1 Introduction

As cells grow and divide, their DNA content is monitored to prevent accumulation of mutations which can lead to the development of cancer (Hartwell & Kastan, 1994). Mutations are the result of DNA damage caused by external sources such as UV light, X-rays, radiation, and chemical carcinogens, or they can be caused by cellular events including stalled DNA replication forks, incompletely replicated DNA, and programmed DNA double-strand breaks. Damaged DNA can be repaired, but mutations are made permanent when the damaged DNA is not repaired accurately or cells divide without fixing the damage. To prevent accumulation of mutations, cells have developed checkpoints to delay cellular growth in the presence of DNA damage or incomplete DNA replication, giving the cell time to repair the DNA before making mutations permanent upon cell division (Weinert, 1998). Abrogation of these checkpoints leads to increased susceptibility to cancer, neurodegeneration, and accelerated aging (Cimprich & Cortez, 2008; Czornak et al, 2008).

Cells can suffer DNA damage at all points in the cell cycle. It is both the type of DNA damage and the stage of the cell cycle that determines which checkpoints are activated as well as the method of repair. During DNA replication in S phase of the cell cycle, cells monitor for both stalled replication forks, which activates the ATR-dependent checkpoint response, and DNA double-strand breaks, which activate the ATM- and DNA-PK dependent checkpoints. The ATM-dependent checkpoint and repair mechanism results in a more accurate repair of double-strand breaks, while the DNA-PK-dependent pathway can be accurate or error-prone repair. The ATM-dependent pathway is the prominent repair pathway for DNA double-strand breaks (DSBs) during S or G2

phase, since this pathway relies on the presence of a sister chromatid which is only present in S and G2 phases, while the DNA-PK pathway is the primary DSB repair pathway in G1 and M phase when a sister chromatid is not available.

The ATR-dependent pathway can be activated in S or G2 phases, while DNA is being replicated and incomplete DNA replication is detected. Some of the same proteins that are necessary for these checkpoint responses are also involved in DNA replication. In S phase, the process of DNA replication is physically coupled to checkpoint mechanisms. Some proteins that are required for checkpoint activation that travel with the replication fork are TopBP1, Claspin, and the Timeless/Tipin complex (Chini & Chen, 2003; Errico et al, 2007; Gotter et al, 2007; Hashimoto & Takisawa, 2003; Kumagai & Dunphy, 2000; Lee et al, 2003; Makiniemi et al, 2001; Unsal-Kacmaz et al, 2007; Van Hatten et al, 2002; Yoshizawa-Sugata & Masai, 2007). Recent work in yeast also highlights this coupling of DNA replication and checkpoints in S phase; Mrc1, the yeast ortholog of Claspin, is required for activation of the S-phase checkpoint and physically interacts with Pol 2, a catalytic subunit of DNA polymerase ε (Alcasabas et al, 2001; Lou et al, 2008). Upon checkpoint activation by stalled replication forks, this interaction is altered but not abolished, potentially altering polymerase activity in response to the replication stress. Alternatively, the change in Mrc1 binding may provide a docking site for additional checkpoint response proteins (Lou et al, 2008). Physically coupling these mechanisms ensures an immediate response when problems arise in DNA replication, so any stalled replication forks can be stabilized quickly to prevent dissociation of replication fork proteins, a signal of fork collapse from which the replication fork can not recover. The coupling of DNA replication and checkpoint

activation is exemplified by the fact that the ATR-dependent DNA replication checkpoint requires the initiation of DNA replication (Stokes et al, 2002). Since these mechanisms are intertwined, both DNA replication and checkpoints are discussed below.

1.1 DNA Replication Initiation

The replication of genomic DNA is a well-conserved and highly regulated process. All chromatin must be replicated once and only once per cell cycle with a minimal rate of mutation. The activity of cell cycle-dependent kinases regulates the process of DNA replication; origins of replication are “marked” for replication in late M phase and early G1 phase, when S-phase CDK (S-CDK) activity is low, and DNA is replicated when S-CDK levels are high. While there are many points of regulation and feedback, this overarching model guides DNA replication in the cell cycle (Diffley, 1996).

When S-CDK levels are low, the Origin Recognition Complex (ORC) first binds chromatin and marks the origins of replication during early G1 phase for the upcoming S phase (Diffley, 2004; Nguyen et al, 2001). Origins are interspersed throughout chromosomes, and not every origin is used every S phase (for review see Natsume & Tanaka, 2009). Cdc6 and Cdt1 subsequently bind origins of replication during G1 phase. Recent results revealed that the Cdt1 that binds origins is bound to Mcm9, a protein with ATPase activity and a helicase domain (Lutzmann & Méchali, 2008). The MCM2-7 complex, which is the replicative helicase, subsequently binds origins of replication, and the binding of this set of proteins signals the formation of the pre-replication complex (pre-RC) and “licensing” origins of replication (Bell & Dutta, 2002; Nishitani &

Lygerou, 2002). Pre-RC complexes must be formed in G1 phase, when S-CDK levels are low, because they can not form when S-CDK levels are high in S phase (Diffley, 2004; Nguyen et al, 2001). Also, at the onset of DNA replication, Cdt1 is bound by geminin, not Mcm9, and is subsequently degraded in a PCNA- and Cul4-dependent manner, preventing the licensing of additional origins of replication (Arias & Walter, 2005; Arias & Walter, 2006; Lutzmann & Méchali, 2008). Cdc6 is also regulated to prevent licensing of additional forks. Upon initiation of DNA replication, yeast Cdc6 is degraded, while human Cdc6 is exported from the nucleus, to prevent additional origin firing (Elsasser et al, 1999; Jallepalli et al, 1998; Jiang et al, 1999; Perkins et al, 2001; Petersen et al, 1999; Saha et al, 1998). These are some of the many points of regulation in which DNA replication can be limited to one round of DNA replication per cell cycle (Arias & Walter, 2007).

It is at this pre-RC stage of replication fork maturation that proteins such as Dna2, required for efficient DNA replication, and Mcm10, responsible for retention of DNA polymerase α at the fork, bind origins of replication (this work and Liu et al, 2000b; Wohlschlegel et al, 2002). TopBP1, which is necessary for the ATR-dependent checkpoint, also binds the developing replication fork at this point (Van Hatten et al, 2002). Binding of the above proteins at this stage of replication fork development is essential; in the absence of Mcm10 and TopBP1, replication forks do not develop further. The presence of Mcm10 at the fork is also required for binding of Cdc45 and formation of the CMG complex, mentioned below. TopBP1 is necessary for both Cdc45 binding and association of the GINS complex, also described below (Im et al, 2009; Kubota et al,

2003; Van Hatten et al, 2002). This ordered binding of proteins is highly conserved from yeast to human, with only minor variations.

Upon entry into S phase, marked by activation of S-CDK, pre-RC complexes are activated by binding of additional proteins, transforming it into what is known as the pre-initiation complex (pre-IC). Activation of both the Cdc7 and CDK2 kinases are required for pre-IC formation, and this step is also well-conserved from yeast to metazoans (Aparicio et al, 1999; Mimura et al, 2000; Walter & Newport, 2000; Zou & Stillman, 2000). Notable pre-IC proteins include Cdc45 and the GINS (Go, Ichi, Nii, and San) complex, and the chromatin binding of Cdc45 and GINS are mutually dependent (Kubota et al, 2003). Cdc45 recruits DNA polymerase α/primase, necessary for initiation of replication, and GINS activates it (De Falco et al, 2007; Mimura et al, 2000). It also may be that both Cdc45 and the GINS complex activate the MCM replicative helicase, as the MCM2-7 helicase can be isolated in the Cdc45-MCM-GINS (CMG) complex and is bound to the same region of DNA as Cdc45 and GINS upon uncoupling of the replicative helicase and polymerase activities in both *Xenopus* and yeast (Gambus et al, 2006; Kubota et al, 2003; Pacek et al, 2006).

DNA can be unwound by the MCM replicative helicase and subsequently bound by RPA, a single-stranded DNA binding protein. It is also at the pre-IC stage of replication fork maturation that additional DNA replication proteins bind the fork, including notable proteins involved in DNA replication, such as the DNA polymerases, Claspin, the Tim/Tipin complex, and the ssDNA-binding protein RPA (Lee et al, 2003; Mimura et al, 2000; Tanaka et al, 2009; Walter & Newport, 2000).

