Integrating DNA damage repair with the cell cycle

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Abstract

DNA is labile and constantly subject to damage. In addition to external mutagens, DNA is continuously damaged by the aqueous environment, cellular metabolites and is prone to strand breakage during replication. Cell duplication is orchestrated by the cell division cycle and specific DNA structures are processed differently depending on where in the cell cycle they are detected. This is often because a specific structure is physiological in one context, for example during DNA replication, while indicating a potentially pathological event in another, such as interphase or mitosis. Thus, contextualising the biochemical entity with respect to cell cycle progression provides information necessary to appropriately regulate DNA processing activities. We review the links between DNA repair and cell cycle context, drawing together recent advances.

Introduction

During the past few decades the DNA repair field moved from considering repair simply as a variety of enzymatic activities and pathways towards understanding of how the relevant DNA transactions act in the context of the cellular environment and the cell division cycle. In simple terms the cell cycle is driven by cyclin dependent kinases (CDKs) that form a negative feedback loop oscillator [1]. The negative feedback is defined by the fact that high CDK activity drives CDK destruction. Mitotic CDKs have two core subunits, a cyclin that is periodically degraded and a kinase that is regulated by phosphorylation. When cells are born into G1, CDK levels are very low because the Anaphase Promoting Complex (APC/C; a specialised ubiquitin ligase) has been activated by the high CDK required for mitosis and has destroyed the mitotic cyclins. APC/C remains active in G1 and the consequent low CDK activity essentially defines G1.

Mammalian cells enter from G1 into S phase in response to the expression of D-type cyclins induced by growth factor signalling pathways. This causes overall CDK levels to increase and consequently the APC/C is inhibited. During S phase, mitotic cyclins are transcribed and translated and CDK activity thus continues to increase [2], although CDK activity is attenuated by phosphorylation of the kinase subunit by WEE1-related enzymes. As S phase reaches completion, CDK levels continue to increase and cells transit into G2. While there is not a defined point where S phase ends and G2 starts, it has historically been considered that the completion of bulk DNA synthesis, as measured by flow cytometry, marks the beginning of G2. More recently, it has become apparent that late replicating regions, including fragile sites, are still being replicated during “G2” in many cells [3].

The decision of when to enter mitosis depends on the continued increase in cyclin levels, the attenuation of WEE1-like kinase activities by CDK and an increase in CdDC25 phosphatase activity (that counteracts WEE1-dependent CDK phosphorylation). These events form positive feedback loops that intersect with the negative feedback mechanism to provide bi-stability to the oscillator. The balance of positive and negative activities, and thus the precise timing of mitosis, are modulated by regulatory mechanisms during which CDK interfaces with other mitotic [4] and checkpoint [5] kinases. Once the bi-stable switch has been thrown [6], cells can be considered to be committed to mitosis. Because high CDK levels activate the APC/C

(the negative feedback loop), high CDK activity programs its own destruction by APC/C –dependent cyclin degradation following the metaphase/anaphase transition. Thus, a new cycle begins [7].

It was first shown in fission yeast that CDK activity was required for efficient double strand break (DSB) repair [8]. Mutants impaired for cyclin B, the sole mitotic cyclin in fission yeast, were observed to be sensitive to DNA damage and unable to appropriately accumulate RPA foci in response to DSBs (see Figure 1). Subsequent work in budding yeast demonstrated that CDK phosphorylation directly regulated resection initiators [9,10] and other enzymatic activities that promote DSB resection [11,12]. Similar phenomena were subsequently shown to be conserved in human cells [13-18].

DSB repair pathway choice: G1 v G2.

DSBs are the most toxic DNA lesions [19]. In a haploid organism, a single unrepaired or misrepaired DSB is generally lethal. In diploid organisms, a single DSB may not be cell lethal, but if unrepaired or misrepaired can result in loss of heterozygosity (LOH) and/or generate gross chromosomal rearrangements (GCRs). There are two main pathways to repair DSBs (Figure 1), homologous recombination (HR), which is generally error-free, and Non Homologous End Joining (NHEJ) that frequently introduces small deletions or insertions. In G1 cells there is no sister chromatid to use as an HR template and cells therefore use NHEJ [20]. Even in diploid cells the use of a homologous chromosome for HR is not favoured because the search for homology is prone to result in non-allelic HR at repeat sequences, potentially generating GCRs and/or LOH.

