The origins and early evolution of DNA mismatch repair genes—multiple horizontal gene transfers and co-evolution

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ABSTRACT

To understand the evolutionary process of the DNA mismatch repair system, we conducted systematic phylogenetic analysis of its key components, the bacterial MutS and MutL genes and their eukaryotic homologs. Based on genome-wide homolog searches, we identified three new MutS subfamilies (MutS3-5) in addition to the previously studied MutS1 and MutS2 subfamilies. Detailed evolutionary analysis strongly suggests that frequent ancient horizontal gene transfer (HGT) occurred with both MutS and MutL genes from bacteria to eukaryotes and/or archaea. Our results further imply that the origins of mismatch repair system in eukaryotes and archaea are largely attributed to ancient HGT from bacteria instead of vertical evolution. Specifically, the eukaryotic MutS and MutL homologs likely originated from endosymbiotic ancestors of mitochondria or chloroplasts, indicating that not only archaea, but also bacteria are important sources of eukaryotic DNA metabolic genes. The archaeal MutS1 and MutL homologs were also acquired from bacteria simultaneously through HGT. Moreover, the distribution and evolution profiles of the MutS1 and MutL genes suggest that they have undergone long-term coevolution. Our work presents an overall portrait of the evolution of these important genes in DNA metabolism and also provides further understanding about the early evolution of cellular organisms.

INTRODUCTION

Mismatched nucleotides are regularly introduced by DNA polymerase during cell division and uncorrected nucleotides will result in mutations. In most cellular organisms, such replication errors are repaired mainly by the DNA mismatch repair (MMR) system that enhances replication fidelity 50- to 1000-folds by repairing mismatched nucleotides, and small insertions and deletions (1–3). The MMR system also prevents recombination between divergent sequences and repairs mismatches on heteroduplex DNA that arise during homologous recombination (4). Therefore, defects in the MMR could lead to highly elevated mutation rates, meiotic defects and infertility (5,6). The function of the MMR system has been thoroughly studied in some model organisms. In Escherichia coli, MMR is initiated when the MutS homodimer proteins bind to mismatched nucleotides on the daughter strand and forms a MutS–DNA complex (1,3). The MutS–DNA complex then interacts with the MutL homodimer proteins in an ATP-dependent manner. The interaction between the MutS and MutL complexes activates the endonuclease MutH to cleave the newly synthesized strand and initiates subsequent DNA repair events, including excision of the incorrect nucleotides and incorporation of the correct nucleotides (1,3).

Homologs of the E. coli MutS have been identified in many bacterial species (7,8). To avoid confusion with other MutS-like genes, here we call them the MutS1 genes. A second MutS homolog, MutS2, is also present in many bacterial species (7), but they are functionally different from MutS1 genes (9–12). In eukaryotes, up to seven different MutS homologs have been identified and designated as MSH1 (MutS Homolog 1) to MSH7 (Table 1). These MSH genes play different roles in MMR as well as meiotic recombination (13–17). In contrast, only limited information is available about MutS homologs in archaea (18,19). Like the MutS gene family, the MutL homologs are also present in most bacterial species and all eukaryotes examined (Table 1) (1,3).

MMR is crucial for maintaining replication fidelity and genome stability in both eukaryotes and prokaryotes. Therefore, it is of great interest to study the evolutionary history of the genes involved in this cellular process. Previous phylogenetic analyses of the MutS gene family

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indicated that the MutS family can be divided into two subfamilies: MutS1 and MutS2 (7,8). However, it was not certain whether the eukaryotic MSH4 and MSH5 genes are members of the MutS1 or MutS2 subfamilies (7,8). Therefore, the evolutionary relationships of MutS homologs are still unclear. In addition, MutS homologs in archaea and their evolutionary relationships with eukaryotic and bacterial counterparts have not been systematically studied. With regard to the MutL genes, although several preliminary phylogenetic trees have been presented (20–22), a detailed evolutionary analysis has not been reported. Therefore, it is necessary to conduct systematic analyses of these MMR genes. Taking advantage of the rapid expansion of sequence data, we searched for homologs of the two gene families from a much broader spectrum of species and systematically investigated their origins and evolutionary history in this study.