Once replication has initiated, DNA replication forks proceed away from the replication origins with coupled leading and lagging strand replication. Data from yeast suggests that DNA polymerase ε is the leading strand polymerase and polymerases α and δ participate in lagging strand replication (McElhinny et al, 2008; Pavlov et al, 2006; Pursell et al, 2007). Claspin, Timeless (Tim), and Tipin are not essential for DNA replication but serve to stabilize replication forks and prevent fork stalling in both metazoans and yeast (Chou & Elledge, 2006; Errico et al, 2007; Katou et al, 2003; Liu et al, 2006b; Noguchi et al, 2003; Noguchi et al, 2004; Petermann et al, 2008; Szyjka et al, 2005; Tourriere et al, 2005; Unsal-Kacmaz et al, 2007; Yoshizawa-Sugata & Masai, 2007).

The termination of DNA replication is less understood than the initiation of replication. In eukaryotic cells, replication forks terminate when two replication forks meet or when one replication fork approaches the end of a telomere. However, most data on the termination of replication are from studies in prokaryotes; the current theory is that as two forks approach each other from opposite directions, one fork stops while the other proceeds (Kaplan, 2006). This process is regulated so forks do not collide and all DNA is replicated.

1.2 Checkpoints

Checkpoints are cellular mechanisms that pause the cell cycle to prevent premature entry into the next phase of the cell cycle. DNA damage and the subsequent repair of that damage activate checkpoints to allow the cell additional time to repair the damage before it is made permanent by cell division. When DNA is not completely replicated in S phase or DNA damage is encountered, cells delay the cell cycle, maintain

the replication fork structure so replication can re-start upon repair of the damage, repair the DNA, inhibit replication from other origins of DNA replication, and alter the transcriptional program of the cell. If checkpoint mechanisms fail, cells suffer from higher mutation rates and potentially cell death, are more likely to become cancerous, and can suffer from accelerated aging (Czornak et al, 2008; Erkko et al, 2008; Hartwell & Kastan, 1994; Karppinen et al, 2006; Kerzendorfer & O'Driscoll, 2009; Singh et al, 2009).

Like DNA replication, the initial steps of checkpoints are better understood than the termination of the checkpoint signal and the recovery from checkpoints. It is likely that checkpoints are down-regulated upon removal of the activating signal once the damaged DNA has been repaired. If DNA damage can not be repaired, an adaptation response, observed in yeast, *Xenopus*, and human cells, can occur in which cells enter mitosis with the DNA damage still present (Leroy et al, 2003; Pellicioli et al, 2001; Sandell & Zakian, 1993; Syljuasen et al, 2006; Toczyski et al, 1997; Vaze et al, 2002; Yoo et al, 2004a).

The type of DNA damage present and the stage of the cell cycle specifies which checkpoint mechanism and method of repair will be utilized by the cell. Checkpoint responses to DNA replication stress and DNA double-strand breaks, both active in S and G2 phases, are described below; other checkpoint responses are present in G1 and M phases. The main checkpoint mechanisms each have a main, apical kinase regulating many aspects of the checkpoint response. It is the ATR kinase that responds to replication stress, while ATM is the prominent kinase that responds to DSBs in S and G2 phases.

1.3 Replication Stress

As cells replicate their DNA, replication stress occurs when replication forks stall upon encountering damaged DNA, protein bound to DNA, abnormal DNA structures, or when the helicase and polymerase activities of the fork become uncoupled. Replication stress triggers the activation of the ATR-dependent checkpoint, and signaling through this pathway retards cell cycle progression, keeping the cell in S phase and providing additional time to complete the replication of genomic DNA. In addition to replication stress, the ATR-dependent checkpoint is also activated by ATM in the presence of DNA double-strand breaks (DSBs), discussed below (Jazayeri et al, 2006; Yoo et al, 2007).

Upon occurrence of replication stress, tracts of single-stranded DNA (ssDNA) are formed at the replication fork, likely due to the activity of the MCM replicative helicase. As with most ssDNA in the cell, this is coated in the ssDNA-binding protein RPA to protect the ssDNA from degradation. RPA-coated ssDNA is believed to be the signal for activation of the ATR-dependent DNA replication checkpoint (Costanzo et al, 2003; Zou & Elledge, 2003). The extent of checkpoint activation depends upon both the length of RPA-coated ssDNA tracts and the type of DNA end exposed, with maximal checkpoint activation when a 5′ end is exposed at a ssDNA-dsDNA (double-stranded DNA) junction (MacDougall et al, 2007; Shiotani & Zou, 2009).

The ATR kinase and its partner ATRIP, henceforth referred to as the ATR-ATRIP complex, are recruited to RPA-coated ssDNA through an RPA-binding domain in ATRIP (Ball et al, 2005; Kim et al, 2005; Zou & Elledge, 2003). Other proteins recruited to the RPA-coated ssDNA are TopBP1, a BRCT-containing protein, and Rad17. Rad17 forms a DNA damage response alternative RFC complex, containing the same small RFC

subunits as the replicative RFC complex but the large subunit is replaced by Rad17. Whereas the RFC complex participates in loading of the PCNA sliding clamp onto DNA for DNA replication fidelity, the Rad17 complex loads the 9-1-1 (Rad9-Rad1-Hus1) clamp onto damaged DNA. The Rad17 complex preferentially loads the 9-1-1 complex onto DNA containing a free 5′ end at a ssDNA-dsDNA junction, the same DNA structure that elicits maximal checkpoint activation (Ellison & Stillman, 2003; MacDougall et al, 2007; Shiotani & Zou, 2009). Once the 9-1-1 complex is loaded onto chromatin, the C-terminal tail of Rad9 in the 9-1-1 complex interacts with both ATRIP in the ATR-ATRIP complex and with TopBP1 (Lee et al, 2007; Yan & Michael, 2009). Both TopBP1 and Rad9 stimulate ATR kinase activity (Delacroix et al, 2007; Kumagai et al, 2006; Mordes et al, 2008). While the signal to activate the checkpoint appears to be RPA-coated ssDNA, its real purpose may be to simply bring these three proteins in close proximity, increasing the local concentration of these proteins. Elegant experiments in both yeast and human cells reveal that simply increasing the local concentration of checkpoint proteins is sufficient to activate the checkpoint response in the absence of DNA damage or replication stress (Bonilla et al, 2008; Soutoglou & Misteli, 2008; Stucki & Jackson, 2006).

Interestingly, ATR-ATRIP binding to RPA-coated ssDNA is not strictly essential for ATR kinase activity stimulation, since ATR can still be activated when bound to ATRIP mutants lacking the ability to bind RPA (Ball et al, 2005; Kim et al, 2005). The reciprocal result was shown in yeast; mutants of Rfa1, the yeast homolog of RPA, that are unable to bind Ddc2, the ATRIP homolog, only suffer minor checkpoint activation defects (Lee et al, 1998; Umezu et al, 1998). Additionally, overexpression of the ATR-

activating protein TopBP1 in human cells results in ATR-dependent phosphorylation of proteins in the absence of RPA-bound ssDNA (Ball et al, 2007; Kumagai & Dunphy, 2006). This also supports the theory that simply increasing the local concentration of checkpoint proteins is sufficient to activate checkpoints, as previously seen in both yeast and human cells (Bonilla et al, 2008; Soutoglou & Misteli, 2008; Stucki & Jackson, 2006).

Active ATR phosphorylates a myriad of proteins, some of which have recently been identified through large-scale screens (Matsuoka et al, 2007; Mu et al, 2007; Smith et al, 2009; Smolka et al, 2007; Stokes et al, 2007). One of the most studied ATR substrates is Chk1, a serine/threonine kinase. Human Chk1 is activated upon ATR-dependent phosphorylation of residues S317 and S345 (Liu et al, 2000a; Zhao & Piwnica-Worms, 2001). Active Chk1 then inhibits the Cdc25 phosphatases, thus preventing CDK activation and entry into mitosis (Furnari et al, 1997; Peng et al, 1997; Sanchez et al, 1997).

Interestingly, TopBP1 and Rad17, proteins responsible for the activation of ATR kinase activity, are also targets of ATR phosphorylation (Bao et al, 2001; Kumagai et al, 2006). ATR-dependent Rad17 phosphorylation is required for 9-1-1 foci formation and checkpoint activation (Bao et al, 2001; Medhurst et al, 2008). Rad9, of the 9-1-1 complex, binds the same basic cleft in RPA that ATRIP binds, and the Rad9 C-terminal tail bridges TopBP1 and the ATR-ATRIP complex (Lee et al, 2007; Xu et al, 2008). This data again suggests that without Rad17 phosphorylation and 9-1-1 accumulation, TopBP1 alone would not activate ATR as efficiently. Therefore, the checkpoint signal would not be amplified and perpetuated, so the checkpoint signal would not be sufficiently activated

in response to ssDNA. Alternatively, the ATR-dependent phosphorylation of Rad17 may serve another purpose in checkpoint signaling.

