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Synergy Between DNA Replication and Repair Mechanisms

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1. Introduction

DNA replication is a fundamental and stringently regulated cellular process that ensures the accurate propagation of the cell's genetic material. An accurate duplication of the genome and segregation to the daughter cells is essential, as any unreplicated genomic regions will result in breaks and deletions during mitosis, including regions containing tumor suppressor genes, while local DNA over-replication will result in gene, and possibly oncogene, amplification (Gonzalez et al., 2005). Several DNA replication proteins, both initiator and replication fork (reviewed in (Hubscher, 2009)) proteins, have been shown to also play an essential role in several DNA repair pathways, such as base excision repair, nucleotide excision repair, and double-strand (ds) break and mismatch repair. Recent work from prokaryotes and eukaryotes has indicated that replication initiator proteins are also directly involved in multiple cellular processes (reviewed in (Scholefield et al., 2011)), coordinating the initiation of DNA replication with other cell cycle-related activities, including DNA repair (Moldovan et al., 2007; Oakley and Patrick, 2010). DNA repair, like all major cellular functions, including transcription and DNA replication, is a tightly regulated process. This review deals with the apparent synergy between the DNA replication and repair mechanisms.

2. Mammalian DNA replication

2.1 Replication origins

Mammalian DNA replication is initiated at multiple sites (estimated to be about 10^4-10^6), termed replication origins, and proceeds bidirectionally (reviewed in (Aladjem, 2007; Arias and Walter, 2007; Rampakakis et al., 2009a; Sclafani and Holzen, 2007; Zannis-Hadjopoulos, 2005)). Clusters of adjacent origins are activated at different times throughout S phase and are replicated in a defined spatial and temporal order. Replication origins are marked by the presence of a mammalian consensus sequence (Di Paola et al., 2006) throughout the genome and the binding of initiator proteins (IPs), which unwind the DNA and recruit additional downstream proteins. Origin activation starts with the binding of an IP to specific recognition sequences, triggering melting at the origin, leading to the formation of a stable pre-replication complex (pre-RC) that contains locally unwound DNA (Bell and Dutta, 2002; Dutta and Bell, 1997) and promoting the assembly of the multienzyme complexes required

for replication. The timing and frequency of initiation may be regulated by the availability of the IP or by topological changes in the DNA that affect the IP's ability to interact with the origin (Kornberg and Baker, 1992), reviewed in (Rampakakis et al., 2010) .

2.2 Pre-replication complex (Pre-RC)

The first initiator protein to bind to the origin and the best characterized is the hexameric origin recognition complex (Orc1-6; reviewed in (Sasaki and Gilbert, 2007)). All ORC subunits, except for ORC6, belong to the superfamily of AAA^+ ATPases (*A*TPases *A*ssociated with various cellular *A*ctivities) with conserved Walker A, B, C and D motifs (Bell and Dutta, 2002; Koonin, 1993). ORC acts as landing pad for the binding of additional replication proteins during G_1-phase, such as Cdc6, another AAA^+-ATPase. ATP binding of Cdc6 leads to a conformational change that promotes its association with chromatin (Tatsumi et al., 2000). Binding of Cdc6 to DNA-bound ORC leads to the activation of the ORC1 ATPase activity (Bell and Dutta, 2002) as well as to a conformational change, which increases the stability and specificity of the ORC-Cdc6-DNA complex [Mizushima, 2000 #5780; Speck, 2005 #9948; Speck, 2007 #9958]. Origin-bound Cdc6 facilitates the recruitment of Cdt1, which physically interacts with components of the putative DNA helicase, the minichromosome maintenance protein complex (MCM2-7), participating in their nuclear translocation and chromatin loading (Cook et al., 2004; Nishitani et al., 2000; Tanaka and Diffley, 2002b; Yanagi et al., 2002) as well as with Cdc6 (Dhar et al., 2001; Nishitani et al., 2000).

Following MCM loading onto ORC-Cdc6, Cdc6 and Cdt1 dissociate from the origins and, finally, ATP hydrolysis by ORC completes the MCM helicase loading reaction (Randell et al., 2006; Speck et al., 2005; Speck and Stillman, 2007). At this stage, origins are primed and awaiting the activity of the cyclin-dependent kinases (CDKs) in order to be activated and S-phase to begin. Activation of the pre-RC to an active initiation complex is regulated by CDKs and other signaling proteins, which promote further protein assembly that eventually leads to the loading of the polymerases and the activation of the MCM helicase.

