Mechanisms of Ageing and Development 129 (2008) 391–407

Contents lists available at ScienceDirect

Mechanisms of Ageing and Development

journal homepage: www.elsevier.com/locate/mechagedev

Review

DNA mismatch repair: Molecular mechanism, cancer, and ageing

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ARTICLE INFO

Article history:
Available online 4 March 2008

Keywords:
Mismatch repair
HNPCC
Ageing
Genome instability
MutS
MutL

ABSTRACT

DNA mismatch repair (MMR) proteins are ubiquitous players in a diverse array of important cellular functions. In its role in post-replication repair, MMR safeguards the genome correcting base mispairs arising as a result of replication errors. Loss of MMR results in greatly increased rates of spontaneous mutation in organisms ranging from bacteria to humans. Mutations in MMR genes cause hereditary nonpolyposis colorectal cancer, and loss of MMR is associated with a significant fraction of sporadic cancers. Given its prominence in mutation avoidance and its ability to target a range of DNA lesions, MMR has been under investigation in studies of ageing mechanisms. This review summarizes what is known about the molecular details of the MMR pathway and the role of MMR proteins in cancer susceptibility and ageing.

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1. Introduction

Ensuring the fidelity of DNA replication is central to preserving genomic integrity, and DNA mismatch repair (MMR) is critical for maintaining the fidelity of replication. We review what is known concerning the molecular mechanism of MMR, its role in DNA damage signalling, and its relationship to cancer and ageing. The recent literature is emphasized; for more comprehensive discussions, please see Harfe and Jinks-Robertson (2000), Jiricny (2006), Kunkel and Erie (2005), Modrich (2006), Schofield and Hsieh (2003).

Replication is an extraordinarily faithful process (Iyer et al., 2006); mutations occur at a frequency of roughly 1 in 10^9 to 10^{10} base pairs per cell division. Nucleotide selection at the base incorporation step and the proofreading function of DNA polymerases collectively result in an error rate of approximately 10^7 per bp per genome. The MMR pathway, a DNA repair pathway conserved from bacteria to humans, targets base substitution mismatches and insertion–deletion mismatches (IDLs) that arise as a result of replication errors that escape the proofreading function of DNA polymerases. In doing so, MMR contributes an additional 50–1000-fold to the overall fidelity of replication. Thus, inactivation of MMR confers a strong mutator phenotype in which the rate of spontaneous mutation is greatly elevated. A hallmark of many MMR-deficient cells is instability at microsatellite regions consisting of mono- and di-nucleotide repeats. Strand slippage during replication through microsatellite regions gives rise to IDLs that are normally repaired by MMR; hence, microsatellite instability (MSI) is widely used as a diagnostic marker for loss of MMR activity in tumour cells (Umar et al., 2004).

1.1. Mutation avoidance and post-replication repair

In *Escherichia coli*, the methyl-directed MMR system has been extensively studied, and the entire pathway has been reconstituted in vitro from purified proteins in the Modrich laboratory (Table 1, reviewed in Kunkel and Erie, 2005; Schofield and Hsieh, 2003). MMR in prokaryotes is initiated when mismatches are recognized by a highly conserved MMR protein, MutS. MutS and a second conserved protein, MutL, act in concert to license the excision repair pathway by activating endonucleolytic cleavage by a third MMR protein, MutH. MutH directs its nicking activity to the unmethylated strand at transiently hemimethylated GATC sites shortly after replication. This methyl-directed nicking by MutH ensures that MMR in *E. coli* is directed to the newly synthesized DNA strand containing the error. In vitro studies helped to establish that MMR is bidirectional with respect to the excision step. In other words, MMR can utilize a nick on either side of the mismatch. With the help of MutL, this nick in the nascent strand acts as a point of entry for helicase II that unwinds the nascent strand, a process that is facilitated by single-strand binding protein (SSB) (Matson and Robertson, 2006; Robertson et al., 2006b). This exposes the strand to digestion by one of four single-strand

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0047-6374/$ – see front matter. Published by Elsevier Ireland Ltd.
doi:10.1016/j.mad.2008.02.012

Table 1
Identity and functions of *E. coli* and eukaryotic proteins involved in MMR of replication errors