In addition to slowing cell cycle progression, as mentioned above, activate ATR kinase also affects other pathways. Upon checkpoint activation, the further firing of DNA replication origins is inhibited until the DNA damage is repaired and the checkpoint mechanism is down-regulated (Costanzo et al, 2003; Maya-Mendoza et al, 2007; Shechter et al, 2004). ATR also participates in the recovery from stalled replication forks, as illustrated by recent work on the ATR-dependent phosphorylation of Mcm2. Mcm2, a member of the MCM replicative helicase complex, can be phosphorylated by either ATM or ATR upon checkpoint activation (Trenz et al, 2008; Yoo et al, 2004b). During checkpoint activating conditions, ATR phosphorylates Mcm2 on S92 of the *Xenopus* protein, and this modification promotes Plx1, the *Xenopus* orthologue of Plk1 (Polo-like kinase 1), binding to Mcm2. This interaction in turn promotes recovery from the checkpoint response by promoting origin firing (Trenz et al, 2008). Plx1 also binds and phosphorylates Claspin, causing it to release from chromatin and thus down-regulate the checkpoint response (Yoo et al, 2004a). In human cells, Plk1-dependent degradation of Claspin in G2 phase of the cell cycle also downregulates the checkpoint mechanism by physically removing proteins that can keep the checkpoint activated (Gewurz & Harper, 2006).

ATR phosphorylates many proteins at the replication fork, including RPA, the MCM2-7 complex, Mcm10, various DNA polymerases, TopBP1, Rad9 of the 9-1-1 complex, Tipin, WRN, BLM, and BRCA1 (Bao et al, 2001; Cortez et al, 2004; Liu et al, 2006a; Matsuoka et al, 2007; Oakley et al, 2001; Yoo et al, 2004b). The function of

many of these phosphorylation events is as yet unknown; they may be related to maintenance of replication fork integrity, DNA repair, checkpoint recovery, protein degradation, or the transcription of genes. Understanding the purpose of these ATR-mediated modifications will shed light on the role of the ATR-dependent checkpoint pathway.

Other unresolved questions surround the topic of replication fork stability upon stalling. Both Claspin and the Tim/Tipin complex are required to maintain replication fork integrity, but results in yeast reveal that they are not equivalent, and forks lacking the respective homologs of these proteins respond differently when encountering a replication fork barrier (Chou & Elledge, 2006; Errico et al, 2007; Katou et al, 2003; Mohanty et al, 2006; Petermann et al, 2008; Unsal-Kacmaz et al, 2007; Yoshizawa-Sugata & Masai, 2007). Many questions remain regarding the similarities and differences between these fork-stabilizing proteins. Also, the effect of ATR-dependent phosphorylation of Tipin is as yet unknown; however, Claspin phosphorylation by ATR is necessary for ATR-dependent checkpoint signaling in response to DSBs, but not in response to replication stress (Yoo et al, 2006).

It is worth noting that there are also ATR-independent responses to stalled replication forks, although these mechanisms are not as well described. One example of an ATR-independent response is the ubiquitylation of PCNA upon induction of stalled replication forks (Chang et al, 2006; Yang et al, 2008). Ubiquitylation of PCNA promotes recruitment of translesion DNA polymerases, believed to be necessary for efficient replication in the presence of damaged DNA (Lopes et al, 2006). This response

is dependent upon both Claspin and the Tim/Tipin complex, but independent of ATR (Yang et al, 2008).

The Cdc7 kinase has been detected in a complex with the Claspin and Tim/Tipin homologs in yeast, may be a target of the ATR-dependent checkpoint, and these proteins participate in checkpoint mechanisms in yeast (Jares et al, 2000; Matsumoto et al, 2005; Shimmoto et al, 2009; Sommariva et al, 2005; Yanow et al, 2003). In yeast, the Cdc7 homolog participates in both the response to stalled replication forks and the response to alkylated DNA (Shimmoto et al, 2009; Sommariva et al, 2005). The human Cdc7/Dbf4 complex is a target of Chk1, and Dbf4 overexpression reverses the checkpoint response (Heffernan et al, 2007). Cdc7 is involved in the metazoan checkpoint response, but the extent of conservation is unresolved. Further research is needed to examine the role of Cdc7 in the response to replication stress and, potentially, the ATR-dependent checkpoint.

1.4 DSB Resection through Homologous Recombination

Unlike DNA replication stress, DNA double-strand breaks (DSBs) can occur at any point during the cell cycle. DSBs can be caused by exogenous sources, such as exposure to ionizing radiation, radiomimetic drugs, or UV light, or by endogenous cellular processes, including meiosis, V(D)J recombination, and immunoglobulin class-switch recombination. Unlike the replication stress checkpoint, there are two kinases that may regulate the checkpoint response to and repair of DSBs. The ATM-dependent checkpoint pathway is coupled to repair by homologous recombination (HR), while the DNA-PK-dependent checkpoint pathway is associated with repair of DSBs by non-

homologous end joining (NHEJ). The repair method of choice is also cell-cycle dependent; homologous recombination relies on the presence of a sister chromatid, only present in S or G2 phases, for use as a template for repair, so HR is the dominant repair mechanism in S and G2. Meanwhile, NHEJ is the predominant DSB repair mechanism in G1 phase, since cells have a 1N DNA content and there is no sister chromatid template available. Repair by HR is an accurate repair, since there is a DNA template for repair, while repair by NHEJ is not necessarily accurate, as DNA ends are joined irrespective of homology (for review see Hartlerode & Scully, 2009). The HR mechanism and ATM-dependent checkpoint response will be reviewed here.

Upon DSB formation, free DNA ends are rapidly bound by the MRN (Mre11-Rad50-Nbs1) complex. Mre11 is a nuclease, Rad50 is a member of the SMC family proposed to hold sister chromatids in close proximity, and Nbs1 physically interacts with the ATM kinase (for review see Lavin, 2007). MRN is also bound to CtIP (CtBP interacting protein), which interacts with BRCA1 and is essential for DNA end joining through homologous recombination (Sartori et al, 2007; Takeda et al, 2007; Yu et al, 2006). Mre11 in the MRN complex first processes the break by degrading the 5′ ends, and this processing releases short single-stranded DNA oligomers capable of activating the ATM kinase (Jazayeri et al, 2008). Inactive ATM dimers are recruited to the DSB ends and activated by the MRN activity. ATM dimers autophosphorylate and dissociate, and the active ATM monomers subsequently phosphorylate proteins involved in the checkpoint response and repair of DSBs (Bakkenist & Kastan, 2003). One protein of interest that is quickly phosphorylated by ATM is the histone variant H2AX. Phosphorylated H2AX, together with the MDC1 adapter protein, recruit DSB checkpoint

and repair proteins to sites of damage and mediate repair by homologous recombination (Hartlerode & Scully, 2009). ATM is also responsible for phosphorylating and activating Chk2, a serine/threonine effector kinase that further phosphorylates proteins in response to DSBs (Matsuoka et al, 1998; Matsuoka et al, 2000).

While the checkpoint signal is perpetuated and amplified by the ATM and Chk2 kinases, the break is also being processed for repair. DSBs are first processed by Mre11 in the MRN/CtIP complex, as mentioned above. This Mre11 processing is both the initial step of repair and an activating step for the checkpoint pathway. DNA ends are further processed by a helicase and nuclease, either WRN and Dna2 or BLM and Exo1 (Liao et al, 2008; Nimonkar et al, 2008). This mechanism of processing is well-conserved and consistent with genetic results in yeast (Budd & Campbell, 2009; Zhu et al, 2008). If the break is processed by the WRN helicase and the Dna2 helicase-nuclease, WRN unwinds the duplex DNA and Dna2 degrades the 5′ strand, lengthening the 3′ ssDNA tail (Liao et al, 2008). Yeast genetics indicate that it is the nuclease, not the helicase, activity of Dna2 that participates in DSB processing (Budd & Campbell, 2009). Exo1 is also capable of processing this 5′ end, thus extending the 3′ ssDNA tail, and the BLM protein stimulates Exo1 activity without requiring BLM helicase activity (Nimonkar et al, 2008). While these two parallel pathways exist, the factors that drive the cell to use one pathway versus another are as yet unknown.

