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Mutation Research xxx (2003) xxx–xxx
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Fundamental and Molecular Mechanisms of Mutagenesis
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Editorial

DNA replication, the cell cycle and genome stability

The 16 reviews and original research articles in this special issue of *Mutation Research* provide an overall view of our current understanding of the relationships between DNA replication, the cell cycle and genome stability in eukaryotic cells. As you will discover as you read these articles, problems with DNA replication—when origins fire inefficiently, or when precursors are in short supply, or when polymerases do not work properly, or when other enzymes of DNA replication are faulty, or when DNA is damaged, or when checkpoint pathways are defective, or even (but less frequently) when all systems are working optimally—are a major source of mutations and genomic rearrangements. Indeed, it seems likely that most of the genomic instability that is necessary for the development of many types of cancer can ultimately be attributed to replication problems.

All of the laboratories represented in this special issue previously made important contributions, published elsewhere, to our understanding of replication and genome stability. Their additional contributions, published here in a single issue, further advance that understanding and provide a convenient single source for readers who wish to be apprised of the current state of the art in this field.

Preparations for DNA replication begin in late M-phase and continue into G1-phase, when the origin recognition complex (ORC), Cdc6, Cdt1 and other proteins cooperate to load the minichromosome maintenance (MCM) proteins onto chromatin to form pre-replication complexes (pre-RCs) at sites that have the potential to become replication origins. At the beginning of S-phase, cyclin-dependent kinase (CDK) and Cdc7 kinase (also called Dbf4-dependent kinase (DDK)) cooperate to signal initiation of DNA replication at a subset of the pre-RCs. In the first article of this special issue, Sidorova and Breeden [1] review the G1 to S transition and point out that successful completion of this transition is essential for genome stability. They suggest that successful completion of S-phase requires the establishment of a dual S-phase surveillance system, which, by measuring both the number of pre-RCs and the number of replication forks, monitors ongoing DNA replication. According to their hypothesis, when the number of pre-RCs and replication forks is below a threshold level, checkpoint activation will not be sufficiently robust to signal in *trans* that late origins should not fire or that mitosis should be restrained until completion of S-phase. Thus, if cells attempt to enter S-phase before an adequate number of pre-RCs has been generated in G1, the number of pre-RCs and forks may fall below the threshold, leading to genomic instability. For this reason, it seems plausible that the acceleration of the G1 to S transition evident in most cancer cells may be directly responsible for some or all of the genomic instability of these cells.

Exactly how the number of pre-RCs and the number of replication forks is monitored by checkpoint surveillance mechanisms is not yet clear. However, considerable evidence, both from yeasts (reviewed by Duncker and Brown [2]) and from mammalian cells (reviewed by Kim et al. [3]) indicates that the DDKs, which are essential for initiation of replication at origins, also play upstream and downstream roles in S-phase checkpoints. Within the DDK regulatory subunit (Dbf4), distinct motifs have been identified that are more important for checkpoint function than for replication function, and it is possible that DDK function may be required to restart stalled replication forks as well as to initiate fork movement in the first place [2]. However, not all checkpoint functions

0027-5107/$ – see front matter
doi:10.1016/j.mrfmmm.2003.09.004

are dependent on DDKs. In murine embryonic stem cells, complete loss of Cdc7 protein leads to apparent checkpoint responses—rapid cessation of DNA synthesis and cell proliferation, followed by p53-dependent apoptosis [3].

Many of the reviews and research articles in this special issue deal with the ways in which cells respond to replication fork blockage by DNA damage, or to replication fork stalling due to weakened DNA polymerases, or to inadequate supply of precursors (deoxynucleoside triphosphates (dNTPs)). Checkpoint responses appear to provide the first line of defense. Longhese et al. [4] provide an extensive overview of these checkpoints, with a focus on checkpoints in *Saccharomyces cerevisiae*. Kai and Wang [5] review S-phase checkpoints in the fission yeast, *Schizosaccharomyces pombe*, and McGowan [6] concentrates on S-phase checkpoints in mammalian cells. Since there is extensive conservation of S-phase checkpoints among all eukaryotic organisms, these three views are both complementary and mutually reenforcing.