**MATERIALS AND METHODS**

**Data mining**

The *Bacillus subtilis* MutS (NP_389586) and MutL (NP_389587) protein sequences were used as queries to search for homologs against complete genome sequences of 461 bacterial and 39 archaeal species (25 May 2007 data) from the National Center for Biotechnology Information (NCBI) databases (23) by TBLASTN. All significant hits with an e-value $<e^{-10}$ were considered as potential MutS and MutL homologs. Domain structures of these potential MutS and MutL homologs were analyzed by searching the Pfam (24) and SMART (25) protein domain databases. Multiple sequence alignments of the MutS or MutL homologs, respectively, were generated for sequence comparison and identification of conserved regions by using MUSCLE version 3.52 (26) (see subsequently). Preliminary neighbor joining (NJ) trees were constructed using MEGA 4.0 (27) to identify major subgroups in these two gene families. Protein sequences from each subgroup were used as queries for the second round search of homologous sequences against protein and genome database of NCBI and JGI (Joint Genome Institute) by using BLASTP and TBLASTN with an e-value $<e^{-10}$ as cutoff. Human MutS and MutL protein sequences were used as queries for searching eukaryotic MutS and MutL homologs from representative eukaryotes by using BLASTP or TBLASTN against the NCBI databases with an e-value $<e^{-10}$ as cutoff. For the following species, common names are shown in figures: Arabidopsis, *Arabidopsis thaliana*; beetle, *Tribolium castaneum*; budding yeast, *Saccharomyces cerevisiae*; chicken, *Gallus gallus*; frog, *Xenopus laevis*; fission yeast, *Schizosaccharomyces pombe*; fruitfly, *Drosophila melanogaster*; humans, *Homo sapiens*; mosquito, *Anopheles gambiense*; rice, *Oryza sativa japonica*; sea urchin, *Strongylocentrotus purpuratus*; and zebrafish, *Danio rerio*.

**Sequence alignment**

Preliminary multiple sequence alignments of all putative MutS and MutL homologs were carried out using MUSCLE version 3.52 with default parameter settings (26). According to NJ trees based on preliminary alignments, we divided MutS and MutL gene families into several major subgroups. A second round of multiple sequence alignments on each subgroup was performed using MUSCLE. These alignments were subsequently inspected and corrected manually by using GeneDoc version 2.6.002 (28). The improved alignments were then combined by using the profile alignment mode of CLUSTALX 1.81 (29).

**Phylogenic analysis**

Because the MutS and MutL homologs from all living organisms have diverged over vast evolutionary distances, synonymous nucleotide substitutions are likely saturated

### Table 1. Known and newly identified MutS and MutL homologs

| Bacteria | Archaea | Eukaryotes |
|----------|---------|------------|
| MutS family genes | MutS1 (MutS) | MutS1 (MutS) |
| | | |
| | MutS2 | MutS3 |
| | | MutS4 |
| MutL family genes | MutL | MutL (MutL?) |
| | | |
| | MLH1 | MLH1 |
| | MLH2 (PMS1) | MLH2 |
| | MLH3 | MLH3 |
| | MLH4 (PMS2) | MLH4 (PMS1) |

The gene names without parentheses are used in this study. Their corresponding old names, if different, are shown in parentheses. The newly identified genes are depicted with an underline.
and DNA sequence analysis would be quite noisy in constructing phylogenetic trees (30). Therefore, we used protein sequences rather than nucleotide sequences in this study. NJ trees were constructed by using MEGA 4.0 (27) and maximum likelihood (ML) trees were constructed by using PHYML version 2.4 (31). The reliability of internal branches for NJ trees was assessed with 1000 bootstrap pseudoreplicates using ‘pairwise deletion option’ of amino acid sequences with Poisson correction (unless indicated otherwise). ML trees were generated in PHYML with 100 replicates of nonparametric bootstrap analysis. The discrete gamma model was used in ML analysis and Gamma shape parameters alpha and proportion of invariable sites were estimated from the data. The JTT (Jones, Taylor & Thornton) amino acid substitution model was used in ML analysis. The ML trees were also inferred by quartet puzzling method for reference (trees are available upon request) (32). Only NJ trees are presented and bootstrap values from both NJ and ML methods are shown on the NJ trees because the two methods yielded very similar tree topologies.