The nuclease degradation of the 5′ strand to reveal a longer 3′ ssDNA tail allows the accumulation of RPA on the ssDNA. Eventually, the bound RPA is replaced with Rad51 recombinase with the help of BRCA1, BARD1, and BRCA2. It is the Rad51-coated ssDNA that is competent for strand invasion of the sister chromatid to search for a

homologous sequence. The cell can then synthesize the remaining missing DNA sequence, and this DNA structure is resolved by one of many enzymes capable of resolving the structure, including BLM and Topoisomerase IIIα, GEN1, SLX4, and the MUS81-EME1 complex (for review see Hartlerode & Scully, 2009).

Checkpoint signaling in response to DSBs is not independent of the repair process. Many DSB repair proteins are substrates of the ATM kinase, including Mre11. It has only recently been discovered that phosphorylation of Mre11 by ATM triggers the dissociation of Mre11 from DNA ends, likely releasing these ends for further processing for repair (Di Virgilio et al, 2009). ATM phosphorylates many additional targets that participate in DSB repair, although the function of many of these modifications is as yet unknown (for review see Czornak et al, 2008). These ATM-dependent phosphorylations may be involved in the recruitment of proteins to DSBs, in processing the DSBs, or in the down-regulation of the checkpoint signal.

Processing of a DSB by HR also activates the ATR-dependent checkpoint pathway. The extended RPA-coated 3′ ssDNA tails generated during processing of DNA ends may serve to recruit ATR-ATRIP (Shiotani & Zou, 2009). Active ATM kinase phosphorylates TopBP1 with MRN as a mediator, which in turn activates the ATR kinase (Yoo et al, 2007; Yoo et al, 2009). Therefore, DSBs activate both the ATM- and ATR-dependent pathways, thus expanding the cellular response to DNA damage. There is also cross-talk between the ATR- and ATM-dependent responses. Chk1, a downstream target of the ATR kinase activated in response to replication stress, is essential for repair of DSBs by HR (Sorensen et al, 2005). Additionally, Exo1, one of the nucleases that participates in the resection of DSBs by HR, was recently found to be a target of the ATR

under conditions of replication stress, with phosphorylation leading to degradation of Exo1 (El-Shemerly et al, 2008). It is as yet unknown if ATM-mediated ATR activation by the DSB checkpoint also causes degradation of Exo1, or if this effect is limited to replication stress. Degradation of Exo1 may signal completion of a given step of repair, or it may function in down-regulation of the checkpoint response. Cross-talk between cellular pathways is a point of interest as we strive to understand the cellular response to DNA damage.

The importance of resolving DSBs is underscored by the multiple redundancies in the DSB processing pathway detected through yeast genetics. In yeast, the nuclease activity of the Mre11 is not essential, although the MRN protein complex itself is necessary for DSB processing. This is likely due to other nucleases, potentially Dna2 and Exo1, compensating for the lack of Mre11 nuclease activity. Yeast lacking Dna2 or Exo1 can still repair DSBs, suggesting that cells can compensate for the lack of either of these proteins and that Dna2 and Exo1 may be able to compensate for each other (Budd et al, 2009; Zhu et al, 2008). Supporting this theory, yeast genetic interactions revealed that it is the nuclease activity of the Dna2 helicase-nuclease that participates in DSB repair; a helicase-dead mutant had no affect on repair efficiencies (Budd & Campbell, 2009). Yeast genetic interactions has revealed much about these functional redundancies, and a current point of interest is understanding these functional redundancies in metazoans.

While much has also been learnt about the response to DSBs, many questions remain regarding the DSB checkpoint and repair. Recent data suggests how the cell cycle may influence the prevalence of DSB repair by HR versus NHEJ at different cell cycle stages. CtIP, which participates in HR, is regulated by CDK-dependent

phosphorylation events, and this may promote repair by HR over NHEJ in S and G2 phases (Chen et al, 2008; Huertas & Jackson, 2009; Yun & Hiom, 2009). There are likely many more points of cell cycle regulation that are not yet understood, including as yet unidentified proteins and modifications to proteins involved in DSB repair.

Cross-talk between checkpoint pathways is also a point of interest. The ATM-dependent activation of ATR was only recently identified, and the extent of cross-talk between other PIKKs, particularly ATR and DNA-PK, is an area of current research. ATM does phosphorylate DNA-PK in irradiated cells, and this ATM-dependent modification acts cooperatively with DNA-PK autophosphoryalation for cooperative activation of the DNA-PK kinase activity (Chen et al, 2007). ATR-dependent activation of ATM upon DNA replication fork stalling or UV has been reported in human cells; however, this effect was not observed in *Xenopus* extract treated ssDNA or dsDNA to induce checkpoint activation (Jazayeri et al, 2008; Stiff et al, 2006).

Finally, the role of MRN in DSB repair is intriguing. Mre11 nuclease activity is not required for DSB resection, but the intact MRN protein complex is required. MRN may serve as a scaffold for binding of other repair proteins, or it may be a domain in Rad50 or Nbs1 that is essential for DSB repair. Understanding the role of MRN past the role of the nuclease, the non-essential role, should yield a greater understanding of DSB repair. Interestingly, MRN also participates in DSB repair by NHEJ (non-homologous end joining). Removal of the MRN complex, or any protein within this complex, greatly reduces the efficiency of NHEJ by both classical and alternative pathways in human cells (Deng et al, 2009; Dimitrova & de Lange, 2009; Dinkelmann et al, 2009; Rass et al, 2009; Xie et al, 2009). The role of MRN in repair of DSBs by both HR and NHEJ is

interesting, and is likely to be one of multiple points in which these pathways are intertwined.

1.5 *Xenopus* Cell-Free System

This work examines the vertebrate homologs of the well-studied *S. cerevisiae* proteins, Dna2, Tof1, and Csm3, in an effort to further the understanding of these proteins in metazoans. Most experiments were performed in *Xenopus laevis* cell-free extract, derived from *X. laevis* eggs arrested in metaphase of meiosis II that were crushed by centrifugation. The addition of calcium stimulates the extract to enter interphase, or S phase, and the further addition of chromosomal DNA and an ATP-regeneration system results in the formation of nuclei and DNA replication in this cell-free extract. The maternal stores of protein from the eggs are sufficient for DNA replication without the need for transcription. This DNA replication is both regulated and synchronized, and cellular responses to DNA damage can be successfully recapitulated in this extract (Blow & Laskey, 1986; Garner & Costanzo, 2009).

In this text, interphase extract refers to S phase extract with no DNA added, and CSF extract is extract that has not been stimulated by addition of calcium, so it is arrested in metaphase II of meiosis. CSF extract is commonly referred to as “M phase” extract, due to its resemblance to M phase extracts. For all experiments involving the addition of DNA to these extracts, *Xenopus laevis* demembranated sperm chromatin was used as the source of chromosomal DNA.

1.6 References

Alcasabas AA, Osborn AJ, Bachant J, Hu F, Werler PJH, Bousset K, Furuya K, Diffley JFX, Carr AM, Elledge SJ (2001) Mrc1 transduces signals of DNA replication stress to activate Rad53. *Nat Cell Biol* **3**(11)**:** 958-965

Aparicio OM, Stout AM, Bell SP (1999) Differential assembly of Cdc45p and DNA polymerases at early and late origins of DNA replication. *Proceedings of the National Academy of Sciences of the United States of America* **96**(16)**:** 9130-9135

Arias EE, Walter JC (2005) Replication-dependent destruction of Cdt1 limits DNA replication to a single round per cell cycle in *Xenopus* egg extracts. *Genes & Development* **19**(1)**:** 114-126

Arias EE, Walter JC (2006) PCNA functions as a molecular platform to trigger Cdt1 destruction and prevent re-replication. *Nat Cell Biol* **8**(1)**:** 84-90

Arias EE, Walter JC (2007) Strength in numbers: preventing rereplication via multiple mechanisms in eukaryotic cells. *Genes & Development* **21**(5)**:** 497-518

Bakkenist CJ, Kastan MB (2003) DNA damage activates ATM through intermolecular autophosphorylation and dimer dissociation. *Nature* **421**(6922)**:** 499-506

Ball HL, Ehrhardt MR, Mordes DA, Glick GG, Chazin WJ, Cortez D (2007) Function of a conserved checkpoint recruitment domain in ATRIP proteins. *Molecular and Cellular Biology* **27**(9)**:** 3367-3377

Ball HL, Myers JS, Cortez D (2005) ATRIP binding to replication protein A-single-stranded DNA promotes ATR-ATRIP localization but is dispensable for Chk1 phosphorylation. *Molecular Biology of the Cell* **16**(5)**:** 2372-2381

Bao S, Tibbetts RS, Brumbaugh KM, Fang Y, Richardson DA, Ali A, Chen SM, Abraham RT, Wang X-F (2001) ATR/ATM-mediated phosphorylation of human Rad17 is required for genotoxic stress responses. *Nature* **411**(6840)**:** 969-974

