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DNA mismatch repair and the DNA damage response

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ABSTRACT

This review discusses the role of DNA mismatch repair (MMR) in the DNA damage response (DDR) that triggers cell cycle arrest and, in some cases, apoptosis. Although the focus is on findings from mammalian cells, much has been learned from studies in other organisms including bacteria and yeast [1,2]. MMR promotes a DDR mediated by a key signaling kinase, ATM and Rad3-related (ATR), in response to various types of DNA damage including some encountered in widely used chemotherapy regimes. An introduction to the DDR mediated by ATR reveals its immense complexity and highlights the many biological and mechanistic questions that remain. Recent findings and future directions are highlighted.

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Contents

1. Introduction

In addition to its roles in editing replication errors and other functions (see other reviews in this issue and [3]), the MMR system is also implicated in the repair and cytotoxicity of a subset of DNA lesions caused by S_N1 DNA alkylators, 6-thioguanine, fluoropyrimidines, cisplatin, UV light and certain environmental carcinogens that form DNA adducts (reviewed in [4–7]). Defining the exact role of MMR in cell killing resulting from exposure to these DNA damaging agents is complicated by the sometimes broad spectrum of DNA damage and the convergence of multiple repair pathways such as base excision repair (BER), nucleotide excision repair (NER) and double-strand break (DSB) repair pathways and attendant DNA damage signaling pathways (see, *e.g.*, [8–11]). The S_N1 DNA alkylators, *e.g.*, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine (MNNG), methylnitrosourea (MNU) and the chemotherapy drug temozolomide, methylate all four DNA bases producing a variety of potentially cytotoxic lesions that are substrates for BER. O^6-methylguanine-DNA methyltransferase (MGMT) directly reverses O^6meG and plays an important role in protecting against cytotoxic effects of S_N1 alkylators and preventing tumor formation *in vivo* [7]. Not unexpectedly, there are numerous clinical implications, and these are discussed in this issue (minireviews by Begum, Heinen, Sijmons in this issue).

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In the case of S_N1 DNA alkylators, the DDR requires components of the MMR system; the loss of functional MMR proteins, *e.g.*, hMutSα (MSH2-MSH6) or hMutLα (MLH1-PMS2) gives rise to tolerance in which the persistence of potentially cytotoxic lesions is no longer linked to cell death. Tolerance to the S_N1 class of DNA alkylating agents was first observed in *Escherichia coli* strains defective in MMR that exhibited greatly increased resistance to cell killing and was subsequently demonstrated in MMR-deficient mammalian cell lines some of which are almost two orders of magnitude more resistant to cell killing than comparable MMR-proficient cells (reviewed in [12]). In a similar vein, rare cells that survive exposure to alkylating agents oftentimes have accrued mutations that inactivate MMR [13]. Despite constituting only a small fraction of total alkylated DNA lesions, O^6me-G is the key contributor to the mutagenic and cytotoxic effects of S_N1 alkylators [14]. Low doses of MNNG induce a G_2/M cell cycle arrest in the second cell cycle after exposure that is dependent on MMR proteins (reviewed in [15,16]). A DDR signaling kinase, ATM and Rad3-related (ATR) is activated and licenses a G_2/M cell cycle arrest mediated by downstream targets including the checkpoint kinases CHK1, CHK2, and SMC1 and cell division control 25 (CDC25) phosphatases. Apoptosis ensues directed in most cases by phosphorylation of p53 that also requires functional MutSα and MutLα [17].

2. The DNA damage response

The cellular responses to DNA damage are collectively termed the DNA damage response. The DDR engages signaling pathways that regulate the recognition of DNA damage, the recruitment of DNA repair factors, the initiation and coordination of DNA repair pathways, transit through the cell cycle and apoptosis [18]. The large number of human diseases and syndromes that arise from defects in components of the DNA damage response reflect the importance of the DDR for health and viability [19].

Three protein kinases, DNA-dependent protein kinase (DNA-PK); ataxia-telangiectasia-mutated (ATM); and ATM and Rad3-related (ATR), have prominent roles in the DDR pathways that respond to genotoxic stress. These master regulator kinases are members of the phosphoinositide three-kinase-related kinase (PIKK) family, a class which also includes suppressor of morphological effect on genitalia family member (SMG1), mammalian target of rapamycin (mTOR), and transformation/transcription domain-associated protein (TRRAP) [20,21]. DNA-PK and ATM are best known for their role in the double-strand DNA breaks (DSB) response though it is increasingly apparent that they function in multiple contexts [22,23]. In contrast to ATM, ATR is essential for the survival of proliferating cells most likely due to its roles in the response to replication stress, *i.e.*, the rescue of stalled or collapsed replication forks and the regulation of replication origin firing. In addition, it is activated by DNA damage that poses a threat to replication including certain base adducts, interstrand cross-links, and DSBs. Despite differences in substrate specificity and activation, the kinases share similar structures and regulatory themes involving localization to sites of damage and reliance on interacting protein partners [24,25]. ATR, like ATM, phosphorylates hundreds of protein targets at Ser/Thr-Gln motifs and other sites. The phosphorylated substrates in turn execute functions affecting DNA repair, replication, transcription, cell cycle checkpoint signaling, and cell fate pathways such as apoptosis or senescence.