Upon entry into S phase, multiple mechanisms ensure that the replication initiation machinery is inactivated so as to avoid re-replication of chromosomal regions and genome instability (Blow and Dutta, 2005; Dorn et al., 2009; Hook et al., 2007; Krasinska et al., 2008; Rampakakis et al., 2009a) and references therein.

2.3 The replisome

Entry into S phase is accompanied by the activation of the replisome, a multiprotein complex that unzips the parental helix and duplicates the separated strands. The core components of the eukaryotic replisome include the putative replicative helicase MCM2-7 complex, which encircles the leading DNA strand, the primase/polymerase α complex, the single-strand DNA (ssDNA) binding protein RPA, the clamp loader replication factor C (RFC; or replication protein C, RPC), the proliferating cell nuclear antigen (PCNA) sliding clamp, and the replicative DNA polymerases δ and ε, as well as the more recently identified Cdc45 and GINS proteins (Sheu and Stillman, 2006; Yabuuchi et al., 2006) (Figure 1).

The structure of the eukaryotic putative MCM helicase has been deduced by using as models the atomic structure of the N-terminus of the MCM protein from *Methanobacterium thermoautotrophicum* (Mth-MCM) and the SV40 T antigen (Fletcher et al., 2003; Gomez-Llorente et al., 2005; Li et al., 2003; Pape et al., 2003; Sclafani et al., 2004). Mth-MCM is a true homologue of the eukaryotic MCM, while the SV40 T antigen is an analogue, resulting from

convergent evolution. Using this reconstructive method the MCM helicase is believed to be a planar, double hexamer in head-to-head conformation. The N-terminal domain is believed to be responsible for oligomerization and DNA binding, while the C-terminal contains the catalytic ATPase and helicase domains. In agreement with this model, using electron microscopy (EM), the eukaryotic MCM complex was shown to have a central large opening between the two hexamers (34 Å), which is thought to accommodate dsDNA participating in its unwinding (Yabuta et al., 2003).

Purification of *in vivo* MCM complexes in human cells led to the identification of a MCM4/6/7 subcomplex with ATPase, ssDNA-binding, dsDNA-binding and helicase activities. This subcomplex is believed to be the catalytic core of the MCM hexamer, while MCM2/3/5 represent the regulatory subunits (Ishimi, 1997; Ishimi et al., 1996). This model was further confirmed by *in vitro* reconstitution experiments using recombinant MCM subunits from yeast, frog and mouse cells (Schwacha and Bell, 2001; Ying and Gautier, 2005; You et al., 2002). However, the helicase activity of the MCM complex was shown to be very weak and not as processive as one would expect from the replicative helicase (Patel and Picha, 2000). This was later explained by the fact that the MCM helicase activity is greatly enhanced by the Cdc45 and GINS co-factors in both *X.laevis* (Masuda et al., 2003; Pacek and Walter, 2004) and *D.melanogaster* (Moyer et al., 2006).

Cdc45 binds onto origins after MCM recruitment, but prior to DNA unwinding and polymerase recruitment [Walter, 2000 #9377;Mimura, 2000 #10138] as well as travels with the replication fork (Aparicio et al., 1999), thus being important for both replication initiation and fork progression [Tercero, 2000 #6212;Zou, 2000 #6499].

GINS is a recently identified member of the replisome composed of the Sld5, Psf1, Psf2 and Psf3 proteins. It has a ring-like structure in the electron microscope and functions interdependently with Cdc45 in the loading of the replisome, including the DNA polymerases and RPA (Aparicio et al., 1999; Kubota et al., 2003; Takayama et al., 2003) and, possibly, the coupling of MCM with other factors at DNA replication forks (Labib and Gambus, 2007).

Upon synthesis of the initial RNA primer by the DNA primase, RFC, an arc-shaped complex of five essential AAA+ type ATPases, recognizes the 3′ ends of the template-primer and loads the proliferating cell nuclear antigen (PCNA) in an ATP-binding dependent manner. PCNA is a homotrimeric ring-shaped complex, which encircles DNA and acts as a sliding clamp able to slide freely in both directions. The PCNA ring tethers polymerases δ and ε firmly to DNA, increasing their processivity from 10-15bp to thousands of nucleotides (Ayyagari et al., 1995), and functions as a moving platform for factors involved in replication-linked processes such as DNA repair, chromatin remodelling and epigenetic inheritance (Moldovan et al., 2007).

3. Interplay between DNA replication and repair proteins

Several proteins that are part of the multi-protein replication complex, but are not a member of the pre-RC, have a dual role in DNA replication and repair, such as PCNA (Dimitrova et al., 1999; Moldovan et al., 2007), the Replication Protein A (RPA)(Chesnokov, 2007) and the multifunctional Ku protein (reviewed in (Rampakakis et al., 2009a).