RESULTS AND DISCUSSIONS

Presence of four MutS subfamilies in bacterial species

Only two MutS subfamilies, MutS1 and MutS2, were identified in previous studies (7,8). In contrast, we found at least four different MutS subfamilies in bacterial genomes (Table 2, Figure 2 and Supplementary Table S1). The two newly identified bacterial MutS subfamilies were designated as MutS3 and MutS4. The MutS1 homologs are present in 86% of bacterial species examined in this study, suggesting that the MMR system is widespread in bacteria (Table 2 and Supplementary Table S1). MutS1 proteins contain four conserved domains that are designated as MutS-I, MutS-II, MutSd and MutSac (Figure 1) (33–36). The MutSac domain is the most conserved domain and plays crucial roles in MMR, including dimerization, ATPase and DNA-binding activities (34). The MutS2 homologs were also found in many bacterial species (36% of bacterial species examined, Table 2 and Supplementary Table S1). Interestingly, MutS2 homologs are usually present in these MutS1-containing species except the ε-Proteobacteria (Table 2). MutS2 proteins lack the MutS-I and MutS-II domains, but share significant similarity with MutS1 proteins in the MutSd and MutSac domains (Figure 1). Furthermore, MutS2 proteins contain an extra ~250 amino acid C-terminal region, which contains a ~90 amino acid conserved domain called SMR (Small MutS Related) (37).

The newly identified MutS3 genes were found only in a limited number of distantly related bacterial species. Many of these species contain two copies of MutS3, denoted as MutS3A and MutS3B (Table 2 and Supplementary Table S1). The MutS3A and MutS3B genes from various bacterial species form two separate clades, suggesting that

| Group          | Representative species | Numbera | MutL | MutS1 | MutS2 | MutS3 | MutS4 |
|----------------|------------------------|---------|------|-------|-------|-------|-------|
| Firmicutes     | Bacillus subtilis      | 83      | +    | +     | +     | +     | +     |
|                | Staphylococcus aureus  | 8       | +    | +     | +     | +     |       |
|                | Thermoanaerobacter tengcongensis | 1 | +    | +     | +     | +     | +     |
|                | Mycoplasma hyopneumoniae | 16     |      |       |       |       |       |
| Proteobacteria | Escherichia coli       | 223     | +    |       |       |       |       |
|                | Geobacter metallireducens | 7     | +    | +     | +     |       |       |
|                | Myxococcus xanthus      | 3       | +    | +     | +     |       |       |
|                | Helicobacter pylori     | 11      |      |       |       |       |       |
| Cyanobacteria  | Synechococcus sp.       | 23      | +    |       |       |       |       |
|                | Gloeobacter violaceus   | 1       | +    | +     | +     |       |       |
| Others         | Cytophaga butchinsonii  | 18      | +    |       |       |       |       |
|                | Chlorobium tepidum      | 19      | +    | +     |       |       |       |
|                | Mycobacterium sp.       | 37      |      |       |       |       |       |
|                | Polaribacter igensii    | 6       | +    | +     | +     |       |       |
|                | Rhodopirellula baltica  | 1       | +    | +     |       |       |       |
|                | Chloroflexus aurantiacus| 4       | +    | +     | +     |       |       |

*+ + indicates the gene is present and blank means that the gene is not present.

aThe number of species that has the pattern of gene distribution.
they were produced by duplication before the divergence of major bacterial groups, and have been lost in most of the bacterial species subsequently (Figure 2). Given the fact that MutS3 genes are present in limited bacterial species, it is also possible that MutS3 might have originated in a specific bacterial lineage and then spread to distantly related bacteria by horizontal gene transfer (HGT, defined as transfer between different species).
Because MutS3A and MutS3B are not closely linked, the HGT hypothesis would involve separate transfers of MutS3A and MutSB to multiple different lineages and more evidence is needed to support the latter scenario. All deduced MutS3 proteins contain the MutSac domain near the C-terminus and some of them also have the MutSd domain (Figure 1).

Members of the fourth bacterial MutS subfamily, MutS4, are also encoding MutSac domain-containing proteins (Figure 1). MutS4 gene were only detected in five distantly related bacterial species and four of them contain two copies, MutS4A and MutS4B (Table 2, Supplementary Table S1). Like the MutS3 genes, the two MutS4 genes could also be generated by duplication in the ancestral bacteria and lost in most bacterial species (Figure 2). Alternatively, HGT of MutS4A and MutS4B between bacterial species is also possible. In these bacterial genomes, the two MutS4 genes are adjacent and the stop codon of MutS4A overlaps with the initiation codon of MutS4B. The conserved gene organization suggests that the two MutS4 genes could be produced by tandem duplication in one lineage, followed by HGT to other distantly related bacteria.