Bell SP, Dutta A (2002) DNA replication in Eukaryotic cells. *Annual Review of Biochemistry* **71**(1)**:** 333-374

Blow JJ, Laskey RA (1986) Initiation of DNA replication in nuclei and purified DNA by a cell-free extract of Xenopus eggs. *Cell* **47**(4)**:** 577-587

Bonilla CY, Melo JA, Toczyski DP (2008) Colocalization of sensors is sufficient to activate the DNA damage checkpoint in the absence of damage. *Molecular Cell* **30**(3)**:** 267-276

Budd M, Campbell J (2009) Interplay of Mre11 nuclease with Dna2 plus Sgs1 in Rad51-dependent recombinational repair. *Plos One* **4**(1)**:** e4267

Budd ME, Cox LS, Campbell JL (2009) Coordination of Nucleases and Helicases during DNA Replication and Double-strand Break Repair. In *Molecular Themes in DNA Replication*, Cox LS (ed). London: RSC Publishing

Chang DJ, Lupardus PJ, Cimprich KA (2006) Monoubiquitination of proliferating cell nuclear antigen induced by stalled replication requires uncoupling of DNA polymerase and mini-chromosome maintenance helicase activities. *Journal of Biological Chemistry* **281:** 32081-32088

Chen BPC, Uematsu N, Kobayashi J, Lerenthal Y, Krempler A, Yajima H, Lobrich M, Shiloh Y, Chen DJ (2007) Ataxia telangiectasia mutated (ATM) is essential for DNA-PKcs phosphorylations at the Thr-2609 cluster upon DNA double strand break. *Journal of Biological Chemistry* **282**(9)**:** 6582-6587

Chen LC, Nievera CJ, Lee AYL, Wu XH (2008) Cell cycle-dependent complex formation of BRCA1.CtIP.MRN is important for DNA double-strand break repair. *Journal of Biological Chemistry* **283**(12)**:** 7713-7720

Chini CCS, Chen J (2003) Human Claspin is required for replication checkpoint control. *Journal of Biological Chemistry* **278**(32)**:** 30057-30062

Chou DM, Elledge SJ (2006) Tipin and Timeless form a mutually protective complex required for genotoxic stress resistance and checkpoint function. *Proceedings of the National Academy of Sciences of the United States of America* **103**(48)**:** 18143-18147

Cimprich KA, Cortez D (2008) ATR: an essential regulator of genome integrity. *Nat Rev Mol Cell Biol* **9**(8)**:** 616-627

Cortez D, Glick G, Elledge SJ (2004) Minichromosome maintenance proteins are direct targets of the ATM and ATR checkpoint kinases. *Proceedings of the National Academy of Sciences of the United States of America* **101**(27)**:** 10078-10083

Costanzo V, Shechter D, Lupardus PJ, Cimprich KA, Gottesman M, Gautier J (2003) An ATR- and Cdc7-Dependent DNA Damage Checkpoint that Inhibits Initiation of DNA Replication. *Molecular Cell* **11**(1)**:** 203-213

Czornak K, Chughtai S, Chrzanowska KH (2008) Mystery of DNA repair: the role of the MRN complex and ATM kinase in DNA damage repair *Journal of Applied Genetics* **49**(4)**:** 383-396

De Falco M, Ferrari E, De Felice M, Rossi M, Hubscher U, Pisani FM (2007) The human GINS complex binds to and specifically stimulates human DNA polymerase alpha-primase. *Embo Reports* **8**(1)**:** 99-103

Delacroix S, Wagner JM, Kobayashi M, Yamamoto K, L.M. K (2007) The Rad9-Hus1-Rad1 (9-1-1) clamp activates checkpoint signaling via TopBP1. *Genes & Development* **21**(12)**:** 1472-1477

Deng YB, Guo XL, Ferguson DO, Chang S (2009) Multiple roles for MRE11 at uncapped telomeres. *Nature* **460**(7257)**:** 914-U157

Di Virgilio M, Ying CY, Gautier J (2009) PIKK-dependent phosphorylation of Mre11 induces MRN complex inactivation by disassembly from chromatin. *DNA Repair* **In Press, Corrected Proof**

Diffley JF (1996) Once and only once upon a time: specifying and regulating origins of DNA replication in eukaryotic cells. *Genes & Development* **10**(22)**:** 2819-2830

Diffley JFX (2004) Regulation of early events in chromosome replication. *Current Biology* **14**(18)**:** R778-R786

Dimitrova N, de Lange T (2009) Cell cycle-dependent role of MRN at dysfunctional telomeres: ATM signaling-dependent induction of nonhomologous end joining (NHEJ) in G(1) and resection-mediated inhibition of NHEJ in G(2). *Molecular and Cellular Biology* **29**(20)**:** 5552-5563

Dinkelmann M, Spehalski E, Stoneham T, Buis J, Wu YP, Sekiguchi JM, Ferguson DO (2009) Multiple functions of MRN in end-joining pathways during isotype class switching. *Nature Structural & Molecular Biology* **16**(8)**:** 808-U825

El-Shemerly M, Hess D, Pyakurel AK, Moselhy S, Ferrari S (2008) ATR-dependent pathways control hEXO1 stability in response to stalled forks. *Nucl Acids Res* **36**(2)**:** 511-519

Ellison V, Stillman B (2003) Biochemical Characterization of DNA Damage Checkpoint Complexes: Clamp Loader and Clamp Complexes with Specificity for 5' Recessed DNA. *PLoS Biol* **1**(2)**:** e33

Elsasser S, Chi Y, Yang P, Campbell JL (1999) Phosphorylation controls timing of Cdc6p destruction: A biochemical analysis. *Molecular Biology of the Cell* **10**(10)**:** 3263-3277

Erkko H, Pylkas K, Karppinen SM, Winqvist R (2008) Germline alterations in the CLSPN gene in breast cancer families. *Cancer Letters* **261**(1)**:** 93-97

Errico A, Costanzo V, Hunt T (2007) Tipin is required for stalled replication forks to resume DNA replication after removal of aphidicolin in *Xenopus* egg extracts. *Proceedings of the National Academy of Sciences of the United States of America* **104**(38)**:** 14929-14934

Furnari B, Rhind N, Russell P (1997) Cdc25 mitotic inducer targeted by Chk1 DNA damage checkpoint kinase. *Science* **277**(5331)**:** 1495-1497

Gambus A, Jones RC, Sanchez-Diaz A, Kanemaki M, van Deursen F, Edmondson RD, Labib K (2006) GINS maintains association of Cdc45 with MCM in replisome progression complexes at eukaryotic DNA replication forks. *Nature Cell Biology* **8**(4)**:** 358-U341

Garner E, Costanzo V (2009) Studying the DNA damage response using in vitro model systems. *DNA Repair* **8**(9)**:** 1025-1037

Gewurz BE, Harper JW (2006) DNA-damage control: Claspin destruction turns off the checkpoint. *Current Biology* **16**(21)**:** R932-R934

Gotter AL, Suppa C, Emanuel BS (2007) Mammalian TIMELESS and Tipin are evolutionarily conserved replication fork-associated factors. *Journal of Molecular Biology* **366**(1)**:** 36-52

Hartlerode AJ, Scully R (2009) Mechanisms of double-strand break repair in somatic mammalian cells. *Biochemical Journal* **423**(2)**:** 157-168

Hartwell LH, Kastan MB (1994) Cell-cycle control and cancer. *Science* **266**(5192)**:** 1821-1828

Hashimoto Y, Takisawa H (2003) *Xenopus* Cut5 is essential for a CDK-dependent process in the initiation of DNA replication. *EMBO Journal* **22**(10)**:** 2526-2535

Heffernan TP, Unsal-Kacmaz K, Heinloth AN, Simpson DA, Paules RS, Sancar A, Cordeiro-Stone M, Kaufmann WK (2007) Cdc7-dbf4 and the human S checkpoint response to UVC. *Journal of Biological Chemistry* **282**(13)**:** 9458-9468

Huertas P, Jackson SP (2009) Human CtIP Mediates Cell Cycle Control of DNA End Resection and Double Strand Break Repair. *Journal of Biological Chemistry* **284**(14)**:** 9558-9565

Im JS, Ki SH, Farina A, Jung S, Hurwitz J, Lee JK (2009) Assembly of the Cdc45-Mcm2-7-GINS complex in human cells requires the Ctf4/And-1, RecQL4, and Mcm10 proteins. *Proceedings of the National Academy of Sciences of the United States of America* **106**(37)**:** 15628-15632