3.1 Proliferating cell nuclear antigen (PCNA)

PCNA, the DNA polymerase processivity factor, associates with replication foci at the onset of S-phase, co-localizes with early-replicating chromatin and is present at initiating

replication forks (Moldovan et al., 2007; O'Keefe et al., 1992). In addition to tethering polymerases δ and ε to DNA, it acts as a landing pad for a large number of factors related to DNA metabolism. Together with its loader RFC (Replication Factor C) they are essential players for processive replication and coordinated DNA repair (Bylund et al., 2006).
Encounter of the replication machinery with DNA lesions can be deleterious as it may result in fork stalling and possibly chromosomal rearrangements or even cell death, if it is prolonged. In response to this, a PCNA-mediated bypass mechanism is activated, named translesion synthesis (TLS). TLS involves the temporary switch from the replicative polymerases δ and ε to error-prone polymerases, such as pol η, with large enough active sites which can accommodate DNA lesions, thus allowing their bypass (Moldovan et al., 2007). Error-free TLS has also been found but its mechanism is still unknown. Hoege et al. showed that post-translational modification of PCNA with ubiquitin is an important process during TLS (Hoege et al., 2002); in fact, a "switch" mechanism was described according to which PCNA mono-ubiquitilation activates the error-prone TLS, whereas PCNA poly-ubiquitilation triggers the error-free TLS. In agreement, human Polη was found to interact specifically with monoubiquitylated PCNA upon UV-induced photodamage (Kannouche et al., 2004).
A role for PCNA in the mismatch repair (MMR) of complementary base mismatches or insertion/deletion loops through direct interaction with the MSH3, MSH6 and MLH1 sensor proteins and exonuclease I (EXOI) has also been shown. The current MMR model involves the recognition of the error-containing newly synthesized DNA strand through the presence of a gap, such as the end of the Okazaki fragment, and the directional orientation of PCNA followed by the excision of the defective strand in the 5′ to 3′ direction by EXOI (Modrich, 2006). A different mode of function of the MMR machinery was also proposed by Kadyrov et al., who showed that MutLα (MLH1/PMS2) is a latent endonuclease activated by MutSα, RFC and PCNA in a mismatch- and ATP-dependent manner. Consequently, a mismatch-containing DNA segment flanked by two strand breaks is removed by EXOI and replaced upon targeting of the DNA synthesis machinery (Kadyrov et al., 2006).
Finally, PCNA functions as a scaffold for factors functioning in base excision repair (BER). More specifically, PCNA has been shown to interact with the UNG2, MPG, and NTH1 DNA glycosylases, as well as the APE2 AP endonuclease, stimulating their ability to generate abasic sites and cleave them in order for repair to take place (Ko and Bennett, 2005; Oyama et al., 2004; Tsuchimoto et al., 2001; Xia et al., 2005). An interaction between PCNA and the structure-specific repair endonuclease xeroderma pigmentosum (XP) G was also found, suggesting a function in nucleotide excision repair (NER) (Gary et al., 1997), but in this case PCNA is recruited by XPG upon nucleotide excision by ERCC1, resulting in the gap filling by polymerase δ (Mocquet et al., 2008).

3.2 Replication protein A (RPA)

RPA is the major eukaryotic single-stranded (ss) DNA binding protein and it is required for DNA replication, recombination and repair. RPA helps recruit DNA primase/polymerase α to the origins, stabilizing ssDNA in the proper extended conformation so that it can be copied by DNA primase, and stimulates its polymerase activity and processivity (Maga et al., 2001). Furthermore, during replication fork progression, RPA stimulates the replicative polymerases δ and ε, possibly through its interaction with PCNA (Dianov et al., 1999; Loor et al., 1997).

Parallel to its function in DNA replication RPA participates in a variety of nuclear metabolism repair processes, involving single-stranded DNA through a complex network of protein-protein interactions. RPA has been shown to play a role in nucleotide excision repair (NER) through its interaction with the XPF-ERCC1 and XPG endonucleases, positioning them at the 5′ and 3′ of the lesions, respectively (Bessho et al., 1997; De Laat et al., 1998; He et al., 1995; Stigger et al., 1998). Furthermore, RPA has been shown to stimulate the base excision repair (BER) of abasic sites in DNA as well as the excision process during mismatch repair (MMR), by binding the human DNA glycosylases UNG2 and hMYH, or the hExoI, respectively (Dianov et al., 1999; Genschel and Modrich, 2003; Nagelhus et al., 1997; Parker et al., 2001). Finally, a role for RPA has also been suggested in the repair of double-strand DNA breaks (DSBs) at stalled replication forks through homologous recombination. More specifically, RPA was shown to protect the ssDNA after DNA strand resection and 3′ DNA overhang generation at DSBs upon hydroxyurea-induced replication stalling, recruit RAD52 through direct interaction and act as a nucleation point for the RAD51 and RAD52 proteins (Sleeth et al., 2007).