A key determinant in the choice between HR and NHEJ is whether the DSB is resected to generate the 3' ssDNA tail essential for strand invasion [21]. Once resected, repair of a DSB by NHEJ is blocked and repair by mechanisms other than HR (for example single strand annealing or microhomology-mediated end joining) are likely to result in loss of genetic material and/or GCRs. To prevent inappropriate attempts at HR, cells thus regulate resection onset (Figure 1). In human cells this pathways choice is controlled by the association of 53BP1 with the chromatin surrounding the break site, which inhibits resection (Figure 2). 53BP1-dependent resection inhibition is counteracted in G2 by BRCA1 and a number of BRCA1-associated factors such as PTIP and RIF1 [20].

Since a defining characteristics of G1 is low CDK and high APC/C it is unsurprising that multiple enzymes required for efficient resection, plus some downstream HR factors, are positively regulated by CDK-dependent phosphorylation. Some may also be degraded directly by APC/C [22]. A key CDK target is CtIP, a regulator of the MRE11/RAD50/NBS1 nuclease. Human CtIP is recruited to DSBs and must be phosphorylated on T847 to be active [14]. However, expressing CtIP-T847E (a phospho-mimetic and thus constitutively activated allele) is insufficient to drive HR. This is because a second mechanism prevents the recruitment of the activator of resection, BRCA1 [23].

BRCA1 associates at DSB sites and promotes HR by counteracting 53BP1-dependent resection inhibition, activating CtIP [24,25] and recruiting BRCA2 (a modulator of the key downstream HR protein RAD51). The recruitment of BRCA2 by

BRCA1 is mediated through an interaction between BRCA1 and the BRCA2-associated protein, PALB2. A Cullin 3 ubiquitin ligase, $CRL3^{KEAP1}$, constitutively targets the BRCA1 interacting domain of PALB2. This ubiquitylation does not promote PALB2 degradation, but prevents the BRCA1-PALB2 interaction. To facilitate BRCA2 recruitment, PALB2 ubiquitylation by $CRL3^{KEAP1}$ must be counteracted by a protease, USP11. To ensure that PALB2 cannot interact with BRCA1 in G1 cells (and thus cannot initiate HR in G1 by recruiting BRCA2) USP11 is degraded in G1 cells. This is enforced by a Cullin 4-dependent Ub ligase that is only active in G1 cells. Therefore, PALB2 ubiquitylation is a second mechanism that inhibits HR in G1 and is complementary to the requirement for CDK phosphorylation of key resection initiation proteins such as CtIP. Combining expression of CtIP-T847E (over-riding CDK regulation of CtIP) with stabilisation of USP11 allows HR in G1 cells.

DNA repair in S phase.

Some aspects of DNA repair in S phase are specific by definition. For example, lesions such as inter-strand crosslinks (ICLs) are mainly detected during S phase when encountered by the replication machinery [26,27]. ICLs detected by the replisome initiate a complex set of enzymatic reactions that, as with many HR events in S phase, are regulated by the Fanconi Anaemia (FA) pathway [28]. Similarly, unrepaired base lesion encountered during DNA replication can be bypassed by the replisome and the resulting gap in the newly synthesised strand filled by post-replication repair (PRR). There are two main PRR pathways; synthesis across the lesion by polymerases with reduced fidelity or an HR-based template switching mechanism that copies the information from the newly synthesised strand of the sister chromatid [29]. PRR pathways are initiated in S phase by the ubiquitylation of PCNA, although it has become clear that many of the gap repair events are occurring in G2 [30,31].

S phase provides the cell with a unique problem: a proportion of DNA is replicated while other segments are not. How does a cell distinguish a lesion in replicated as opposed to unreplicated chromatin? In G1 cells Histone H4 in unreplicated chromatin is dimethylated on Lysine 20 (H4K20me2). During replication, newly incorporated H4 is unmethylated on this residue [32]. This provides a mark (H4K20Me0) that allows the association of the TONSL protein (Figure 1). Along with MMS22L, TONSL can load RAD51 and is a facilitator of HR [33,34]. Thus, newly replicated DNA is HR-competent. In contrast, H4K20me2 cannot associate with TONSL, but can facilitate the binding of 53BP1: in response to a DNA lesion H2A is ubiquitylated on lysine 15 (H2AK15Ub) by RNF168 [35] and co-operates with (constitutive) H4K20me2 to provide a docking platform for 53BP1. Because H4K20Me2 is diluted by a factor of two in newly replicated DNA, the affinity of 53BP1 at DNA damage sites is decreased, attenuating the ability of 53BP1 to block the HR. This likely synergises with TONSL/MMS22L association to facilitate HR and limit NHEJ when damage occurs in newly replicated chromatin [36]. Thus, cells can distinguish replicated from unreplicated DNA in S phase, helping the appropriate processing of DNA lesions.