Although the biological functions of MutS3 and MutS4 genes have not been studied, the presence of the MutSac domain in these proteins suggests that they might be involved in DNA metabolism in these species. However, their absence from most bacteria suggests that since they are not essential, they gradually became diversified or lost during evolution. Furthermore, presence of two duplicate MutS3 or MutS4 genes might have accelerated their diversification in some species, as supported by the relatively long branches associated with the duplicates. This could partially explain why they are not as conserved as the MutS1 genes.

### Presence and origins of MutS homologs in archaeal genomes

The MutS1 orthologs were only detected from nine archaeal species, which all belong to the Phylum Euryarchaeota (Supplementary Table S1). These nine species could be classified into two groups: halophiles and methanogens. Notably, two similar MutS1 genes were present in the halophiles. The archaeal MutS1 proteins shared identical domain structure with the bacterial MutS1 proteins, suggesting that they are closely related to their bacterial counterparts. Surprisingly, according to the phylogenetic trees based on the MutSac domain (Figure 2), the archaeal MutS1 genes were nested within bacterial MutS1 genes and formed two separated groups instead of a monophyletic group. This topology was further confirmed by another phylogenetic analysis using all four domains shared by all prokaryotic MutS1 proteins (Figure 3A). One of archaeal MutS1 group, including all methanogen MutS1 genes, are closely related to MutS1 from Firmicutes, a group of Gram-positive bacteria, with strong bootstrap supports. The halophile MutS1A and MutS1B genes were likely produced by gene duplication before divergence of these halophiles, formed the other archaeal group (Figure 3A).

Considering the fact that the archaeal MutS1 genes are only present in nine species, and the phylogenetic topology is incongruent with the universal tree of life based on the small subunit rRNA (38), it is likely that HGT has occurred with MutS1 genes. The special affinity of MutS1 genes between the methanogen archaea and Firmicutes strongly supports a possible HGT from Firmicutes to the methanogens. However, it is unclear about the bacterial donor of the two halophile MutS1 genes due to insufficient phylogenetic evidence. The uncertain origin of the halophile MutS1 genes could be because of the sequence divergence following the duplication in ancestral halophiles and the consequent reduction of sequence similarity to their bacterial donor genes. The halophile MutS1 genes were most similar to Firmicutes MutS1 among bacterial species in BLASTP searches, suggesting a possible Firmicutes origin of halophile MutS1 genes.

We did not detect any gene that is significantly similar to the bacterial MutS2 and MutS3 genes from archaeal genomes. However, two copies of MutS4-like genes were found in each of the two closely related thermophilic archaeal species Thermoplasma volcanium and Ferroplasma acidarmanus. The two archaeal MutS4-like genes were grouped into MutS4A and MutS4B subgroup, respectively (Figure 2). Therefore, each of the MutS4A and MutS4B groups contains both bacterial and archaeal members, suggesting a possible duplication event prior to the divergence of bacteria and archaea. Alternatively, the archaeal species might have acquired the MutS4 genes from bacteria through HGT or vice versa, because archaeal MutS4A and MutS4B are also neighboring genes similar to their bacterial counterparts. The highly similar gene organization between bacterial and archaeal species strongly suggests HGT between them. Because the four MutS4-containing bacterial species are distributed in distantly related taxonomic groups, the MutS4 genes should exist in bacteria prior to their divergence, if we do not consider HGT in this case. In contrast, the two MutS4-containing archaeal species are taxonomically closely related, suggesting a more recent origin of MutS4. Therefore, HGT of MutS4 from bacteria to archaea is more favored under the parsimonious assumption. However, since the distance between the two archaeal species are similar to or larger than that of bacteria, the opposite scenario is also possible if these genes have evolved with similar rates.

In addition to the MutS1 and MutS4 genes, a novel type of MutS genes were identified in 14 archaeal species and designated here as the MutS5 subfamily. The deduced archaeal MutS5 proteins share significant similarity with other MutS-like proteins in the MutSac domain (Figure 1). The Pyrococcus furiosus MutS5 gene was previously regarded as a MutS2-like gene (19). PfMutS5 encodes a protein possessing thermostable ATPase and nonspecific DNA-binding activities, but no detectable mismatch-specific DNA-binding activity, suggesting that the MutS5 genes might be involved in other DNA metabolic activities in archaea. The MutS5 genes formed a separate clade in the tree shown in Figure 2, suggesting that MutS5 genes have diverged from other MutS-like genes during early cellular evolution.
Origin and evolution of eukaryotic MutS1-like genes

