DNA Repair xxx (2015) xxx–xxx

Contents lists available at ScienceDirect

DNA Repair

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

Visualization of mismatch repair complexes using fluorescence microscopy

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ARTICLE INFO

Article history:
Received 30 June 2015
Received in revised form
30 September 2015
Accepted 30 November 2015
Available online xxx

Keywords:
Visualization of DNA Mismatch repair complexes
MMR
Microsatellite instability
DNA repair

ABSTRACT

DNA mismatch repair (MMR) is a surveillance mechanism present in most living organisms, which repairs errors introduced by DNA polymerases. Importantly, loss of MMR function due to inactivating mutations and/or epigenetic silencing results in the accumulation of mutations and as consequence increased cancer susceptibility, as observed in Lynch syndrome patients.

During the past decades important progress has been made in the MMR field resulting in the identification and characterization of essential MMR components, culminating in the *in vitro* reconstitution of 5′ and 3′ nick-directed MMR. However, several mechanistic aspects of the MMR reaction remain not fully understood, therefore alternative approaches and further investigations are needed.

Recently, the use of imaging techniques and, more specifically, visualization of MMR components in living cells, has broadened our mechanistic understanding of the repair reaction providing more detailed information about the spatio-temporal organization of MMR *in vivo*. In this review we would like to comment on mechanistic aspects of the MMR reaction in light of these and other recent findings. Moreover, we will discuss the current limitations and provide future perspectives regarding imaging of mismatch repair components in diverse organisms.

Contents

1. Introduction

1.1. DNA replication and mismatch repair

Replication of eukaryotic DNA requires the function of DNA polymerases Polα, Polδ and Polε [61]. Biochemical evidence supports the idea that Polα, which lacks proofreading activity and possesses a low processivity, initiates DNA synthesis at replication origins as well as at every Okazaki fragment. Synthesis of the lagging strand requires Polδ to further elongate the DNA strand up to 200–300 bases [95]. On the other hand, synthesis of the leading strand occurs in a continuous manner; however, it is less clear if Polε [88,98] or Polδ [53], or a combination or both, are mainly required during this process [114].

Imaging studies in several eukaryotic organisms revealed that DNA replication occurs within the nucleus at well-defined clusters, referred to as “replication factories”, in which several DNA

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http://dx.doi.org/10.1016/j.dnarep.2015.11.014
1568-7864/

replication origins are firing in close proximity, most likely sharing components of the DNA replication machinery [11,23,47,86,87].

In order to safeguard the stability of the genome Polε and Polδ harbor a 3′-5′ proofreading exonuclease activity that increases DNA replication fidelity by about 100-fold [49,63,101]. In addition, most living organisms possess a post-replicative correction mechanism, called mismatch repair (MMR) pathway that further increases DNA replication fidelity by an additional 1000-fold [52,63,102]. In general, MMR recognizes DNA replication errors (*i.e.*, mispairs, insertions or deletions) and introduces a nick at the strand containing the misincorporated base, which is used as entry point during an excision reaction (see article by Kadyrova and Kadyrov, in this issue of DNA repair). Next, the excised DNA track is resynthesized and ligated.

About 30 years ago, the first MMR genes were cloned from *Salmonella typhimurium* [38,89] as well as from *Streptococcus pneumoniae* [94,97]. Inactivation of these genes was associated with a mutator phenotype, and they were thus referred to as "mut" genes. A break-through came in 1993, when it was found that the elevated mutation rates caused by the inactivation of MMR genes, result in the predisposition to an early-onset of cancer in humans, a disorder called hereditary non-polyposis colorectal cancer (HNPCC) or Lynch syndrome [20,66,92].

The MMR system is best understood in *Escherichia coli* (*E. coli*), in which the mismatch recognition complex, represented by the MutS homodimer, detects the mispaired base and recruits the MutL homodimer to the site of damage. MutL interacts and activates the endonuclease MutH, resulting in nicking of the newly replicated strand. In *E. coli*, hemi-methylated d(GATC) sites act as strand discrimination signal during the nicking reaction, as MutH endonuclease only nicks the unmethylated newly replicated strand [76,119] (reviewed by Putnam, in this issue of DNA repair). This strand discrimination signal disappears when the DNA adenine methylase (Dam) modifies d(GATC) sequences shortly after the DNA has been replicated, in this way creating a window of time during which MMR can discriminate between parental and daughter strand. It has been proposed that nicks introduced by MutH are used as entry point by exonucleases during an excision reaction. After the strand excision reaction, the parental strand is used as template for DNA re-synthesis [49,63].

