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Review

Insights from a decade of biophysical studies on MutL: Roles in strand discrimination and mismatch removal

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ABSTRACT

DNA mismatch repair (MMR) is a conserved pathway that safeguards genome integrity by correcting replication errors. The coordinated actions of two proteins (MutS and MutL) initiate the mismatch repair response and defects in the genes encoding for these proteins have been linked to sporadic and hereditary cancers. The basic steps to repair a mismatch include recognizing the mismatch, discriminating the newly synthesized from the parental strand, removing and re-synthesizing the erroneous strand. Although the DNA mismatch repair pathway has been extensively studied over the last four decades, the strand discrimination mechanism has remained elusive in most organisms. Work over the last decade has brought significant progress onto this step of the pathway, in turn ascribing new and critical roles to the MutL protein. In this review, we describe biochemical, biophysical and structural analyses that have clarified how MutL aids at discriminating the newly synthesized strand from its template and marking it for removal.

Contents

Abbreviations: MMR, mismatch repair; MSI, microsatellite instability; MLH, MutL homolog; PMS, post-meiotic segregation; IDL, insertion/deletion loop; GHKL, Gyrase/Hsp90/Histidine kinase/MutL; AFM, atomic force microscopy; NTD, N-terminal domain; CTD, C-terminal domain; β, sliding β-clamp; PCNA, proliferating cell nuclear antigen; PIP-box, PCNA-interacting protein box.

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1. Introduction

DNA mismatch repair (MMR) corrects errors that have escaped polymerase proofreading, thereby enhancing replication fidelity by two to three orders of magnitude (Arana and Kunkel, 2010; Iyer et al., 2006; Jiricny, 2013). Defects in this pathway results in accumulation of point mutations, strand slippage replication errors and microsatellite instability (MSI). Identification of germline MMR gene mutations in Lynch syndrome —the most common form of hereditary colorectal cancer— confirmed the link between the mismatch repair pathway and hereditary cancer (Lynch et al., 2009). Furthermore, many oncogenes and tumor suppressor genes have repetitive sequences within their coding region and, hence, inactivation of mismatch repair genes is also a potential factor leading to some sporadic cancers.

Two ubiquitous proteins are responsible for targeting mismatches for repair (Kunkel and Erie, 2005). MutS surveys newly replicated DNA and identify the presence of mismatched base pairs, as well as small insertion/deletions loops on the DNA. Once MutS has found a mismatch, it recruits MutL. In turn, MutL recruits downstream factors to target the newly synthesized strand of the duplex for repair. Discriminating the newly synthesized strand is the most critical step, and yet the most obscure, of the pathway. *Escherichia coli* uses a devoted latent endonuclease (MutH) that recognizes transiently hemi-methylated GATC sequences and nicks the unmethylated strand of the duplex. However, homologs of MutH have only been found in a small subset of gamma-proteobacteria. Most prokaryotes and all eukaryotes lack a *mutH* gene and use a different process of strand discrimination. Work from several groups over the last decade has identified new critical roles of the MutL proteins in organisms that lack MutH, emphasizing their central role in mismatch repair. Here, we review recent biochemical, biophysical and structural work that has delineated how MutL compensates for the lack of MutH and orchestrates critical protein–protein interactions during the early steps of DNA mismatch repair.

2. MutL is conserved from bacteria to humans

Prokaryotes encode a single MutL polypeptide that self-associates to form a functional MutL dimer. Conversely, eukaryotes encode several MutL paralogs (MLH and PMS proteins) that form multiple heterodimers. MutLα, the heterodimer formed by the association of yeast Mlh1 and Pms1 (MLH1 and PMS2 in humans), carries the main mismatch repair activity in eukaryotes. There are two additional MLH heterodimers in eukaryotic cells: MutLβ (MLH1-PMS1) and MutLγ (MLH1-MLH3). Human MutLγ can partially compensate for the lack of MutLα *in vitro* (Cannavo et al., 2005). *Saccharomyces cerevisiae* MutLγ (Mlh1-Mlh3) has also a minor role in the correction of insertion-deletions loops (IDL) (Flores-Rozas and Kolodner, 1998). However, the main function of MutLγ in mammals and yeast is the resolution of recombination intermediates during meiosis (Wang et al., 1999; Zakharyevich et al., 2010, 2012). The role of the third heterodimer, MutLβ (MLH1-PMS1 in humans and Mlh1-Mlh2 in yeast), is less understood. Being the common subunit to all MutL dimers, MLH1 defects are associated with severe phenotypes. Mutations inactivating *MLH1* account for 50% of all Lynch syndrome cases, whereas mutations in hPMS2 and hMLH3 are rare contributors to Lynch syndrome. In yeast, *mlh2* mutants cause a weak phenotype and display partial redundancy with Mlh3 in Msh3-dependent manner to repair frameshift intermediates (Harfe et al., 2000).