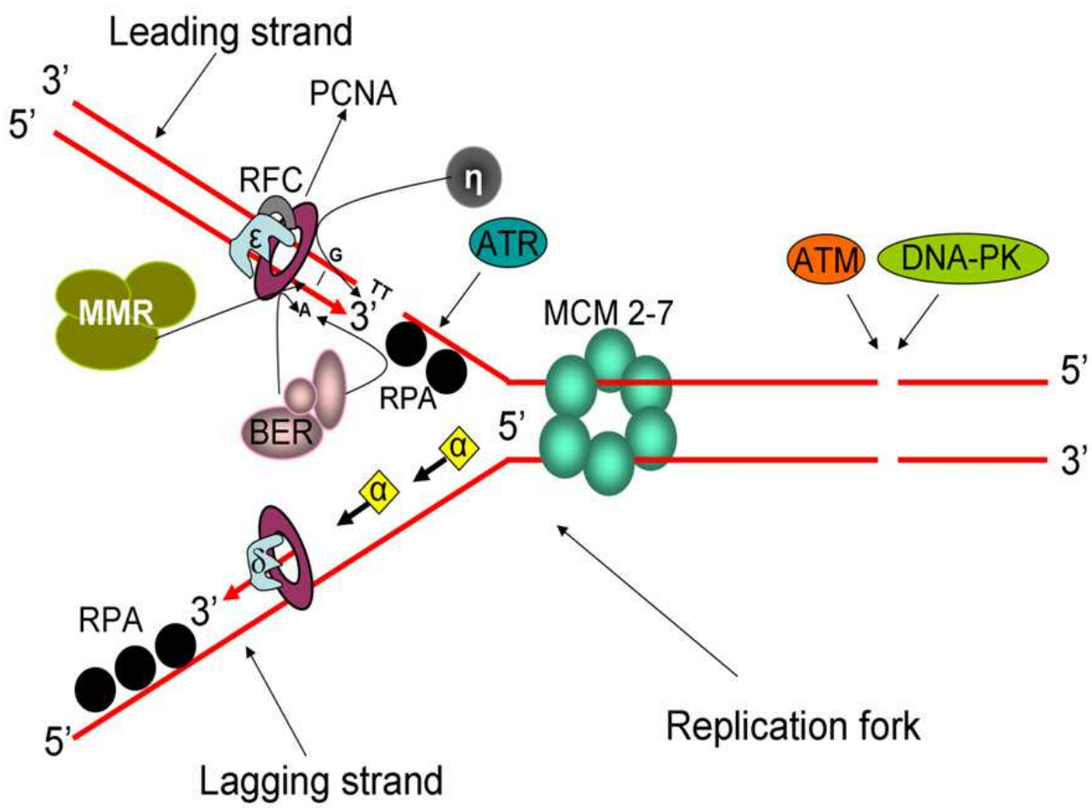

Fig. 1. Interplay between the DNA replication and DNA repair machineries. Encounter of the replication fork with various types of damaged DNA results in the recruitment of DNA repair enzymes and triggers the activation of cell cycle checkpoints, cell cycle arrest or apoptosis.

3.3 The Ku protein

The heterodimeric Ku protein (Ku70/Ku80; reviewed in (Tuteja and Tuteja, 2000)) is a multifunctional guard of the genome, participating in DNA replication and repair, recombination, telomeric maintenance, and the suppression of chromosomal rearrangements (Downs and Jackson, 2004; Zannis-Hadjopoulos et al., 2004). Ku is a member of the non-homologous end-joining (NHEJ) machinery, participating in the repair of double-strand DNA breaks (DSBs) by recruiting and allosterically activating the DNA-dependent protein kinase (DNA-PK) (Collis et al., 2005), as well as of the DNA replication licensing machinery, binding onto mammalian DNA replication origins at the end of G1-phase (Novac et al., 2001) and recruiting the DNA replication machinery (Rampakakis et al., 2009a; Rampakakis et al., 2008; Sibani et al., 2005b).

