DNA Repair 12 (2013) 257–264

Contents lists available at SciVerse ScienceDirect

DNA Repair

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

Mismatch recognition function of *Arabidopsis thaliana* MutSγ

Rodrigo Gómez, Claudia P. Spampinato*

Centro de Estudios Fotosintéticos y Bioquímicos (CEFOBI), Facultad de Ciencias Bioquímicas y Farmacéuticas, Universidad Nacional de Rosario, Suipacha 531, 2000 Rosario, Argentina

ARTICLE INFO

Article history:
Received 6 December 2012
Received in revised form 9 January 2013
Accepted 10 January 2013
Available online 4 February 2013

Keywords:
Mismatch repair
Genome stability
Mutation rate
Arabidopsis thaliana
Saccharomyces cerevisiae

ABSTRACT

Genetic stability depends in part on an efficient DNA lesion recognition and correction by the DNA mismatch repair (MMR) system. In eukaryotes, MMR is initiated by the binding of heterodimeric MutS homologue (MSH) complexes, MSH2–MSH6 and MSH2–MSH3, which recognize and bind mismatches and unpaired nucleotides. Plants encode another mismatch recognition protein, named MSH7. MSH7 forms a heterodimer with MSH2 and the protein complex is designated MutSγ. We here report the effect the expression of Arabidopsis MSH2 and MSH7 alone or in combination exert on the genomic stability of *Saccharomyces cerevisiae*. AtMSH2 and AtMutSγ proteins failed to complement the hypermutator phenotype of an *msh2* deficient strain. However, overexpressing AtMutSγ in MMR proficient strains generated a 4-fold increase in *CAN1* forward mutation rate, when compared to wild-type strains. Can^r mutation spectrum analysis of AtMutSγ overproducing strains revealed a substantial increase in the frequency of base substitution mutations, including an increased accumulation of base pair changes from G:C to A:T and T:A to C:G, G:C or A:T. Taken together, these results suggest that AtMutSγ affects yeast genomic stability by recognizing specific mismatches and preventing correction by yeast MutSα and MutSβ, with subsequent inability to interact with yeast downstream proteins needed to complete MMR.

1. Introduction

DNA mismatch repair (MMR) system maintains genome stability by correcting single base–base mismatches and unpaired nucleotides in template or nascent DNA strands (deletion or insertion loops, respectively). Proteins involved in this pathway have been conserved from bacteria to plants [1] although both the nature and number of orthologues have become more complex throughout evolution. MutS and MutL proteins function as homodimers in *Escherichia coli* and as multiple heterodimers (mainly MutSα, MutSβ and MutLα) in yeast and humans. In addition, plants contain a unique MutS protein named MutSγ.

MutSα, MutSβ and MutSγ are heterodimers of MSH2 complexed with MSH6, MSH3 or MSH7, respectively [2–5]. MutSα is mainly required to correct single mispairs and short insertion/deletion loops (IDLs), whereas MutSβ is predominantly involved in the removal of large IDLs (2–12 nucleotides) [2,4,6,7]. Cristal structures of human MutSα in complex with a series of DNA substrates [8] confirmed the responsible motifs for mismatch recognition, previously observed in prokaryotic proteins [8–10]. A conserved FXE motif, only present in the MSH6 subunit, plays critical roles in mismatch interaction. MSH3 lacks these conserved phenylalanine and glutamate residues suggesting that protein binding to IDLs occurs through different contacts [11].

MutSγ is by far much less characterized than MutSα and MutSβ. So far, studies performed with AtMSH2 and AtMSH7 proteins, products of *in vitro* transcription and translation techniques, have suggested that MutSγ preferentially recognizes certain base–base mismatches [5,12]. In addition, MutSγ has a specific role in meiotic recombination [13,14]. In fact, MSH7, as well as MSH6 and prokaryotic MutS, contains the conserved FXE motif required for mismatch interaction [1]. To further characterize the *in vivo* role of MutSγ, we performed functional analyses in yeast. We used three chromosomal markers, in particular *his7-2*, *lys2::InsE-A$_{14}$* and *CAN1*. The *his7-2* allele, contains a T:A deletion in a stretch of 8 T:A in the *HIS7* gene [15]. Frameshift reversion, largely due to +1 bp insertions and −2 bp deletions, is evaluated by cell growth on medium lacking histidine. The second marker used, the allele *lys2*, contains a mononucleotide run of 14 As in the *LYS2* gene (*lys2::InsE-A$_{14}$*) [16]. Reversion by −1 bp deletions of the *lys2* allele enables the strain to grow on medium lacking lysine. Finally, the *CAN1* gene codes for arginine permease. This protein transports arginine and its toxic analog canavanine into the cell. When inactivating mutations, such as base substitutions, deletions, insertions, and large chromosomal rearrangements, occur in the *CAN1* gene, strains lose permease activity and become resistant to media containing canavanine [17].