DNA repair in Mitosis.

Early experiments showed that introducing DNA damage into early mitotic cells resulted in a transient reversal of mitosis [37]. However, until quite recently it was considered that once the decision to enter mitosis has been made DNA damage

responses became largely irrelevant. It is now recognised that, when cell transit into mitosis, DNA damage responses are significantly modified. For example, in response to DSBs ATM activation and H2AX phosphorylation still occur (in fact more so because some counteracting phosphatases are downregulated by APC/C), but RNF8/RNF168-dependent ubiquitylation is decreased [38] and DSB end resection prevented by mechanisms that include CtIP hyper-phosphorylation [39,40]. NHEJ is also attenuated in mitosis by via XRCC4 phosphorylation [41] and a range of key downstream HR proteins, including BRCA2 and RPA, are also phosphorylated, altering their properties [42].

It has recently become clear that a small number of stalled (or ongoing) replication events are present in many cells as they enter mitosis. These correlate to common fragile sites (CFS), genomic loci that have a tendency to break during mitosis. At least a proportion of CFS are defined by being late replicating regions of the genome with a paucity of potential replication initiation zones [3]. To minimise the potential for these late replication events to generate genetic instability specific mechanisms act during mitosis to repair failed replication events and/or minimise the consequent genetic instability. Attempts to complete replication as cells enter mitosis have recently been recognised by the incorporation of pulsed BrdU label into mitotic chromosomes. This requires the activity of MUS81, POLD3 (a subunit of Polymerase delta required for its full efficiency) and RAD52, but not RAD51. [43,44]. The DNA synthesis that occurs in early mitotic cells is clearly distinct from the canonical synthesis observed during S phase and contributes to maintaining genome stability.

In mammalian cells mitosis-specific DNA replication is initiated by the association of the structure-specific nuclease MUS81/EME1 (an obligate heterodimer) with SLX1/SLX4. MUS81/EME1 and SLX1/SLX4 exist as separate complexes in G1/S but come together in late G2/M [45]. This is controlled by MUS81's association with the SAP domain of SLX4, which requires multiple CDK phosphorylation events. The MUS81/EME1 - SLX1/SLX4 interaction is likely to modulate function by changing MUS81 substrate specificity: SLX4 enhances MUS81/EME1 activity against fork structures *in vitro* by relaxing its substrate specificity [46]. Consistent with a mitotic function for MUS81/EME1 against replication structures, inhibition of WEE1 (thus promoting premature CDK hyper-activation in S phase) results in catastrophic MUS81/EME1-dependent pulverisation of chromosomes. The cell cycle regulation of MUS81 nuclease activity appears to be particularly important: MUS81 can also bind an alternative EME1-related subunit, EME2. MUS81-EME2 complex only forms in G1/S phase and has a distinct function in remodelling replication structures to promote fork restart [47,48].

Additional mitotic-specific repair events have been identified, including the resolution of Holiday junctions (HJs) that persist from recombination events that occurred during S phase and G2 [49]. Double HJs are, by preference, dissolved by BLM/TOP3 in G2 – a process that avoids crossovers and thus minimises potential LOH [50]. However, single HJs and unprocessed double HJs can persist into mitosis and are resolved by either MUS81/EME1-SLX1/SLX4 or Gen1, a HJ resolvase that is also activated in mitosis in a CDK-dependent manner [51,52].

Conclusion

The study of the mechanisms that regulate DNA repair and other DNA damage responses through the cell cycle is evolving rapidly. There is an immense amount of detail in the literature concerning the regulation of repair and checkpoint signalling [53] by cell cycle dependent phosphorylation and ubiquitylation. In the next few years we will likely have a clearer understanding of how DNA repair pathway choice for DSBs, the most toxic of DNA lesions, is integrated with, and fine-tuned by, the cell cycle. We will also develop a more complete understanding of the causes and consequences of the genetic instability that results from DNA lesions that persist into mitosis and how the intricacies of DNA structure-specific DNA metabolic enzymes are contextualised minimise these events.