E. coli protein	Function	Homologues	Function
MutS	Binds mismatches	MSH2–MSH6 (MutSα)	Repairs single base–base and 1–2 base IDL mismatches
		MSH2–MSH3 (MutSβ)	Repair of some single base IDLs and IDLs ≥2 bases Partially redundant with Msh2–Msh6
MutL	Matchmaker that coordinates multiple steps in MMR	MLH1–PMS2 (yPMS1) (MutLα)	Matchmaker for coordinating events from mismatch binding by MutS homologues to DNA repair synthesis Endonuclease (PMS2)
		MLH1–MLH2 (hPMS1) (MutLβ)	Function of human heterodimer unknown Suppresses some IDL mutagenesis in yeast
		MLH1–MLH3 (MutLγ)	Suppresses some IDL mutagenesis Participates in meiosis
MutH	Nicks nascent unmethylated strand at hemimethylated GATC sites	None	
γ–δ Complex	Loads β-clamp onto DNA	RFC complex	Loads PCNA, modulates excision polarity
β-Clamp	Interacts with MutS and may recruit it to mismatches and/or the replication fork Enhances processivity of DNA pol III	PCNA	Interacts with MutS and MutL homologues Recruits MMR proteins to mismatches Increases MM binding specificity of Msh2–Msh6 Participates in excision and probably in signalling Participates in DNA repair synthesis Participates in DNA re-synthesis
Helicase II	Loaded onto DNA at nick by MutS and MutL Unwinds DNA to allow excision of ssDNA	None	
ExoI, ExoX	Perform 3′–5′ excision of ssDNA	EXOI (Rth1)	Excision of dsDNA
RecJ	Perform 5′–3′ excision of ssDNA (also 3′–5′ excision by ExoVII)	3′ exo of polδ	Excision of ssDNA
ExoVII		3′ exo of polε	Synergistic mutator with Exo1 mutant
DNA pol III	Accurate re-synthesis of DNA	DNA polδ	Accurate repair synthesis
SSB	Participates in excision and DNA synthesis	RPA	Participates in excision and in DNA synthesis
DNA ligase	Seals nicks after completion of DNA synthesis	DNA ligase	Seals nicks after completion of DNA synthesis

Adapted from Kunkel and Erie (2005) with permission.

exonucleases having either 3′–5′ or 5′–3′ polarity: ExoI, ExoVII, ExoX, or RecJ (Burdett et al., 2001). The resulting DNA gap is repaired in a reaction involving pol III and ligase thereby restoring the duplex to its intact parental genotype.

MMR in eukaryotes follows the broad outline described above for the *E. coli* methyl-directed MMR pathway (see Fig. 1 and discussion below), and reconstitutions in the Li and Modrich laboratories of MMR reactions from purified proteins possess many of the key features associated with MMR in vivo (Constantin et al., 2005; Dzantiev et al., 2004; Zhang et al., 2005). These studies were predicated on a large body of earlier work that identified individual components from active fractions of cell extracts and characterized partial reactions (reviewed in Jiricny, 2006). Zhang et al. (2005) have demonstrated MMR of a G-T mismatch in 5′-directed repair reactions containing MutSα, MutLα, RPA, EXO1, PCNA, RFC, HMGB1, DNA polymerase δ, and DNA ligase I (Yuan et al., 2004). Substitution of MutSβ for MutSα allowed repair of a 3 nt IDL. MutLα was not required for 5′-directed repair, but did regulate EXO1-catalysed excision. 3′-Directed repair was not supported in this system. Constantin et al. (2005) reconstituted a nick-directed, bidirectional reaction involving seven components: MutSα, MutLα, RPA, EXO1, PCNA, RFC, and DNA polymerase δ. Again, MutLα was not required for 5′-directed repair, but was essential for 3′-directed repair. EXO1 was required for both 3′- and 5′-directed repair.

Some notable differences between MMR in *E. coli* and MMR in eukaryotes are readily apparent (reviewed in Modrich, 2006). First, whereas bacterial MutS and MutL proteins function as homodimeric proteins, their eukaryotic counterparts are invariably heterodimers composed of two related, but distinct protein subunits. In fact, eukaryotic cells possess several MutS and MutL homologues, and the choice of subunit partner dictates substrate specificity and cellular function (see Table 1; Kunkel and Erie, 2005). MSH2–MSH6, or MutSα, targets base–base mispairs and +1 IDLs; MSH2–MSH3, or MutSβ, targets primarily IDLs though recent genetic and biochemical data support a role for yMsh3 in the repair of certain base–base mispairs in vivo (Harrington and Kolodner, 2007). Second, although the *E. coli* methyl-directed MMR system has been completely defined, a minimal human system has only been recently reconstituted with purified proteins (see below), and many aspects of the pathway remain unclear. Third, while *E. coli* and closely related Gram-negative bacteria can take advantage of dam methylation to direct strand-specific repair, such signals are not available to other prokaryotic or eukaryotic cells.

A number of key issues concerning the molecular mechanism of MMR remain unresolved. Chief among them are: (i) How are MMR proteins recruited to newly replicated DNA most likely in the context of the replication machinery, and how does MutS recognize mismatches? (ii) What is the nature of the MutS (MutSα)–MutL (MutLα)–heteroduplex DNA complex that licenses MMR? (iii) How does MMR couple the mismatch recognition step with a strand-specific excision step? The last is critical as the gapped DNA intermediate formed during MMR, if not repaired,