Our data show that AtMSH2 is unable to complement the hypermutator phenotype of an *msh2* deficient strain. However, expression of AtMSH2–AtMSH7 protein complex in a MMR

* Corresponding author. Tel.: +54 341 4371955x113; fax: +54 341 54 341 4371955x103.
E-mail address: spampinato@cefobi-conicet.gov.ar (C.P. Spampinato).

1568-7864/$ – see front matter © 2013 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.dnarep.2013.01.002

Table 1
Primers used for vector constructions. Restriction sites and the ATG initiation codon are in italics and underlined, respectively.

Name	Sequence
y5pSac-MSH7	ATCT*GAGCTC*ACC<u>ATG</u>GTGCAGCGCCAGAGAT
y3pSph-MSH7	CGTG*GCATGC*TTATTTGGGAACACAGTAAGAGG
y5pKpn-MSH2	CG*CGGTACC*<u>ATG</u>GAGGGTAATTTCGAGGAA
y3pCsp-MSH2	CTACAT*CGGTCCG*TTATCACAGAAACTGCCT

proficient strain leads to a clear increase in *CAN1* mutator rate, indicating that the expression of the plant DNA repair protein affects yeast MMR mechanism. Analysis of Can^r mutation spectrum indicate a high frequency of G:C to A:T, T:A to G:C, T:A to C:G and T:A to A:T base substitutions. These studies suggest that MutSγ is important in ensuring genome stability by recognizing mismatches that arise by spontaneous deamination or environmental stresses, such as solar UV light and reactive oxygen species.

2. Materials and methods

2.1. Yeast strains and growth conditions

Saccharomyces cerevisiae haploid strains E134 (*MATα ade5 lys2::InsEA$_{14}$ trp1-289 his7-2 leu2-3, 112 ura3-52*) and DAG60 (*msh2*Δ derivative of E134) have been previously described [18]. Yeast were grown in YPDA [1% (w/v) yeast extract, 2% (w/v) peptone, 2% (w/v) glucose, 20 mg/l adenine] or SC [19] containing 2% (w/v) glucose without leucine (SCDL). When appropiate, 2% (w/v) galactose was included instead of glucose (SCGL) for inducing genes cloned downstream *GAL1-10* promoters. To measure forward mutation rates to canavanine resistance, SC medium without arginine containing 60 mg/l L-canavanine was used. Solid media were also supplemented with 1.5% (w/v) agar.

2.2. Vector constructions

Yeast expression plasmids were constructed in YEp181SPGAL [18] as follows. At*MSH7* was PCR amplified by the proofreading Vent DNA polymerase using the pGEM3Z-AtMSH7 plasmid [5] as template and the y5pSac-MSH7 and y3pSph-MSH7 oligonucleotides as primers (Table 1). PCR reactions were carried out as described by Gomez et al. [23] except elongation time was increased up to 3.5 min. The At*MSH7* gene was then inserted into the YEp181SPGAL vector after digestion with *Sac*I and *Sph*I. This construction places the At*MSH7* coding sequence downstream the *GAL1* promoter, generating the YEp-AtMSH7 plasmid (Fig. 1). Then, the At*MSH2* was inserted into this plasmid downstream the *GAL10* promoter as follows. The At*MSH2* sequence was amplified by PCR using the above conditions except for the template (pGEM3Z-AtMSH2, [20]) and primers used (y5pKpn-MSH2 and y3pCsp-MSH2, Table 1). The PCR fragment was digested with *Kpn*I and *Csp*I and ligated into the digested *Kpn*I/*Pst*I YEp-AtMSH7 plasmid. The resulting linear vector was then blunt-ended with Klenow DNA polymerase and afterwards ligated to generate YEp-AtMSH7/AtMSH2 plasmid (Fig. 1). Finally, the At*MSH7* gene was released from the YEp-AtMSH7/AtMSH2 plasmid by digestion with *BamH*I and *Sph*I followed by blunt-ended ligation. The resulting construction, named YEp-AtMSH2, contains the At*MSH2* cDNA downstream the *GAL10* promoter (Fig. 1). The absence of random mutations introduced by the DNA polymerase in all the PCR amplified fragments was determined by DNA sequencing (Macrogen, Korea).