3.3.1 Ku and mammalian DNA replication

There has been a lot of accumulated evidence implicating the Ku protein in the initiation of mammalian DNA replication. Ku was initially identified as the DNA-dependent ATPase purified from HeLa cells (Cao et al., 1994), which co-fractionated with a 21S multiprotein complex that is able to support SV40 *in vitro* DNA replication (Vishwanatha and Baril, 1990). It was subsequently shown to co-immunoprecipitate with well characterized DNA replication proteins involved in either the initiation or the elongation phase, such as DNA polymerases α, δ and ε, PCNA, topoisomerase II, RF-C, RP-A, and ORC-2 (Matheos et al., 2002). In agreement with and corroborating the previous studies, a proteomic analysis using a TAP affinity purification procedure, identified Ku as part of a complex with MCM2-7 proteins, the putative replicative DNA helicase (Burckstummer et al., 2006). Furthermore, Ku was identified as part of a human protein initiation complex, important for the replication of Kaposi's sarcoma associated HSV (KHSV) (Wang et al., 2008).

Ku is an origin binding protein, binding to several replication origins, among them the adenovirus type 2 origin (de Vries et al., 1989), the Herpes Simplex Virus Type 1 (HSV1) origin (Murata et al., 2004), the B48 human origin (Toth et al., 1993), the mammalian replication origin consensus sequence, A3/4 (Price et al., 2003; Ruiz et al., 1999), the Chinese hamster dihydrofolate reductase (DHFR) replication origin, oriβ, and the monkey replication origins ors8 and ors12 (Novac et al., 2001), as well as the human origins lamin B2, β-globin, c-myc (Sibani et al., 2005a, b) and dnmt1 (DNA-methyltransferase) (Araujo et al., 1998). Ku was shown to associate *in vivo* with replication origins in a cell cycle dependent manner (Novac et al., 2001; Ruiz et al., 1999; Sibani et al., 2005a) and its differential binding to DNA is a determining factor in its involvement in DNA replication, exhibiting distinct origin DNA binding properties from its association with DNA ends or other internal DNA sequences (Schild-Poulter et al., 2003).

The role of Ku in DNA replication is believed to be two-fold. First, with regard to the initiation of DNA replication, Sibani et al. showed that Ku binds to human replication origins prior to the ORC assembly and Ku-deficiency results in decreased origin usage and initiation of DNA replication (Sibani et al., 2005a, b). A possible mechanism for this was recently proposed, involving the DNA topology machinery. Topoisomerases I and II, the major constituents of the DNA topology machinery, were previously found to interact with the lamin B2 origin and participate in their activation (Abdurashidova et al., 2007). Recently, Rampakakis et al. showed that the binding of Ku and Topo IIβ to the human replication origins lamin B2 and hOrs8 (in a complex also containing DNA-PK and PARP-1) is

associated with a transient, site-specific dsDNA break at these origins, which leads to local topological changes and recruitment of the replication initiator machinery (Rampakakis et al., 2009a). As the DNA topology and NHEJ machineries have reverse enzymatic activities, generating and repairing DNA DSBs, respectively, their functional synergy in replication origin activation is striking. A possible scenario is that Ku functions in tethering Topo IIβ onto replication origins, thus increasing the sequence specificity of its cleaving enzymatic activity (Figure 2), in a manner similar to that shown for RAG recombinases, which have similar enzymatic properties to DNA topoisomerases (Sawchuk et al., 2004). Alternatively, recruitment of DNA-PK by Ku and repair of the DSBs through NHEJ may function as a backup mechanism, ensuring chromosomal stability in cases of Topo II malfunction.