3. Upstream events and activation of ATR

Recruitment of ATR and its constitutively interacting partner, ATR interacting protein (ATRIP), to damaged DNA was observed to be dependent on an interaction between ATRIP and replication protein A (RPA) bound to single-stranded DNA (ssDNA) [26]. Subsequent work has supported a model in which processing of DNA damage by various repair systems yields a common intermediate consisting of RPA-ssDNA, that, together with a ssDNA–dsDNA junction, serves to activate ATR [24,27]. Such structures are generated at stalled replication forks in S phase where fork reversal or uncoupling of replication factor leads to exposure of ssDNA. They can also be generated by resection at ends of DSBs during break repair. Excision repair pathways such as nucleotide excision repair (NER) acting in G_1 and G_2/M similarly can generate RPA-ssDNA structures that elicit ATR activation.

Activation of ATR requires not only localization to sites of DNA damage but also a combination of interacting partners. Recognition and recruitment to RPA-ssDNA requires ATR interacting protein (ATRIP), an obligate partner of ATR that interacts directly with RPA [26,28,29]. In addition, RPA stimulates the Rad17-replication factor C (RFC) clamp loader complex, directing it to load the Rad9-Rad1-Hus1 (9-1-1) clamp complex at the 5′ end of the ssDNA-dsDNA junction [30–32]. The 9–1–1 complex bound to DNA recruits topoisomerase binding protein 1 (TopBP1) that activates ATR through interactions with ATRIP [33–36]. The recruitment to damage sites of ATR and its key activator TopBP1 utilize distinct interactions within multiple protein complexes helping to ensure that ATR is activated only when appropriate [27].

Detailed mechanisms of each step in the activation pathway as well as the ways in which activation may differ under different damage contexts or physiological conditions remain poorly understood. A growing list of posttranslational modifications reveals their important roles in coordinating the assembly and activity of signaling complex components at sites of damage [27,24,37]. After binding of ATR–ATRIP to RPA-ssDNA, ATR undergoes trans-autophosphorylation. This phosphorylation is essential for further ATR activation as it generates a docking site for TopBP1 [38,39]. Phosphorylation of TopBP1 by ATM further enhances ATR-TopBP1 interaction and thereby ATR activity [40,41]. RPA phosphorylation by DNA-PK has also been implicated in the ATR checkpoint response [42–44]. RPA is a direct substrate of ATR in executing the DDR [45–47]. Other proteins necessary for ATR activation, including Rad17 and TopBP1, have also been identified as substrates of ATR. Other post-translational modifications of ATR and activation complex proteins are important for signaling regulation. SUMOylation of ATRIP, for instance, has been shown to enhance ATR activation by promoting interaction of ATRIP with other proteins in the pathway, including ATR, RPA, and TopBP1 [48]. The ubiquitin ligase PRP19 is recruited to RPA-ssDNA, where it enhances signaling by ubiquitinating RPA and potentially other substrates at the signaling complex [49].

Recent work also reveals new players that promote ATR activation, many of which still warrant further characterization. The Triple T complex (TTT) acts with Hsp90 to chaperone PIKKs, including ATR, promoting PIKK maturation and checkpoint signaling [50–52]. The 9–1–1 interacting nuclear orphan (RHINO) protein is an enhancer of ATR activation through interactions with the 9-1-1 clamp and TopBP1 [53,54]. Other recently identified examples include CDK2-interacting protein (CINP), which interacts with ATRIP, and the Mre11-Rad50-Nbs1 (MRN) complex that functions in DSB repair, that is required for recruitment of TopBP1 to ss-dsDNA junctions in *Xenopus* nuclear extracts [55,56]. Regulation may be fine-tuned to respond to specific types of damage or specific contexts such as cell cycle control [57,58].

4. Downstream signaling from ATR

Following activation, ATR acts locally at sites of damage and more globally to phosphorylate a range of substrates that execute

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