In eukaryotes, only MutS1 and MutS2 orthologs were detected (Figure 2 and Supplementary Table S2). The eukaryotic MutS1-like genes include MMR genes MSH1, MSH2, MSH3, MSH6, as well as nonMMR genes MSH4 and MSH5. MSH4 and MSH5 are the most divergent eukaryotic MutS1 genes in terms of both functions and sequences and thus were classified as MutS2 genes in a previous study (7). Our phylogenetic analysis indicated that all MSH genes, including MSH4 and MSH5, were grouped within the MutS1 subfamily by both NJ and ML methods with strong bootstrap supports (98 and 86%, respectively). The MSH4 and MSH5 genes were located at the most basal position in the MutS1 subfamily (Figure 2), probably because of long-branch attraction. Without a third group, the MSH4 and MSH5 could be mistakenly considered to be closely related to the MutS2 genes, and this problem was resolved in this study when the other three MutS subfamilies were included.

Because the phylogenetic tree shown in Figure 2 was reconstructed based on the ~200 amino acid MutSac domain that is the only domain shared by all MutS proteins, the sequence information could be insufficient to resolve interior branches of the MutS1 subfamily. To gain further understanding about the origin and evolution of eukaryotic MutS1-like genes, additional phylogenetic analysis of the MutS1 subfamily was performed. To maximize available information, a new phylogenetic tree was generated based on MutSd and MutSac domains that were shared by all MutS1 proteins (Figures 1 and 4A). As shown in Figure 4A, eukaryotic MSH genes formed six major paralogous groups (MSH1–MSH6). MSH2–MSH6 genes were found in all major eukaryotic lineages, including animals, plants, fungi and protists. MSH1 was previously found only in fungal species, but we also detected an MSH1 ortholog in the slime mold Dictyostelium discoideum. Therefore, all six MSH genes are present in multiple eukaryotic lineages, suggesting that they were generated by duplication before the divergence of major eukaryotic lineages and the MSH1 genes were likely lost in animals and plants (Figure 4A).

In addition to the six major MSH genes, a number of other MutS1-like genes have been identified in some specific lineages, such as the plant MSH1 and MSH7 genes and the coral mtMSH1 gene (39–41). The MSH7 genes were only detected in plants and are highly similar to the MSH6 genes. As shown in Figure 4A, the MSH7 genes were likely resulted from duplication of the plant MSH6 gene, consistent with a previous preliminary
phylogenetic analysis (40). Due to considerably diverged sequences of plant MSH1 (distinct from the fungal MSH1 genes) and coral mtMSH1 genes, their origins and evolutionary relationships with other MutS1 genes were not elucidated in this study (results not shown).

Notably, the fungal/protist MSH1 genes are closely related to the prokaryotic MutS1 genes (Figure 4A), suggesting that MSH1 genes were likely the most primitive eukaryotic MutS1 members. Specifically, the MSH1 genes grouped with the α-proteobacterial MutS1 in the phylogenetic tree containing prokaryotic MutS1 and MSH1 genes using all four conserved MutS1 domains (Figure 3A). The unusual affinity between eukaryotic and α-proteobacterial MutS1 homologs strongly suggests
the occurrence of HGT between them. It is widely accepted that the eukaryotic mitochondria originated from an α-proteobacterium-like endosymbiont (42). To test whether the other eukaryotic MS genes (MSH2–MSH7) originated from MSH1 or another bacterial lineage, a separate phylogenetic analysis was conducted using all four MutS domains with representative prokaryotic MutS1 genes and eukaryotic MS genes (Figure 4B). Relatively strong support was obtained for the hypothesis that the other eukaryotic MS genes were derived from MSH1, not one of the other bacterial lineages. Thus, it is reasonable to postulate that ancestral eukaryotes acquired MutS1-type genes from the α-proteobacterium-like precursor of mitochondria. This scenario is further supported by the findings that the fungal MSH1 is involved in repairing mitochondrial DNA mismatches (13). The ancestral MutS1/MSH1 gene, similar to many other organelle genes (43), was translocated from the mitochondrial genome to the nuclear chromosome during early eukaryotic evolution. Multiple gene duplication events on the ancestral eukaryotic MutS1 (MSH1) occurred, probably after its integration into nuclear genome, and produced at least six additional MSH genes.