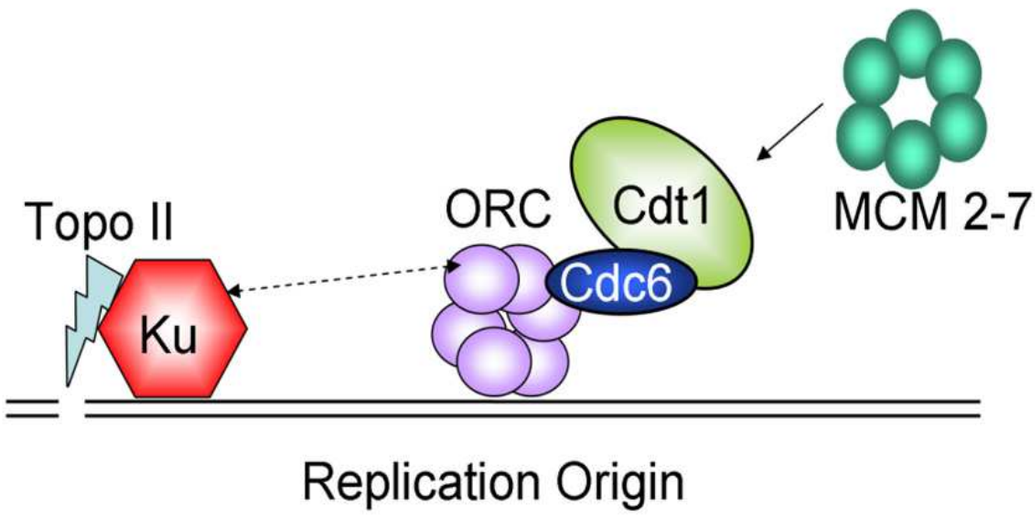

Fig. 2. Model for the role of Ku in pre-RC assembly. Targeting of Ku and Topoisomerase II onto chromatin during G1 phase leads to topologic changes in the chromosomal regions that correspond to replication origins, facilitating the assembly/stability of the ORC hexamer.

Second, at the replication fork progression level, Park et al. showed that upon IR-induced DNA damage, Ku-, but not DNA-PKcs-, deficient cells exhibited significantly slow S phase progression due to collapse of PCNA from the replication fork (Park et al., 2004). These results led the authors to suggest a role for Ku in maintaining the sliding clamp on chromatin at chromosomal breaks, thus facilitating efficient resumption of DNA replication. In agreement with a role for Ku in the replication fork progression, Hoek et al. showed that Ku directly associates with the chromatin assembly factor 1 (CAF-1) (Hoek et al., 2011), the primary DNA replication-coupled histone deposition factor, which is attached to the replication fork through PCNA (Shibahara and Stillman, 1999). Although no functional evidence was shown, the authors suggested that the significance of this interaction may involve the recruitment of CAF-1 to sites of DSBs in order to establish the appropriate local chromatin structure, which would allow cell cycle progression. Finally, a DNA-PKcs dependent role for Ku was also shown during DNA replication (Shimura et al., 2007). Using the DNA replication inhibitor aphidicolin to transiently perturb DNA replication, Shimura et al. showed that persistent DNA breaks accumulated in DNA-PKcs deficient cells, resulting in the activation of an ATR-mediated S-phase checkpoint and blockage of cell cycle

progression. In contrast, their wild-type cells continued to synthesize DNA and were able to promptly repair the DNA breaks, suggesting a role of DNA-PK in immediately repairing DNA breaks following deceleration of DNA replication.
Altogether these results suggest that, in addition to its role in repairing dsDNA breaks that occur during replication fork progression (Shimura et al., 2007), Ku is also involved in the prevention of DNA breaks caused by replication fork collapse by: i) binding onto DNA replication origins at G1 phase (Novac et al., 2001; Ruiz et al., 1999), recruiting the DNA replication machinery (Rampakakis et al., 2008; Rampakakis and Zannis-Hadjopoulos, 2009; Sibani et al., 2005b) and ensuring genomic duplication and maintenance (Toth et al., 1993) (progression into S phase without the appropriate number of activated replication origins would lead to an increase of the average replicon size, resulting in stalled replication forks and chromosomal instability (Ekholm-Reed et al., 2004; Tanaka and Diffley, 2002a)); and ii) maintaining the DNA polymerase processivity factor PCNA on chromatin following ionizing radiation (Park et al., 2004).

3.4 DNA damage checkpoints

Accurate and precise genome duplication and segregation to the daughter cells is essential, as small unreplicated regions will result in breaks and deletions during mitosis, including in tumor suppressor genes, while local over-replication would result in gene, and possibly oncogene, amplification (Gonzalez et al., 2005). Thus, the cell has evolved surveillance mechanisms (cell cycle checkpoints) to monitor the proper succession of events throughout the cell cycle. The checkpoint proteins are activated following DNA lesions (Branzei and Foiani, 2008; Hakem, 2008) or insufficient replication initiator proteins (Lau and Jiang, 2006; Machida and Dutta, 2005) and arrest cells in the cell cycle in order for DNA-repair to take place.