The checkpoint kinases ATR and ATM (in mammalian cells), Mec1 (in budding yeast) and Rad3 (in fission yeast) play essential roles in sensing problems with DNA molecules and in transducing signals to downstream checkpoint pathways. These kinases can be effectively inhibited in vitro by caffeine in the millimolar concentration range. Kaufmann et al. [7] report that, consistent with previous observations, 2 mM caffeine reversed ATM- and ATR-dependent S and G2 checkpoints in immortalized human fibroblasts. Mysteriously, however, 2 and even 5 mM caffeine were unable to reverse ATM-dependent checkpoint function in G1-phase [7]. One possible interpretation of this observation is that caffeine may not be able to inhibit ATM or ATR inside living cells, even though it can inhibit these kinases in vitro, and the observed inhibition of S and G2 checkpoints by caffeine may indicate that caffeine is able to inhibit other steps (not ATM or ATR) in these checkpoint pathways. While caffeine was able to inhibit the S checkpoint response to UVC, its effect on UVC genotoxicity was rather small in normal human fibroblasts. In contrast, in *xeroderma pigmentosum* variant cells, which lack the damage-bypass polymerase DNA pol η, caffeine markedly enhanced UVC genotoxicity, suggesting that there may be a mechanism for repair of DNA replication forks arrested at cyclobutane pyrimidine dimers that is inhibited by caffeine.

Some of the major pathways by which cells restore stalled forks employ homologous recombination. Helleday [8] elegantly summarizes how homologous recombination can be used to restore both collapsed replication forks, where one of the parental strands has been broken to create a “1-end” double-strand break, and stalled forks, that may have re-wound to generate branched “chicken-foot” structures.

Major contributions to replication fork preservation and re-starting are also made by the set of pathways that, for historic reasons, has been called “post-replication repair”. Now we know that these pathways employ several mechanisms for bypassing DNA lesions that would otherwise stall replication forks. Since the lesions are bypassed and not repaired, perhaps a better name for these pathways would be “DNA damage bypass”. Smirnova and Klein [9] and Barbour and Xiao [10] describe the differences between the “error-free” bypass pathways, which in most cases employ recombination mechanisms, and the “error-prone” pathways, which in most cases involve a small amount of non-faithful DNA synthesis by one or more translesion polymerases. Smirnova and Klein [9] point out that even in the absence of external sources of DNA damage, the error-free bypass pathways cooperate with checkpoint and homologous recombination pathways in diploid yeast cells to prevent genomic instability. Barbour and Xiao [10] emphasize that, when both damage bypass and recombination pathways are defective, an alternative damage avoidance pathway, which involves the protein Mgs1, permits lesion bypass in some cases.

Replication forks can stall when DNA polymerases have difficulties, even in the absence of DNA damage. Kai and Wang [5] describe an experimental system in which polymerase difficulties are induced by growing fission yeast cells that harbor a temperature-sensitive polymerase α at an elevated, but not lethal, temperature. Under these conditions, the mutation frequency is increased. The higher mutation frequency appears to be due, at least in part, to a small amount of DNA synthesis by an error-prone translesion polymerase that is loaded onto replication forks by the checkpoint clamp loader (Rad17) and the checkpoint clamp complex of Rad9, Rad1 and Hus1 [5]. It seems likely that this translesion polymerase activity allows restart of

replication forks that have stalled due to weak polymerase α [5]. Note that a "translesion" polymerase is employed in this case even though no known "lesion" is generated when a replication fork stalls due to a defect in polymerase α.

Replication forks naturally stall at a "replication fork barrier" located downstream of the ribosomal RNA genes in budding yeast ribosomal DNA. Weitao et al. [11] provide evidence that this natural stalling is enhanced by deletion of the gene encoding the Sgs1 helicase, suggesting that Sgs1 is normally important for restarting stalled forks. Interestingly, budding yeast Sgs1 is similar to the human Bloom's and Werner's syndrome helicases, suggesting that some of the symptoms of these syndromes may be due to inability to appropriately restart replication forks.

One of the most efficient means of blocking replication forks is by inhibiting the re-ligation of topoisomerase I-generated nicks. Pommier et al. [12] thoroughly review the ways in which the resulting topoisomerase I cleavage complexes can be generated (both naturally and by topoisomerase I poisons), bypassed and repaired. Cancer cells are frequently defective in one or more of the pathways responsible for repair of topoisomerase I cleavage complexes, and this may explain the frequent hypersensitivity of cancer cells to topoisomerase I poisons [12].