Jallepalli PV, Tien D, Kelly TJ (1998) sud1(+) Targets cyclin-dependent kinase-phosphorylated Cdc18 and Rum1 proteins for degradation and stops unwanted diploidization in fission yeast. *Proceedings of the National Academy of Sciences of the United States of America* **95**(14)**:** 8159-8164

Jares P, Donaldson A, Blow JJ (2000) The Cdc7/Dbf4 protein kinase: target of the S phase checkpoint? *EMBO Reports* **1**(4)**:** 319-322

Jazayeri A, Balestrini A, Garner E, Haber JE, Costanzo V (2008) Mre11-Rad50-Nbs1-dependent processing of DNA breaks generates oligonucleotides that stimulate ATM activity. *EMBO J* **27**(14)**:** 1953-1962

Jazayeri A, Falck J, Lukas C, Bartek J, Smith GCM, Lukas J, Jackson SP (2006) ATM- and cell cycle-dependent regulation of ATR in response to DNA double-strand breaks. *Nature Cell Biology* **8**(1)**:** 37-U13

Jiang W, Wells NJ, Hunter T (1999) Multistep regulation of DNA replication by Cdk phosphorylation of HsCdc6. *Proceedings of the National Academy of Sciences of the United States of America* **96**(11)**:** 6193-6198

Kaplan DL (2006) Replication termination: mechanism of polar arrest revealed. *Current Biology* **16**(17)**:** R684-686

Karppinen SM, Erkko H, Reini K, Pospiech H, Heikkinen K, Rapakko K, Syvaoja JE, Winqvist R (2006) Identification of a common polymorphism in the TopBP1 gene associated with hereditary susceptibility to breast and ovarian cancer. *European Journal of Cancer* **42**(15)**:** 2647-2652

Katou Y, Kanoh Y, Bando M, Noguchi H, Tanaka H, Ashikari T, Sugimoto K, Shirahige K (2003) S-phase checkpoint proteins Tof1 and Mrc1 form a stable replication-pausing complex. *Nature* **424**(6952)**:** 1078-1083

Kerzendorfer C, O'Driscoll M (2009) Human DNA damage response and repair deficiency syndromes: Linking genomic instability and cell cycle checkpoint proficiency. *DNA Repair* **8**(9)**:** 1139-1152

Kim SM, Kumagai A, Lee J, Dunphy WG (2005) Phosphorylation of Chk1 by ATM- and Rad3-related (ATR) in *Xenopus* egg extracts requires binding of ATRIP to ATR but not the stable DNA-binding or coiled-coil domains of ATRIP. *Journal of Biological Chemistry* **280**(46)**:** 38355-38364

Kubota Y, Takase Y, Komori Y, Hashimoto Y, Arata T, Kamimura Y, Araki H, Takisawa H (2003) A novel ring-like complex of *Xenopus* proteins essential for the initiation of DNA replication. *Genes & Development* **17**(9)**:** 1141-1152

Kumagai A, Dunphy WG (2000) Claspin, a novel protein required for the activation of Chk1 during a DNA replication checkpoint response in *Xenopus* egg extracts. *Molecular Cell* **6**(4)**:** 839-849

Kumagai A, Dunphy WG (2006) How cells activate ATR. *Cell Cycle* **5**(12)**:** 1265-1268

Kumagai A, Lee J, Yoo HY, Dunphy WG (2006) TopBP1 activates the ATR-ATRIP complex. *Cell* **124**(5)**:** 943-955

Lavin MF (2007) ATM and the Mre11 complex combine to recognize and signal DNA double-strand breaks. *Oncogene* **26**(56)**:** 7749-7758

Lee J, Kumagai A, Dunphy WG (2003) Claspin, a Chk1-regulatory protein, monitors DNA replication on chromatin independently of RPA, ATR, and Rad17. *Molecular Cell* **11**(2)**:** 329-340

Lee J, Kumagai A, Dunphy WG (2007) The Rad9-Hus1-Rad1 checkpoint interaction of TopBP1 with ATR. *Journal of Biological Chemistry* **282**(38)**:** 28036-28044

Lee SE, Moore JK, Holmes A, Umezu K, Kolodner RD, Haber JE (1998) *Saccharomyces* Ku70, Mre11/Rad50, and RPA proteins regulate adaptation to G2/M arrest after DNA damage. *Cell* **94**(3)**:** 399-409

Leroy C, Lee SE, Vaze MB, Ochsenbein F, Guerois R, Haber JE, Marsolier-Kergoat MC (2003) PP2C Phosphatases Ptc2 and Ptc3 are required for DNA checkpoint inactivation after a double-strand break. *Molecular Cell* **11**(3)**:** 827-835

Liao S, Toczylowski T, Yan H (2008) Identification of the *Xenopus* DNA2 protein as a major nuclease for the 5'->3' strand-specific processing of DNA ends. *Nucl Acids Res* **36**(19)**:** 6091-6100

Liu JS, Kuo SR, Melendy T (2006a) Phosphorylation of replication protein A by S-phase checkpoint kinases. *DNA Repair* **5**(3)**:** 369-380

Liu Q, Guntuku S, Cui X-S, Matsuoka S, Cortez D, Tamai K, Luo G, Carattini-Rivera S, DeMayo F, Bradley A, Donehower LA, Elledge SJ (2000a) Chk1 is an essential kinase that is regulated by Atr and required for the G2/M DNA damage checkpoint. *Genes & Development* **14**(12)**:** 1448-1459

Liu QQ, Choe WC, Campbell JL (2000b) Identification of the *Xenopus laevis* homolog of *Saccharomyces cerevisiae* DNA2 and its role in DNA replication. *Journal of Biological Chemistry* **275**(3)**:** 1615-1624

Liu S, Bekker-Jensen S, Mailand N, Lukas C, Bartek J, Lukas J (2006b) Claspin operates downstream of TopBP1 to direct ATR signaling towards Chk1 activation. *Mol Cell Biol* **26**(16)**:** 6056-6064

Lopes M, Foiani M, Sogo JM (2006) Multiple mechanisms control chromosome integrity after replication fork uncoupling and restart at irreparable UV lesions. *Molecular Cell* **21**(1)**:** 15-27

Lou H, Komata M, Katou Y, Guan Z, Reis CC, Budd M, Shirahige K, Campbell JL (2008) Mrc1 and DNA Polymerase epsilon function together in linking DNA replication and the S phase checkpoint. *Molecular Cell* **32**(1)**:** 106-117

Lutzmann M, Méchali M (2008) MCM9 Binds Cdt1 and is required for the assembly of prereplication complexes. *Molecular Cell* **31**(2)**:** 190-200

MacDougall CA, Byun TS, Van C, Yee MC, Cimprich KA (2007) The structural determinants of checkpoint activation. *Genes & Development* **21**(8)**:** 898-903

Makiniemi M, Hillukkala T, Tuusa J, Reini K, Vaara M, Huang DQ, Pospiech H, Majuri I, Westerling T, Makela TP, Syvaoja JE (2001) BRCT domain-containing protein TopBP1 functions in DNA replication and damage response. *Journal of Biological Chemistry* **276**(32)**:** 30399-30406

Matsumoto S, Ogino K, Noguchi E, Russell P, Masai H (2005) Hsk1-Dfp1/Him1, the Cdc7-Dbf4 kinase in *Schizosaccharomyces pombe*, associates with Swi1, a component of the replication fork protection complex. *Journal of Biological Chemistry* **280**(52)**:** 42536-42542

Matsuoka S, Ballif BA, Smogorzewska A, McDonald ER, III, Hurov KE, Luo J, Bakalarski CE, Zhao Z, Solimini N, Lerenthal Y, Shiloh Y, Gygi SP, Elledge SJ (2007)

ATM and ATR substrate analysis reveals extensive protein networks responsive to DNA damage. *Science* **316**(5828)**:** 1160-1166

Matsuoka S, Huang MX, Elledge SJ (1998) Linkage of ATM to cell cycle regulation by the Chk2 protein kinase. *Science* **282**(5395)**:** 1893-1897

Matsuoka S, Rotman G, Ogawa A, Shiloh Y, Tamai K, Elledge SJ (2000) Ataxia telangiectasia-mutated phosphorylates Chk2 in vivo and in vitro. *Proceedings of the National Academy of Sciences of the United States of America* **97**(19)**:** 10389-10394

Maya-Mendoza A, Petermann E, Gillespie DAF, Caldecott KW, Jackson DA (2007) Chk1 regulates the density of active replication origins during the vertebrate S phase. *EMBO Journal* **26**(11)**:** 2719-2731