Although the origin of eukaryotes is still controversial, it has been shown that archaea are more closely related to eukaryotes with regard to genes involved in DNA replication and repair, transcription and translation, all of which are called informational genes (44). Genome-wide comparisons between yeast, bacteria and archaea also suggested that informational genes of eukaryotes were derived almost exclusively from archaea (45). For example, among DNA repair genes, the eukaryotic recombinational repair gene RAD51 is more closely related to the archaean RADA than to the bacterial recA (46). Remarkably, as another major group of DNA repair genes, the eukaryotic MutS1 (MSH) genes apparently originated from bacteria, instead of archaea. Therefore, our study provides a prominent example for an alternative origin for eukaryotic informational genes.

Functional diversification of MS genes and implication on eukaryotic evolution

As described above, the eukaryotic MHS genes experienced multiple gene duplication events (Figure 4A). Duplicated gene copies provide extra genetic materials for functional specialization and innovation (47–49). In E. coli, the MutS1 proteins form asymmetric homodimers for DNA repair, suggesting that the two subunits play non-identical roles in MMR (35,36). In eukaryotes, different types of DNA mismatches are repaired by two different heterodimers, MSH2/MSH3 and MSH2/MSH6, instead of asymmetric homodimers (3). Therefore, the expansion of the MutS1 subfamily in eukaryotes probably allowed functional specialization of the duplicated MSH genes in two ways. First, the asymmetric MutS1 homodimer was replaced by MSH heterodimers, allowing additional freedom to specialize in each subunit of the heterodimers. Second, the MutS1 homodimer was replaced by two different heterodimers, making it possible for each heterodimer to evolve functionally to repair specific type of DNA errors. The duplication and subsequent functional divergence might have enhanced the efficiency of MMR in eukaryotes.

Furthermore, MSH4 and MSH5 are not required for MMR, but are indispensable for stabilizing heteroduplex formation between nonidentical homologous sequences during meiotic recombination (50,51). The emergence of MSH4 and MSH5 might have facilitated the evolution of meiosis by allowing the interaction of homologous, yet mismatched, sequences. Therefore, the specialization and innovation of MSH gene functions could have contributed to evolution of MMR and meiosis, which are critical for the evolutionary success of eukaryotes.

Interestingly, the evolution of eukaryotic MSH genes is similar to that of the recombinational repair gene RAD51 in several regards (46). First, both gene families have experienced multiple gene duplication in the ancestral eukaryote. Second, each duplicate gene has been maintained as single copy over vast evolutionary distances after the divergence of major lineages of eukaryotes, suggesting a very strong selection for a single copy. Third, meiosis-specific genes were generated in both the gene families. These similarities suggest that a certain class of eukaryotic multiple-gene families, which are important for DNA metabolism, might have evolved through similar mechanism(s).

Origin and evolution of plant MutS2 homologs

The eukaryotic MutS2 genes are only found in chloroplast-containing species, such as plants and green algae (Supplementary Table S2). We detected two copies of MutS2-like genes in each of the nuclear genomes of the flowering plants Arabidopsis thaliana and rice (Oryza sativa japonica), and three copies of MutS2-like genes from the genome of the moss Physcomitrella patens. Phylogenetic trees of MutS2 subfamily show that all eukaryotic MutS2 genes, except for the moss MutS2C gene, formed a well-supported clade and were most closely related to the cyanobacterial MutS2 (Figure 5). It is well

Figure 5. A phylogenetic tree of MutS2 subfamily from representative species showing horizontal gene transfer between bacteria and plants. Phylogenetic trees were constructed using full-length MutS2 protein sequences. The methods used in tree reconstruction and percent bootstrap values are given as in Figure 2.
accepted that the plant chloroplasts were derived from an ancestral endosymbiont related to cyanobacteria (42). Therefore, the eukaryotic MutS2 gene was apparently transferred from the ancestral chloroplast genome to the nuclear genome and it also explains the presence of eukaryotic MutS2-like genes only in chloroplast-contain-
ing species. Furthermore, the MutS2 gene was duplicated before the divergence of land plants and green algae, producing two similar paralogs, MutS2A and MutS2B (Figure 5).