3.4.1 Initiation of DNA replication and checkpoint activation

Low levels of replication initiator proteins, were shown to induce a blockage of cells to late G_1 phase, due to Cyclin E/Cdk2 inactivation (Machida and Dutta, 2005; Rampakakis et al., 2008), or apoptosis (Feng et al., 2003). Blockage of pre-RC assembly by overexpressing a stable form of geminin in primary fibroblasts resulted in G1 arrest with reduced Cyclin E levels and hypophosphorylated pRB (Shreeram et al., 2002). Altogether, these results suggest the existence of a G1/S checkpoint overseeing the efficient pre-RC formation. Although the significance of this checkpoint is still obscure, it is thought to protect cells from DNA replication crisis and possible aberrant genome duplication, since premature progression into S phase without the appropriate number of activated replication origins would lead to an increase of the average replicon size, resulting in stalled replication forks and chromosomal instability (Ekholm-Reed et al., 2004; Tanaka and Diffley, 2002b). In agreement with this scenario, deregulation of Cyclin E was shown to impair pre-RC formation and cause chromosome instability in human cancer cells (Ekholm-Reed et al., 2004).
Origin re-replication due to erroneous pre-RC inactivation upon S-phase entry activates a different type of cell cycle checkpoint acting at the G2/M border. Overexpression of Cdt1 or Cdc6 induces an ATM/ATR– and p53-dependent checkpoint pathway preventing re-replication (Vaziri et al., 2003). Similarly, re-replication induced by geminin depletion resulted in the activation of a G2/M checkpoint which, however, was p53-independent, but Chk1-dependent (Melixetian et al., 2004; Zhu et al., 2004). Blow et al. showed that the underlying mechanism behind this checkpoint involves the generation of short re-replicated

dsDNA strands due to head-to-tail collision of replication forks (Davidson et al., 2006). As a result, cell cycle arrest prevents cells from entry into M phase and mitotic catastrophe.

3.4.2 Replication fork progression and checkpoint activation

Replication errors in S-phase trigger changes in the cdk cycle, either blocking the cells in specific stages or causing them to succumb to apoptosis, in case of extensive damage. Inhibition of fork progression by topoisomerase inhibitors (Clifford et al., 2003; Downes et al., 1994; Mikhailov et al., 2004) or by double-strand breaks (Kastan and Bartek, 2004) leads to the activation of a G_2/M checkpoint before mitotic entry.

Due to its complexity, DNA replication during S phase is often accompanied by various types of DNA damage (Branzei and Foiani, 2008). In most cases this damage is detected by cellular surveillance mechanisms, resulting in the activation of cell cycle checkpoints and DNA repair mechanisms. Unrepaired dsDNA breaks (DSBs) or DNA lesions during G1 phase may result in the collapse of replication forks, whereas DNA lesions or gaps may induce fork stalling. ATM and DNA-PK are the main effectors of the dsDNA break-induced checkpoints, whereas ATR is mainly activated by ssDNA and stalled replication forks. DSB resection, also leads to the ATR activation due to the generation of intermediate RPA-covered ssDNA (Jazayeri et al., 2006). Recruitment of DNA-PK, ATM and ATR at damaged DNA sites induces the activation of a complex network of downstream effectors, including checkpoint kinases 1 and 2 (Chk1 and Chk2, respectively), and resulting in DNA repair (Matsuoka et al., 2007; Shrivastav et al., 2008).

3.4.3 DNA damage checkpoints and cancer

A number of studies have shown that the DNA damage and DNA replication checkpoints represent a tumorigenesis barrier and that deregulation of their constituents occurs during transformation to the malignant phenotype, allowing genomic instability and progression towards uncontrolled cellular proliferation (Bartkova et al., 2005; Bartkova et al., 2006; Holland and Cleveland, 2009; Lau et al., 2007). DSBs are considered to be among the most detrimental forms of DNA damage and can arise both from exogenous stimuli (i.e., DNA damaging agents, ionizing radiation) and endogenous processes (i.e., base oxidation due to reactive oxygen species, DNA depurination due to hydrolysis, and replication fork collapse (Branzei and Foiani, 2008). In such cases, cells elicit a DNA damage response (DDR), which consists of a biochemical cascade leading to p53 activation (Halazonetis et al., 2008). The nature of the DDR response depends on the extent of damage and can either involve repair of the damage, or cell growth arrest in the form of senescence or apoptosis (Bartkova et al., 2006; Gorgoulis and Halazonetis, 2010; Gorgoulis et al., 2005). The DDR represents an early inducible barrier in carcinogenesis that can be activated by compromised DNA replication (Halazonetis et al., 2008), which commonly coincides with oncogenic factor overexpression. Such factors include a variety of oncogenes, such as traditional ones that promote cellular growth as well as replication licensing ones (Bartkova et al., 2006; Liontos et al., 2007). Sustained production of DSBs can eventually lead to increased activation of the DDR pathway and a selective pressure for p53 inactivation. Eventually, a loss of the anti-tumor barriers takes place, leading to the emergence of genomic instability. Normal cells, on the other hand, maintain these checkpoints intact, being able to arrest in the cell cycle in response to genotoxic stress, and this disparity is an obvious target for therapeutic exploit (Lau and Jiang, 2006). Thus: i) DNA repair inhibitors represent a promising therapeutic target, either as single agents or in combination with DNA-damaging agents, depending on

the tumor genetic background with regard to the DNA repair machinery status (Antoni et al., 2007), and ii) the status of the various constituents of the DNA repair machinery could be used as a prognostic factor in many cases.