McElhinny SAN, Gordenin DA, Stith CM, Burgers PMJ, Kunkel TA (2008) Division of labor at the eukaryotic replication fork. *Molecular Cell* **30**(2)**:** 137-144

Medhurst AL, Warmerdam DO, Akerman I, Verwayen EH, Kanaar R, Smits VAJ, Lakin ND (2008) ATR and Rad17 collaborate in modulating Rad9 localisation at sites of DNA damage. *J Cell Sci* **121**(23)**:** 3933-3940

Mimura S, Masuda T, Matsui T, Takisawa H (2000) Central role for Cdc45 in establishing an initiation complex of DNA replication in *Xenopus* egg extracts. *Genes to Cells* **5**(6)**:** 439-452

Mohanty BK, Bairwa NK, Bastia D (2006) The Tof1p-Csm3p protein complex counteracts the Rrm3p helicase to control replication termination of *Saccharomyces cerevisiae*. *Proceedings of the National Academy of Sciences of the United States of America* **103**(4)**:** 897-902

Mordes DA, Glick GG, Zhao RX, Cortez D (2008) TopBP1 activates ATR through ATRIP and a PIKK regulatory domain. *Genes & Development* **22**(11)**:** 1478-1489

Mu JJ, Wang Y, Luo H, Leng M, Zhang JL, Yang T, Besusso D, Jung SY, Qin J (2007) A proteomic analysis of ataxia telangiectasia-mutated (ATM)/ATM-Rad3-related (ATR) substrates identifies the ubiquitin-proteasome system as a regulator for DNA damage checkpoints. *Journal of Biological Chemistry* **282**(24)**:** 17330-17334

Natsume T, Tanaka T (2009) Spatial regulation and organization of DNA replication within the nucleus. *Chromosome Research*

Nguyen VQ, Co C, Li JJ (2001) Cyclin-dependent kinases prevent DNA re-replication through multiple mechanisms. *Nature* **411**(6841)**:** 1068-1073

Nimonkar AV, Ozsoy AZ, Genschel J, Modrich P, Kowalczykowski SC (2008) Human exonuclease 1 and BLM helicase interact to resect DNA and initiate DNA repair. *Proceedings of the National Academy of Sciences of the United States of America* **105**(44)**:** 16906-16911

Nishitani H, Lygerou Z (2002) Control of DNA replication licensing in a cell cycle. *Genes to Cells* **7**(6)**:** 523-534

Noguchi E, Noguchi C, Du LL, Russell P (2003) Swi1 prevents replication fork collapse and controls checkpoint kinase Cds1. *Molecular and Cellular Biology* **23**(21)**:** 7861-7874

Noguchi E, Noguchi C, McDonald WH, Yates JR, Russell P (2004) Swi1 and Swi3 are components of a replication fork protection complex in fission yeast. *Molecular and Cellular Biology* **24**(19)**:** 8342-8355

Oakley GG, Loberg LI, Yao JQ, Risinger MA, Yunker RL, Zernik-Kobak M, Khanna KK, Lavin MF, Carty MP, Dixon K (2001) UV-induced hyperphosphorylation of replication protein A depends on DNA replication and expression of ATM protein. *Molecular Biology of the Cell* **12**(5)**:** 1199-1213

Pacek M, Tutter AV, Kubota Y, Takisawa H, Walter JC (2006) Localization of MCM2-7, Cdc45, and GINS to the site of DNA unwinding during eukaryotic DNA replication. *Molecular Cell* **21**(4)**:** 581-587

Pavlov YI, Frahm C, McElhinny SAN, Niimi A, Suzuki M, Kunkel TA (2006) Evidence that errors made by DNA polymerase alpha are corrected by DNA polymerase delta. *Current Biology* **16**(2)**:** 202-207

Pellicioli A, Lee SB, Lucca C, Foiani M, Haber JE (2001) Regulation of *Saccharomyces* Rad53 checkpoint kinase during adaptation from DNA damage-induced G2/M arrest. *Molecular Cell* **7**(2)**:** 293-300

Peng CY, Graves PR, Thoma RS, Wu ZQ, Shaw AS, PiwnicaWorms H (1997) Mitotic and G(2) checkpoint control: Regulation of 14-3-3 protein binding by phosphorylation of Cdc25C on serine-216. *Science* **277**(5331)**:** 1501-1505

Perkins G, Drury LS, Diffley JFX (2001) Separate SCFCDC4 recognition elements target Cdc6 for proteolysis in S phase and mitosis. *EMBO Journal* **20**(17)**:** 4836-4845

Petermann E, Helleday T, Caldecott KW (2008) Claspin promotes normal replication fork rates in human cells. *Mol Biol Cell* **19**(6)**:** 2373-2378

Petersen BO, Lukas J, Sorensen CS, Bartek J, Helin K (1999) Phosphorylation of mammalian CDC6 by Cyclin A/CDK2 regulates its subcellular localization. *EMBO Journal* **18**(2)**:** 396-410

Pursell ZF, Isoz I, Lundstrom EB, Johansson E, Kunkel TA (2007) Yeast DNA polymerase epsilon participates in leading-strand DNA replication. *Science* **317**(5834)**:** 127-130

Rass E, Grabarz A, Plo I, Gautier J, Bertrand P, Lopez BS (2009) Role of Mre11 in chromosomal nonhomologous end joining in mammalian cells. *Nature Structural & Molecular Biology* **16**(8)**:** 819-U838

Saha P, Chen JJ, Thome KC, Lawlis SJ, Hou ZH, Hendricks M, Parvin JD, Dutta A (1998) Human CDC6/Cdc18 associates with Orc1 and cyclin-cdk and is selectively eliminated from the nucleus at the onset of S phase. *Molecular and Cellular Biology* **18**(5)**:** 2758-2767

Sanchez Y, Wong C, Thoma RS, Richman R, Wu RQ, PiwnicaWorms H, Elledge SJ (1997) Conservation of the Chk1 checkpoint pathway in mammals: Linkage of DNA damage to Cdk regulation through Cdc25. *Science* **277**(5331)**:** 1497-1501

Sandell LL, Zakian VA (1993) Loss of a yeast telomere- arrest, recovery, and chromosome loss. *Cell* **75**(4)**:** 729-739

Sartori AA, Lukas C, Coates J, Mistrik M, Fu S, Bartek J, Baer R, Lukas J, Jackson SP (2007) Human CtIP promotes DNA end resection. *Nature* **450**(7169)**:** 509-U506

Shechter D, Costanzo V, Gautier J (2004) ATR and ATM regulate the timing of DNA replication origin firing. *Nature Cell Biology* **6**(7)**:** 648-655

Shimmoto M, Matsumoto S, Odagiri Y, Noguchi E, Russell P, Masai H (2009) Interactions between Swi1-Swi3, Mrc1 and S phase kinase, Hsk1 may regulate cellular responses to stalled replication forks in fission yeast. *Genes to Cells* **14**(6)**:** 669-682

Shiotani B, Zou L (2009) Single-stranded DNA orchestrates an ATM-to-ATR switch at DNA breaks. *Molecular Cell* **33**(5)**:** 547-558

Singh DK, Ahn B, Bohr VA (2009) Roles of RECQ helicases in recombination based DNA repair, genomic stability and aging. *Biogerontology* **10**(3)**:** 235-252

Smith E, Dejsuphong D, Balestrini A, Hampel M, Lenz C, Takeda S, Vindigni A, Costanzo V (2009) An ATM- and ATR-dependent checkpoint inactivates spindle assembly by targeting CEP63. *Nat Cell Biol* **11**(3)**:** 278-285

Smolka MB, Albuquerque CP, Chen SH, Zhou H (2007) Proteome-wide identification of in vivo targets of DNA damage checkpoint kinases. *Proceedings of the National Academy of Sciences of the United States of America* **104**(25)**:** 10364-10369

Sommariva E, Pellny TK, Karahan N, Kumar S, Huberman JA, Dalgaard JZ (2005) *Schizosaccharomyces pombe* Swi1, Swi3, and hskl are components of a novel S-phase

response pathway to alkylation damage. *Molecular and Cellular Biology* **25**(7)**:** 2770-2784

Sorensen CS, Hansen LT, Dziegielewski J, Syljuasen RG, Lundin C, Bartek J, Helleday T (2005) The cell-cycle checkpoint kinase Chk1 is required for mammalian homologous recombination repair. *Nature Cell Biology* **7**(2)**:** 195-U121

Soutoglou E, Misteli T (2008) Activation of the cellular DNA damage response in the absence of DNA lesions. *Science* **320**(5882)**:** 1507-1510