Acknowledgments
The authors acknowledge funding from the Wellcome Trust (AMC; 110047/Z/15Z) and the Medical Research Council (JMM; MR/P018955/1). The funders have had no role in the preparation of this work or the decision to submit for publication.

Figure Legends

Figure 1
The two main pathways of DSB repair. Left: DSB repair by NHEJ. the Ju70/80 dimer binds to DNA ends and protects them from nucleases. DNA-PKcs binds to Ku and orchestrates end processing and subsequent ligation by DNA ligase IV (Lig4). Right: in S phase and G2 cells can repair DSBs through homologous recombination (HR) using the sister chromatid as a template. CtIP and MRN are rapidly recruited and can initiate resection by removing Ku. Resection is continued by a combination of two pathways involving the nucleases, Exo1 or DNA2. The decision whether to initiate resection is the key control point between HR and NHEJ (see text for details). As DNA is resected, RPA (yellow) is loaded and subsequently Rad51 forms a filament on the ssDNA. This performs a homology search and establishes a D-loop which allows the initiation of DNA synthesis.

Figure 2
Simplified schematic of key cell cycle dependent DNA damage responses. Before replication (blue background) 53BP1 binds to chromatin at sites of DNA damage and inhibits resection and thus HR. This facilitates repair by NHEJ. After a region has been replicated (red background) the newly deposited H4 remains unmethylated on K20 and allows the binding of TONSL/MMS22L, which helps promote recombination. In addition, BRCA1 becomes competent to associate with sites of DNA damage and counteracts the inhibitory function of 53BP (see text for mechanistic details). If unresolved replication or recombination structures remain when cells enter mitosis (green background) the high activity of CDK and other mitotic kinases (such as Polo-like kinases) modify the function of multiple enzymes to attempt their resolution. Thus, a wide variety of DNA damage response and repair enzymes are regulated to facilitate distinct responses to otherwise biochemically identical lesions.