4. The role of chromatin structure

The architecture of chromatin is of central importance in cellular processes such as DNA replication, DNA repair and gene expression (reviewed in (Winkler and Luger, 2011)). Chromatin reconfiguration that occurs during embryonic DNA replication has a direct effect on reactivation of gene expression (Forlani et al., 1998), while remodeling of chromatin structure is necessary for enabling eukaryotic cell DNA repair (Groth et al., 2007). Furthermore, chromatin structure affects the selection, activation and temporal program of replication origins (Rampakakis et al., 2009b). Chromatin dynamics are directly influenced by histone modifications, affecting the association of various chromatin modifying, DNA replication, repair and transcription factors to chromatin. It was also recently shown that PCNA affects the epigenetic landscape by influencing the composition of histone modifications on chromatin (Miller et al., 2010). PCNA also recruits a large number of chromatin-modifying enzymes to DNA replication sites, including the maintenance DNA methyltranseferase DNMT1, the chromatin assembly factor CAF-1, histone deacetylases (HDACs), and WSTF-SNF2h (reviewed in (Groth et al., 2007)), thus connecting DNA replication with epigenetic inheritance (Zhang et al., 2000). Recent studies indicate that the ubiquitination and SUMOylation of PCNA regulate the manner by which eukaryotic cells respond to different types of DNA damage as well as the selection of the appropriate repair pathways (reviewed in (Chen et al., 2011)).

In view of the fact that the chromatin dynamics during DNA repair are distinct from those seen during DNA replication (Groth et al., 2007), it is very likely that high order chromatin structure also influences the activity of those proteins with a dual role in DNA replication and repair. Thus, the temporal regulation of both the expression and proper targeting of chromatin modifiers to specific DNA loci may be responsible for directing these proteins toward one or the other of their dual functions (i.e., DNA replication or repair), depending on the cellular requirements of the moment.

5. Conclusion

Accumulated evidence points to a synergy between the DNA replication and repair machineries, as several proteins are involved in both pathways. The functional significance of the synergy between DNA replication and repair proteins lies in the fact that several proteins are strategically located on the DNA and poised to carry both replication and repair functions, depending on the local environment and cellular requirements for normal functioning and survival. The existence of proteins with a dual role in DNA replication and repair is logical, economical and beneficial for the cell, allowing it to coordinate the two important processes of replication and repair, thus optimizing its likelihood of accurate genome duplication and survival.

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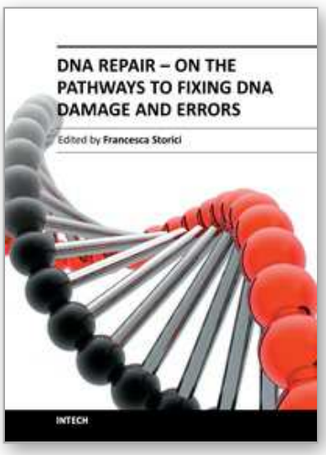

DNA Repair - On the Pathways to Fixing DNA Damage and Errors
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DNA repair is fundamental to all cell types to maintain genomic stability. A collection of cutting-edge reviews, DNA Repair - On the pathways to fixing DNA damage and errors covers major aspects of the DNA repair processes in a large variety of organisms, emphasizing foremost developments, questions to be solved and new directions in this rapidly evolving area of modern biology. Written by researchers at the vanguard of the DNA repair field, the chapters highlight the importance of the DNA repair mechanisms and their linkage to DNA replication, cell-cycle progression and DNA recombination. Major topics include: base excision repair, nucleotide excision repair, mismatch repair, double-strand break repair, with focus on specific inhibitors and key players of DNA repair such as nucleases, ubiquitin-proteasome enzymes, poly ADP-ribose polymerase and factors relevant for DNA repair in mitochondria and embryonic stem cells. This book is a journey into the cosmos of DNA repair and its frontiers.

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