Stiff T, Walker SA, Cerosaletti K, Goodarzi AA, Petermann E, Concannon P, O'Driscoll M, Jeggo PA (2006) ATR-dependent phosphorylation and activation of ATM in response to UV treatment or replication fork stalling. *EMBO Journal* **25**(24)**:** 5775-5782

Stokes MP, Rush J, MacNeill J, Ren JM, Sprott K, Nardone J, Yang V, Beausoleil SA, Gygi SP, Livingstone M, Zhang H, Polakiewicz RD, Comb MJ (2007) Profiling of UV-induced ATM/ATR signaling pathways. *Proceedings of the National Academy of Sciences of the United States of America* **104**(50)**:** 19855-19860

Stokes MP, Van Hatten R, Lindsay HD, Michael WM (2002) DNA replication is required for the checkpoint response to damaged DNA in *Xenopus* egg extracts. *J Cell Biol* **158**(5)**:** 863-872

Stucki M, Jackson SP (2006) gamma H2AX and MDC1: Anchoring the DNA-damage-response machinery to broken chromosomes. *DNA Repair* **5**(5)**:** 534-543

Sung P, Klein H (2006) Mechanism of homologous recombination: mediators and helicases take on regulatory functions. *Nature Reviews Molecular Cell Biology* **7**(10)**:** 739-750

Syljuasen RG, Jensen S, Bartek J, Lukas J (2006) Adaptation to the ionizing radiation-induced G(2) checkpoint occurs in human cells and depends on checkpoint kinase 1 and Polo-like kinase 1 kinases. *Cancer Research* **66**(21)**:** 10253-10257

Szyjka SJ, Viggiani CJ, Aparicio OM (2005) Mrc1 is required for normal progression of replication forks throughout chromatin in *S. cerevisiae*. *Molecular Cell* **19**(5)**:** 691-697

Takeda S, Nakamura K, Taniguchi Y, Paull TT (2007) Ctp1/CtIP and the MRN complex collaborate in the initial steps of homologous recombination. *Molecular Cell* **28**(3)**:** 351-352

Tanaka H, Kubota Y, Tsujimura T, Kumano M, Masai H, Takisawa H (2009) Replisome progression complex links DNA replication to sister chromatid cohesion in *Xenopus* egg extracts. *Genes Cells* **14**(8)**:** 949-963

Toczyski DP, Galgoczy DJ, Hartwell LH (1997) CDC5 and CKII control adaptation to the yeast DNA damage checkpoint. *Cell* **90**(6)**:** 1097-1106

Tourriere H, Versini G, Cordon-Preciado V, Alabert C, Pasero P (2005) Mrc1 and Tof1 promote replication fork progression and recovery independently of Rad53. *Molecular Cell* **19**(5)**:** 699-706

Trenz K, Errico A, Costanzo V (2008) Plx1 is required for chromosomal DNA replication under stressful conditions. *EMBO Journal* **27**(6)**:** 876-885

Umezu K, Sugawara N, Chen C, Haber JE, Kolodner RD (1998) Genetic analysis of yeast RPA1 reveals its multiple functions in DNA metabolism. *Genetics* **148**(3)**:** 989-1005

Unsal-Kacmaz K, Chastain PD, Qu PP, Minoo P, Cordeiro-Stone M, Sancar A, Kaufmann WK (2007) The human Tim/Tipin complex coordinates an intra-S checkpoint response to UV that slows replication fork displacement. *Molecular and Cellular Biology* **27**(8)**:** 3131-3142

Van Hatten RA, Tutter AV, Holway AH, Khederian AM, Walter JC, Michael WM (2002) The *Xenopus* Xmus101 protein is required for the recruitment of Cdc45 to origins of DNA replication. *Journal of Cell Biology* **159**(4)**:** 541-547

Vaze MB, Pellicioli A, Lee SE, Ira G, Liberi G, Arbel-Eden A, Foiani M, Haber JE (2002) Recovery from checkpoint-mediated arrest after repair of a double-strand break requires Srs2 helicase. *Molecular Cell* **10**(2)**:** 373-385

Walter J, Newport J (2000) Initiation of eukaryotic DNA replication: Origin unwinding and sequential chromatin association of Cdc45, RPA, and DNA polymerase alpha. *Molecular Cell* **5**(4)**:** 617-627

Weinert T (1998) DNA damage and checkpoint pathways: Molecular anatomy and interactions with repair. *Cell* **94**(5)**:** 555-558

Wohlschlegel JA, Dhar SK, Prokhorova TA, Dutta A, Walter JC (2002) *Xenopus* Mcm10 binds to origins of DNA replication after Mcm2-7 and stimulates origin binding of Cdc45. *Molecular Cell* **9**(2)**:** 233-240

Xie AY, Kwok A, Scully R (2009) Role of mammalian Mre11 in classical and alternative nonhomologous end joining. *Nature Structural & Molecular Biology* **16**(8)**:** 814-U831

Xu X, Vaithiyalingam S, Glick GG, Mordes DA, Chazin WJ, Cortez D (2008) The basic cleft of RPA70N binds multiple checkpoint proteins, including RAD9, to regulate ATR signaling. *Molecular and Cellular Biology* **28**(24)**:** 7345-7353

Yan S, Michael WM (2009) TopBP1 and DNA polymerase-alpha directly recruit the 9-1-1 complex to stalled DNA replication forks. *Journal of Cell Biology* **184**(6)**:** 793-804

Yang XH, Shiotani B, Classon M, Zou L (2008) Chk1 and Claspin potentiate PCNA ubiquitination. *Genes & Development* **22**(9)**:** 1147-1152

Yanow SK, Gold DA, Yoo HY, Dunphy WG (2003) *Xenopus* Drf1, a regulator of Cdc7, displays checkpoint-dependent accumulation on chromatin during an S-phase arrest. *Journal of Biological Chemistry* **278**(42)**:** 41083-41092

Yoo HY, Jeong SY, Dunphy WG (2006) Site-specific phosphorylation of a checkpoint mediator protein controls its responses to different DNA structures. *Genes & Development* **20**(7)**:** 772-783

Yoo HY, Kumagai A, Shevchenko A, Dunphy WG (2004a) Adaptation of a DNA replication checkpoint response depends upon inactivation of Claspin by the Polo-like kinase. *Cell* **117**(5)**:** 575-588

Yoo HY, Kumagai A, Shevchenko A, Dunphy WG (2007) Ataxia-telangiectasia mutated (ATM)-dependent activation of ATR occurs through phosphorylation of TopBP1 by ATM. *Journal of Biological Chemistry* **282**(24)**:** 17501-17506

Yoo HY, Kumagai A, Shevchenko A, Dunphy WG (2009) The Mre11-Rad50-Nbs1 complex mediates activation of TopBP1 by ATM. *Molecular Biology of the Cell* **20**(9)**:** 2351-2360

Yoo HY, Shevchenko A, Dunphy WG (2004b) Mcm2 is a direct substrate of ATM and ATR during DNA damage and DNA replication checkpoint responses. *Journal of Biological Chemistry* **279**(51)**:** 53353-53364

Yoshizawa-Sugata N, Masai H (2007) Human Tim/Timeless-interacting protein, Tipin, is required for efficient progression of S phase and DNA replication checkpoint. *Journal of Biological Chemistry* **282**(4)**:** 2729-2740

Yu XC, Fu SA, Lai MY, Baer R, Chen JJ (2006) BRCA1 ubiquitinates its phosphorylation-dependent binding partners CtIP. *Genes & Development* **20**(13)**:** 1721-1726

Yun MH, Hiom K (2009) CtIP-BRCA1 modulates the choice of DNA double-strand-break repair pathway throughout the cell cycle. *Nature* **459**(7245)**:** 460-U184

Zhao H, Piwnica-Worms H (2001) ATR-mediated checkpoint pathways regulate phosphorylation and activation of human Chk1. *Mol Cell Biol* **21**(13)**:** 4129-4139

Zhu Z, Chung WH, Shim EY, Lee SE, Ira G (2008) Sgs1 helicase and two nucleases Dna2 and Exo1 resect DNA double-strand break ends. *Cell* **134**(6)**:** 981-994

Zou L, Elledge SJ (2003) Sensing DNA damage through ATRIP recognition of RPA-ssDNA complexes. *Science* **300**(5625)**:** 1542-1548

Zou L, Stillman B (2000) Assembly of a complex containing Cdc45p, replication protein A, and Mcm2p at replication origins controlled by S-phase cyclin-dependent kinases and Cdc7p-Dbf4p kinase. *Molecular and Cellular Biology* **20**(9)**:** 3086-3096