In eukaryotes, DNA replication errors are recognized by two, partially redundant MutS homolog (MSH) heterodimeric complexes: MutSα (Msh2–Msh6) and MutSβ (Msh2–Msh3) [2,60]. Upon mismatch recognition, the MutSα or MutSβ complexes promote the recruitment of MutL homolog (MLH) complexes to the mismatch site. Three different heterodimeric MLH complexes have been identified: MutLα (Mlh1–Pms1 in *Saccharomyces cerevisiae* (*S. cerevisiae*) or Mlh1-Pms2 in humans), which is essential for MMR, MutLβ (Mlh1–Mlh2 in *S. cerevisiae* or Mlh1–Pms1 in humans), for which the biological function is less clear; and MutLγ (Mlh1–Mlh3), which plays an important role during meiotic recombination [124,125] (reviewed by Manhart and Alani, in this issue of DNA repair).

Remarkable, eukaryotes and most bacteria (with the exception of a subset of gammaproteobacteria including *E. coli*) do not use DNA-methylation as a MMR strand discrimination signal [24] (reviewed by Putnam, in this issue of DNA repair). The nature of the strand discrimination signal has remained unknown for decades, although accumulating evidence suggests that nicks at the 3′ or 5′ ends of Okazaki fragments [90] or nicks that result after removal of misincorporated ribonucleotides introduced during DNA replication [30,77], might be used to discriminate the parental from daughter strand. Moreover, an additional factor that likely plays a role during strand discrimination is Proliferating Cell Nuclear Antigen (PCNA), which not only promotes the processivity of DNA polymerases at the replication fork, but also interacts with MMR components like Msh3, Msh6 [10,21] and Mlh1 [14,68].

Although eukaryotic MMR lacks a MutH homolog protein, it has been found that the MutLα complex possesses endonuclease activity, which is stimulated in a PCNA-dependent manner [54,93] (reviewed by Kadyrova and Kadyrov, in this issue of DNA repair). Based on *in vitro* reconstitution experiments, it has been proposed that PCNA is loaded onto DNA by Replication Factor C (RFC) at a pre-existing nick, and due to the asymmetric nature of loaded PCNA, MutLα is directed to nick the nicked strand by a mechanism that is only partially understood.

Biochemical and genetic evidence indicates that after the nicking reaction, excision of the strand containing the misincorporated base occurs in either a fast exonuclease1-dependent (Exo1-dependent) or a slower Exo1-independent reaction (recently reviewed by [32] (see also Kadyrova and Kadyrov, in this issue of DNA repair) in which most likely multiple rounds of PCNA-stimulated MutLα nicking are followed by excision and DNA re-synthesis.

Recently, visualization of MMR components by fluorescence microscopy has provided additional insights into the kinetics and spatio-temporal patterns of MMR complexes and has questioned current mechanistic models of the MMR repair reaction. In order to visualize the MMR repair process *in situ*, MMR genes have been fluorescently tagged in *E. coli* [15,16], *Bacillus subtilis* [69,70,111,112], *S. cerevisiae* [8,33,44] and mammalian cells [46,58,74,103]. However, visualization of functional fluorescently labeled MMR proteins under the expression of their native promoters in living cells has only been achieved in *B. subtilis* [69,70,111,112] and *S. cerevisiae* [8,33,44].

In this review, we would like to summarize recent findings related to the visualization of MMR components and discuss their consequences for our current understanding of the mechanism of MMR. For this purpose, we will focus on the spatio-temporal coupling of mismatch recognition to replication factories, the identification and characterization of MMR recognition and repair intermediates, and current limitations and future perspective of imaging MMR components in different model organisms.

1.2. Spatiotemporal coupling of MMR with DNA replication

1.2.1. MutS/MutSα foci as a mismatch recognition intermediate

The MMR machinery has to detect infrequent DNA replication errors [62] that escaped the proofreading activity of DNA polymerases. Mismatch recognition, strand discrimination and incision need to take place during a brief window of time, which in *E. coli* correspond to the time the newly synthesized DNA strand remains unmethylated at the d(GATC) sites. In *S. cerevisiae*, these repair-associated events also occur with temporal constraints, most likely defined by the presence of a DNA replication-associated signal, which is used during strand discrimination [45]. Therefore, having the mismatch recognition machinery at the place where errors are being generated is expected to facilitate repair. The idea of coupling DNA synthesis and mismatch recognition is supported by the finding that Msh3 and Msh6 interact with PCNA via a PCNA interacting protein motif (or PIP-box) located at the Msh3 and Msh6 N-terminus [10,21,58]. Moreover, it has been shown that *in vitro* reconstituted MMR reactions require the presence of a single strand break on the DNA [49,63], suggesting that *in vivo* the MMR machinery may take advantage of transiently existing nicks on newly replicated DNA.

Visualization of mismatch repair and DNA replication components by fluorescence microscopy revealed that indeed the mismatch recognition complex, MutS in *B. subtilis* [70,111] as well as Msh2-Msh6 in *S. cerevisiae* [44] or human cells [58,74], does

Please cite this article in press as: T.T. Schmidt, H. Hombauer, Visualization of mismatch repair complexes using fluorescence microscopy, DNA Repair (2015), http://dx.doi.org/10.1016/j.dnarep.2015.11.014