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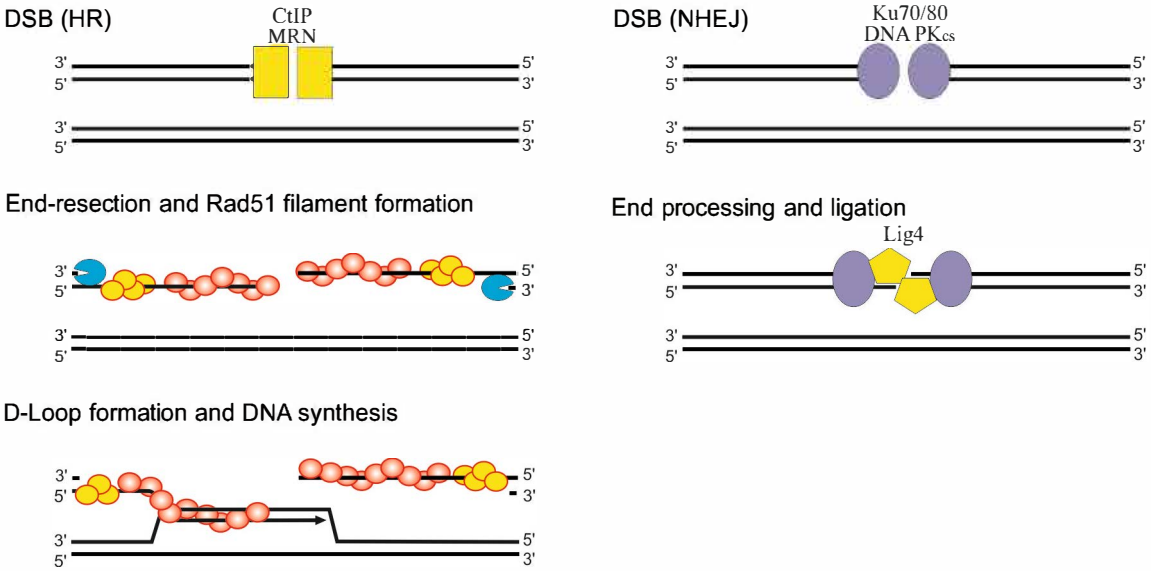
Figure 1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DSB (NHEJ)
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D-Loop formation and DNA synthesis
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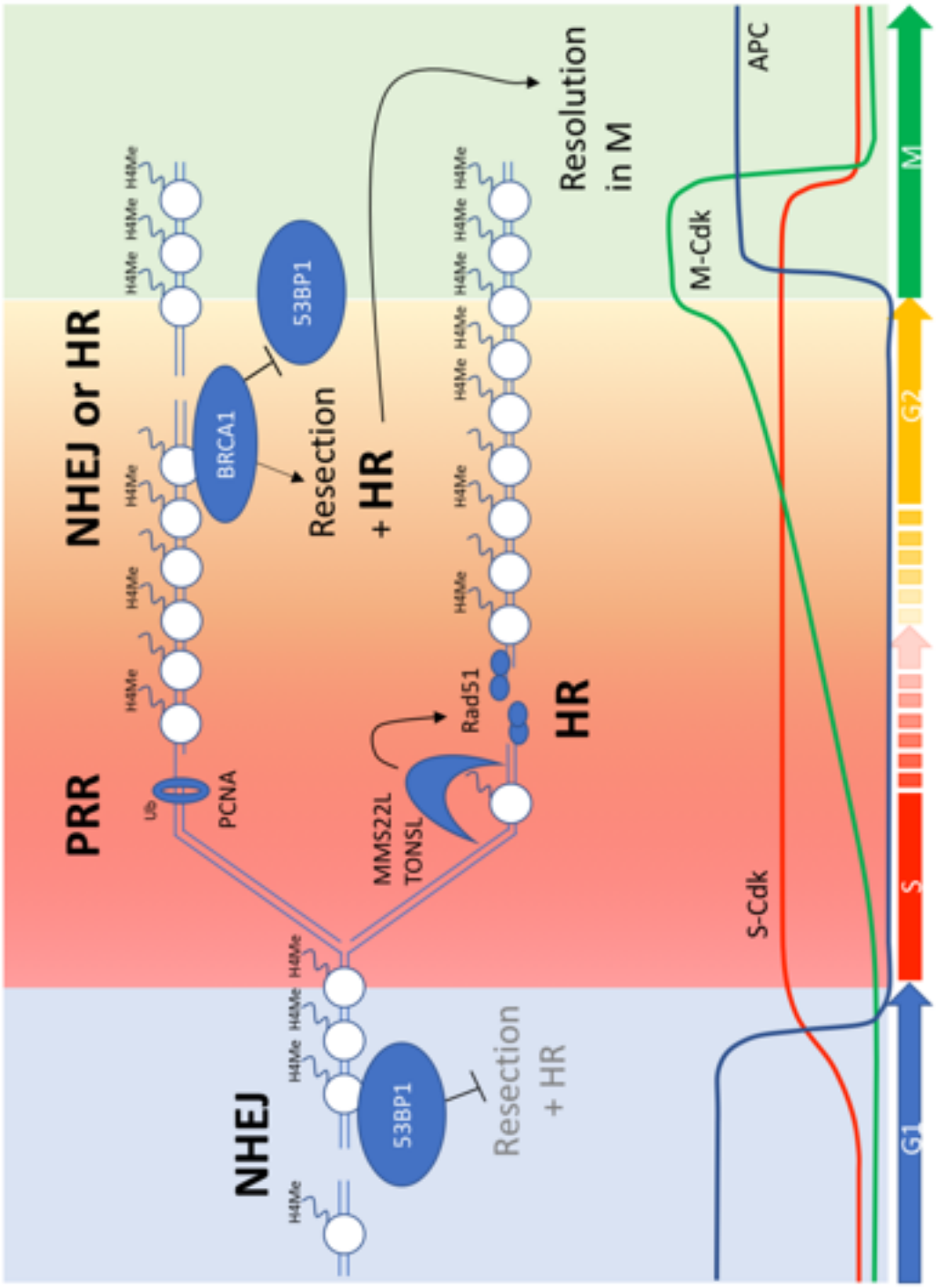
Figure 2


PRR
NHEJ or HR
NHEJ
HR
H4Me
Ub
PCNA
BRCA1
53BP1
Resection
+ HR
MMS22L
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Rad51
Resolution
in M
S-Cdk
M-Cdk
APC
G1
S
G2
M