2.3. Expression of MMR proteins

For MMR protein expression in yeast strains, electrocompetent cells were prepared and transformed as described previously [21] with some modifications. The electroporated cells were diluted in 1 ml YPDA and maintained 1 h at 30 °C without agitation. The cells were collected by centrifugation at 3000 × *g* for 3 min and the resulting pellet was resuspended in 50 μl of SCDL and spread on SCDL plates. Transformant colonies appeared within 3–4 days at 30 °C. Single colonies were grown in liquid SCDL media overnight at 30 °C. An aliquot of the culture was harvested and washed three times with distilled water to remove glucose medium. Cells were then inoculated into SCGL medium to give an initial $OD_{600\,nm} < 0.01$ and allowed to grow until an $OD_{600\,nm}$ of 0.2–0.3 was reached. Cells were harvested and immediately stored at −70 °C.

2.4. Yeast extract preparation

Cells ($2–3 \times 10^8$) were resuspended in 0.1 ml buffer containing 40 mM Tris–HCl, 1 mM EDTA, 1 mM PMSF, 0.1% (v/v) β-mercaptoethanol, pH 6.8. For denaturing extract preparation, buffer was also supplemented with 5% (w/v) SDS. Glass beads were added and 6 series of 1 min vortex-mixing alternating with 1 min incubation on ice were performed. After cell-lysis, samples were centrifuged in order to pellet glass beads and cell debris. The supernatants were stored at −70 °C.

2.5. Immunological analyses

Denaturing gels containing 50 μg of yeast extract were electrotransferred to nitrocellulose membranes (Bio-Rad) at 50 V for 1 h for immunoblotting [22]. Affinity purified anti-AtMSH2 polyclonal antibodies (6.5 μg/ml) were used for detection [23]. Bound primary antibodies were recognized by goat anti-rabbit IgG conjugated to alkaline phosphatase and subsequently developed with 5-bromo-4-chloro-3-indolyl phosphate and nitro blue tetrazolium [22].

2.6. Mutation rate measurement

Reversion rates of *lys2::InsEA$_{14}$* and *his7-2* and forward mutations to canavanine resistance were measured by fluctuation analysis as previously described [18,24]. Briefly, a single colony carrying a particular vector was grown to saturation in SCDL. Cultures were pelleted, washed twice in sterile distilled water to remove glucose, inoculated into the SCGL medium such that the initial inoculum size contained 1000 cells/ml, and dispensed (200 μl) into 96-well plates. Cultures were grown to saturation at 30 °C without shaking. Cells from 12 to 24 cultures were pooled and diluted to determine growth by measuring $OD_{600\,nm}$ and by plating into SCDL agar medium. Cells from 12 to 36 cultures were plated after appropriate dilutions onto selective medium lacking lysine or histidine to count for revertant mutants or lacking arginine but containing canavanine to count resistant mutants. Data were analyzed by the Lea-Coulson method of the median using the Fluctuation anALysis CalculatOR program [25]. The 95% confidence intervals were determined as previously described [26,27] while the significance of differences between mutation rates (*p*-value) was estimated using Kruskal–Wallis and Mann–Whitney tests, where appropriate.

2.7. DNA annealing and labeling

Oligodeoxyribonucleotides were purchased from Integrated DNA Technologies. A 61-bp G-T heteroduplex was prepared by mixing equal concentrations of top strand (5′-TCGCCAGAATCGCCGAATTGCTAGCAAGCTTTCGAGTCTAGAAATTCGGCGAATCCCGTCA-3′)