In addition to the HGT from cyanobacteria to plants, a separate HGT event might have occurred from Firmicutes to Physcomitrella, resulting in the presence of MutS2C in its genome (Figure 5). An intron is present in the MutS2C genomic sequence, so it is not likely that MutS2C is a microbial contaminant. Although HGT between Firmicutes to moss is unusual, it is not unique to the MutS2C gene, because a similar HGT event has been reported for the MIP gene (52). It is not clear how HGT occurred from Firmicutes to the moss and what biological roles MutS2C plays, but it is worthwhile to postulate that HGT could occur more frequently and play more important roles than previously recognized during the evolution of multicellular organisms.

A model for the early evolution of the MutS gene family

The evolutionary history of each MutS subfamily was elucidated after detailed analyses of their distributions and phylogenies. One of the key points is that HGT events apparently have frequently occurred in several MutS1 subfamilies from bacteria to eukaryotes and/or archaea. These included separate events for MutS1 from bacteria to eukaryotes and archaea, and MutS2 from bacteria to plant and algae via the endosymbiosis of the chloroplast. Furthermore, the MutS3 and MutS4 genes might also be transferred between different lineages of bacteria or between bacteria and archaea. MMR genes (MutS1 and MutL) have been suggested to be frequently transferred between different strains of E. coli (53,54). Such frequent HGT between closely related bacteria could also serve as an alternative to gene duplication in the production of new copies in a gene family. Our study further indicates that HGT of MutS family genes also have frequently occurred between distant related species through various pathways. The MutS1 gene was involved in preventing homologous recombination between divergent sequences (1). As a consequence, we could expect elevated recombination rates in those lost of MutS1 prokaryotic species, consistent with the observation that many E. coli populations experience frequent losses and reacquisitions of MMR genes (53). We could speculate that certain archaea lineages reacquired MutS1 from bacteria after the loss of MutS1 in anarchaeal archaea. Similarly, there could also be HGT of MutS1 between distantly related bacteria groups. Nevertheless, the possible HGT of MutS1 between different bacteria lineages would have no impact on our results and discussion about the origins of eukaryotic and archaeal MutS1 genes.

If we assume that the MutS4 genes were transferred from bacteria to archaea and then exclude genes produced by HGT from the phylogenetic tree shown in Figure 2, it can be significantly simplified as a tree including four bacterium-specific groups (MutS1-4) and one archaea-specific group (MutS5). Because each of MutS1-4 subfamilies is present in divergent bacterial groups, it is reasonable to postulate that they were produced by several gene duplication events before the divergence of bacteria. However, it is difficult to determine the evolutionary relationships among the five subfamilies without knowing the true root of the phylogenetic tree. Theoretically, the root could be designated at any point between two major sister clades. Among these possibilities, the most parsimonious scenario is to root the tree between MutS5 and the joint clade of the other four subfamilies. According to this hypothesis, the ancestral MutS gene was present in the common ancestor of bacteria and archaea. Since the split of bacteria and archaea, the MutS gene evolved differently in the two groups (Figure 6). The ancestral bacteria MutS were duplicated and produced four subfamilies (MutS1-4). In contrast, the ancestral archaeal MutS gene was maintained as single copy (MutS5). MutS5 was lost in most archaeal species possibly due to appearance of new mismatch repair gene (55) or acquisition of MMR from bacteria (this study). As a result of the fading of the archaeal MutS5 gene, the eukaryotes, which are believed to share a last common ancestor with archaea, did not inherit the MutS1 gene from an archaea-like ancestor, but from their bacterial endosymbionts.

In addition, other scenarios are also theoretically possible. One of the hypotheses is to locate the root between MutS1 and the other four subfamilies. In this case, if the gene duplication generating the MutS1 and the ancestor of the other four subfamilies occurred before the divergence of archaea and bacteria, multiple gene loss events of MutS1-3 should have occurred in archaea. If the gene duplication occurred after the divergence of archaea

Figure 6. A model of the evolutionary history of the MutS gene family. Multiple gene duplication events have occurred on the ancestral MutS gene before the divergence of bacteria, producing four lineages, MutS1, MutS2, MutS3 and MutS4. The ancestral MutS gene evolved to MutS5 gene in archaea. Eukaryotic MutS1 homologs were acquired from ancient 2-proteobacteria through endosymbiosis of mitochondria. The archaeal MutS1 genes were likely originated from Firmicutes by HGT. The plant and green algae MutS2 genes were obtained from ancient cyanobacteria by endosymbiosis of chloroplasts. Some archaeal species acquired the MutS4 gene from bacteria by HGT.
and bacteria, it means that the MutS genes were present only in ancestral bacteria and all archaeal MutS genes, including MutS5, were acquired by HGT from bacteria. However, the MutS5 genes do not share significant affinity to any of the bacterial MutS genes; therefore, it is unreasonable to propose that MutS5 originated from a specific subfamily of bacterial MutS genes by HGT. Although other rooting possibilities cannot be ruled out, the first scenario is most favored according to the current data. Furthermore, the position of the root does not affect our major conclusions that the MutS family has five subfamilies and multiple HGT have occurred from bacteria to eukaryotes and archaea.