Despite its different roles, all MutL homologs are composed of two structurally conserved domains joint by a linker that varies in length and sequence. The sequence of the N-terminal domain is highly conserved from bacteria to humans and this domain has the characteristic fold of the GHKL family of ATPases (Ban and Yang, 1998). The sequence of the C-terminal domain is not conserved, but recent structural studies have revealed that it also has a conserved structure (Guarné et al., 2004; Gueneau et al., 2013; Namadurai et al., 2010; Pillon et al., 2010). While original studies presumed that the sole role of this domain was mediating protein dimerization, work in the last decade has revealed the many diverse roles of this region of MutL. The modular architecture of MutL is critical to its functions as it imposes a conformational regulation induced by the interaction with MutS, the processivity clamp, and downstream mismatch repair factors.

3. The multifaceted N-terminal domain of MutL

The ATPase activity of MutL proteins is weak, but can be stimulated in the presence of DNA (Ban and Yang, 1998; Guarné et al., 2001; Hall et al., 2002). Binding of ATP triggers the self-association of the two ATPase domains of the dimer. Therefore, it was originally presumed that the dimer was the active ATPase form (Ban et al., 1999; Ban and Yang, 1998). However, studies with the N-terminal domains of human and yeast MutL homologs demonstrated that dimerization is not required for the ATPase activity of MutL (Guarné et al., 2001; Hall et al., 2002). The structures of the N-terminal regions of *E. coli* MutL, hMLH1, hPMS2 and yPMS1 have been determined ((Arana et al., 2010; Ban et al., 1999; Ban and Yang, 1998; Guarné et al., 2001) and PDB ID: 4P7A). This region of MutL is made up of two subdomains. The first subdomain contains the four conserved motifs characteristic of the GHKL family, while the second presumably mediates DNA binding (Ban et al., 1999). The structures of *E. coli* MutL and human PMS2 were determined in the presence and absence of nucleotide. Comparison of these structures reveals that the relative orientation between the ATPase and DNA-binding subdomains changes upon nucleotide binding. However, the two subdomains have the same relative orientation in the structures of the N-terminal domains of *E. coli* MutL, hMLH1, hPMS2 and yPMS1 bound to nucleotide (reviewed in (Guarné, 2012)). The nucleotide-induced conformational changes within the ATPase domain could help progress the repair reaction from the recognition to the strand discrimination step, thereby establishing the hierarchy of the interactions mediated by MutL.

The N-terminal domain of MutL mediates the interaction with MutS. Indeed, this interaction can be recapitulated *in vitro* using this fragment of MutL (Lenhart et al., 2013; Winkler et al., 2011). The presence of ATP and a mismatch-containing duplex DNA promote the interaction (Winkler et al., 2011). The residues of MutS defining the binding interface are not conserved among different species, but in all cases MutL seems to recognize the connector region of MutS (Lenhart et al., 2013; Mendillo et al., 2009; Winkler et al., 2011). The N-terminal domain of MutL also mediates in *E. coli* the interaction with MutH, the latent endonuclease that discriminates the new from the template strand in the *E. coli* mismatch repair system (Ban et al., 1999), as well as the vsr endonuclease in very-short patch repair (Heinze et al., 2009). In both cases, only the ATP-bound form of MutL interacts with MutH or Vsr. Therefore, it is tempting to speculate that ATP binding to MutL is the signal that helps transition from mismatch recognition by MutS to strand discrimination. Implicitly, this suggests that MutS could regulate the strand discrimination step by enhancing nucleotide binding to MutL.

Recent work using functional fluorescently labeled *E. coli* MutS and MutL *in vivo* reported that MutL foci were 2–3 times more intense than MutS foci co-localized on individual mismatches, however a steric block on the DNA provided by a variant of MutH able to bind, but not cut, DNA decreased MutL foci fluorescence 3-fold. These results indicate that MutL accumulation coordinates

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