions.

Similarly, the data regarding the role of upstream signalling factors does not lend itself well to a straightforward single model. This is almost certainly due to the fact that not all events will take place at every break. The location, timing, and complexity of the break are just a few of the factors that might influence which events are carried out and in what order.

Another critical question is about how the pre-existing chromatin environment influences pathway usage so that the cell uses the optimal choice. The core process of DNA non-homologous end-joining (c-NHEJ) represents a compact process, demanding little chromatin opening. There may, in fact, be a significant benefit from a highly compacted environment distal to a DSB undergoing c-NHEJ to restrict the possibility of translocation events, which can potentially occur readily by c-NHEJ due to the lack of requirement for sequence homology for rejoining. HR, in contrast, necessitates extensive end-resection and histone changes if branch migration also occurs and the extensive homology requirements restrict the opportunity for translocation formation. However, paradoxically, the extensive chromatin changes

necessitated by HR may be a significant factor restricting its usage in higher organisms, where the precise epigenetic code is complex but critical, since the precision of this code needs to be reconstituted after the completion of repair. Recent studies have suggested that, at least in late S/G2 phase, homologous recombination (HR) is exploited to repair DSBs within transcriptionally active regions, a possibility that appears rational given the potential enhanced accuracy of HR compared to NHEJ [30]. As discussed in the review by Irene Chiolo and colleagues, there is also evidence, though with less obvious rationality, that DSBs within repeat sequences may be preferably repaired by HR. If correct, then what determines how the optimal pathway is chosen and how do these signals interface with damage-induced chromatin modifications?

If HR repairs transcription associated DSBs in late S/G2 phase, then what happens to such DSBs in G1 phase? Recent studies have revealed that the slow component of DSB repair in G0/G1 phase cells occurs via a resection-mediated process of c-NHEJ [31], which arises in a manner akin to HR in late S/G2 phase cells [32]. This process will most likely require a greater degree of chromatin relaxation than the fast process of c-NHEJ, which occurs without the requirement for resection nucleases. Significantly, many of the reporter constructs for NHEJ are likely to monitor this resection-mediated NHEJ process, since resection-independent c-NHEJ will predominantly reconstitute the restriction site. Thus, an important future question is how damage induced chromatin modifications and chromatin remodelling influence the usage of these two forms of c-NHEJ (resection-independent or resection-dependent) versus HR (dependent upon extensive resection). However, to address such questions it is vital to understand the factors influencing which repair pathway is optimally utilised, which may itself be determined by pre-existing (ie non-DNA damage induced) chromatin modifications or structure.

Collectively, our reviews demonstrate the significance of the nucleosome as a central hub that organises the recruitment of repair and signalling factors in a co-ordinated fashion to achieve optimal DSB repair. Such optimal DSB repair may itself however, be determined, at least in part, by the chromatin environment prior to DNA damage. The optimal DSB repair process at these distinct sites has possibly been evolutionary determined by the route limiting genomic instability. This encompasses a range of endpoints including the avoidance of junctional deletions or missense mutations, translocations and longer term epigenetic changes in the DSB vicinity.

Moreover, the ability to interface DSB repair with the arrest and subsequent recovery of DNA transactions, such as repair and replication, is clearly important. This likely involves a complex network of changes in chromatin structure that arise in a temporal, spatial and context dependent manner. The future challenge lies in unravelling this complex web.

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