Interference with DNA replication by inhibition of polymerases (for example with aphidicolin, a specific inhibitor of DNA polymerases α, δ and ε), by various types of DNA damage, by depletion of dNTP pools with hydroxyurea (HU; an inhibitor of ribonucleotide reductase), or by hypoxia, gives rise to distinct cellular responses. The variety of such responses is emphasized in the papers from Hammond et al. [13] and Liu et al. [14]. Hammond et al. [13] compared the effects on mammalian cells of inducing replication arrest with hypoxia to the effects of inducing arrest with HU or aphidicolin. They found that HU- and aphidicolin-induced arrest were accompanied by detectable DNA damage, while hypoxia-induced arrest was not, and they suggest that the cellular responses to the damage-free replication arrest by hypoxia are mediated entirely by ATR, while responses to the damage-accompanied arrest induced by HU or aphidicolin are mediated by both ATR and ATM [13]. Liu et al. [14] compared the effects on mammalian cells of using HU, aphidicolin, or DNA-damaging chemicals—adozelesin, a bulky alkylating agent, or methyl methane sulfonate (MMS), a methylating agent—to interfere with replication. They found that these four treatments differ from each other with regard to extents of Chk1, RPA32, and H2AX phosphorylation, RPA and γ-H2AX focus formation, and induction of apoptosis. Their results suggest that much additional research is needed before we will fully understand the variety of cellular responses to different DNA-damaging and replication-fork-stalling agents [14]. Their results and those of Hammond et al. [13] also indicate that caution is needed when attempting to compare results obtained with different agents.

One of the possible consequences of severe replication problems in metazoan cells is apoptosis. Until recently, it seemed likely that this response was confined to multicellular organisms. However, a growing body of research—reviewed in this issue by Burhans et al. [15]—now indicates that programmed cell death occurs in all organisms, prokaryotic and eukaryotic, unicellular and multicellular. Furthermore, many of the phenomena of mammalian apoptosis, including production of reactive oxygen species (ROS), activation of caspases, and inversion of cellular membranes—are conserved in yeasts. Production of ROS can be detected in both budding and fission yeasts under conditions that produce replication stress [15].

What is the fate of cells in which S-phase checkpoints and repair pathways fail? As reviewed by Andreassen et al. [16], these cells move into G2 and then into mitosis, where the G2 and mitotic checkpoints slow the cell cycle to provide an opportunity for the lesions to be repaired. In mammalian cells, the G2 and mitotic checkpoints are transient, however, and there is a possibility that the damage engendered in S-phase will persist though mitosis into the subsequent G1-phase [16]. In many cases, mitosis with damaged DNA is faulty, resulting in G1 tetraploidy. In these cases, the G1 tetraploidy checkpoint permanently arrests the cells or induces apoptosis, depending on cell type [16]. Thus, the G2, mitotic and G1 tetraploidy checkpoints provide a triple backup to the S-phase checkpoint, helping to ensure that problems arising during S-phase will not be propagated, because damage will be repaired or the cell carrying the damage will be prevented from multiplying [16].

Although it is too early to predict the impact that this special issue of *Mutation Research* will have on the

fields of DNA replication, the cell cycle, and genome stability, in my opinion the high quality of each of the individual articles guarantees that the issue as a whole will make an important contribution. The high quality of the articles is a consequence of the thought and care of the authors and also of the helpful suggestions provided by the reviewers, whom I thank here en masse. Another factor contributing to the likely success of this special issue is the fact that the authors were unusually cooperative in meeting publication deadlines, and the publishers were unusually efficient in preparing these articles for publication. As readers can judge from the dates of receipt of initial and revised manuscripts, this collection of review and original research articles was published more promptly than most single research or review articles. As a result, the references contained in these review and research articles are unusually up-to-date at the time of publication. I would like to thank all of the authors, and also Peter Stambrook, the *Mutation Research* Editor-in-Charge of this special issue, and his Secretary, Kathleen Gouge, for their cooperation, encouragement and patience. The success of this special issue is a consequence of their combined efforts.

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