Evolution of the MutL gene family

Our searches of MutL homologs uncovered a most intriguing result that they are only present strictly in MutS1-containing species and vice versa (Table 2 and Supplementary Tables S1 and S2). All MutL proteins share two highly conserved domains, the HATPase and DNA mismatch repair domains (Supplementary Figure S1). Therefore, the phylogeny of the MutL gene family was reconstructed based on these two domains (Figure 4C). As shown in Figure 4C, the eukaryotic MutL homologs formed four well-supported clades, and archaeal and bacterial MutL genes formed the fifth clade. Three of the eukaryotic subgroups contain sequences from fungi, plants and animals, indicating that the four eukaryotic MutL homologs were generated by gene duplication prior to the divergence of major eukaryotic lineages.

Our phylogenetic analysis showed that plant and fungal PMS1 genes are grouped with animal PMS2, indicating that plant and fungal PMS1 genes are the orthologs of the animal PMS2, rather than the animal PMS1 genes. This is consistent with previous functional studies on these genes (21,56). In addition, another clade contains the fungal MLH2 and animal PMS1 genes. To avoid confusion between gene names and orthologous relationships, we designated the group with the fungal MLH2 and animal PMS1 genes as the MLH2 group, and the group with plant and fungal PMS1 and animal PMS2 genes as the MLH4 group (Figure 4C). The MLH2 genes can only be identified in vertebrate animals and some fungal species, indicating that their orthologs were lost in many eukaryotic organisms.

Like the MSH genes, the available functional data of MLH genes support the idea that the duplicated MLH genes have experienced functional specialization and innovation. For example, MLH1 and MLH4 proteins form heterodimers (MutLz) and function in MMR during the mitotic cycle, analogous to the prokaryotic MutL homodimers (57,58). Furthermore, the MutLz are also required for MMR of the heteroduplex formed by the meiotic recombination (59,60), indicating that the MutLz have acquired new meiotic roles during evolution. In addition, MLH3 is important for meiotic recombination, particularly the formation of double Holliday junction (61). In summary, the generation and functional diversification of multiple eukaryotic MLH genes might also have contributed significantly to the evolution of eukaryotes and meiosis, similar to their functional partner MutS1 genes.

Similar to the archaeal MutS1 genes, archaeal MutL genes did not form a monophyletic clade (Figure 3B). The MutL genes from archaeal methanogens were grouped with Firmicutes with strong bootstrap supports, indicating that methanogen MutL genes might also have originated from Firmicutes by HGT. In addition, halophile MutL genes form the other archaeal MutL clade and were nested in the bacterial MutL groups. Therefore, it is likely that halophile MutL were acquired from bacteria through HGT, although we were unable to infer their bacterial donor based on current data. Therefore, all MutL genes present in archaea were likely transferred from bacteria, suggesting that the MutL genes were not present or were lost in ancestral archaea. As a consequence, the eukaryotic MLH genes were apparently originated from bacteria, but not archaea. It is reasonable to postulate that the first eukaryotic MutL homolog was transferred from x-proteobacterium-like endosymbionts along with the MutS1 gene, although mitochondria-targeted MutL homologs were not detected in eukaryotes. One explanation is that the mitochondria-targeted MutL homologs have been lost in eukaryotes, consistent with the observation that the mitochondria-targeted MSH1 genes have been lost in most eukaryotes.

Co-transfer of MutS1 and MutL genes from bacteria to archaea

To further elucidate the origin of archaeal MutS1 and MutL genes, their genomic locations were compared between bacteria and archaea. The positions of MutS1 and MutL genes were obtained for each species from NCBI genomic database. In most groups of bacteria, the two genes are distantly located, separating by at least 10000 nucleotides (Figure 7). In contrast, MutS1 and MutL are neighboring genes in most Firmicutes. Coincidentally, the physical proximity of MutS1 and MutL genes was also found in the methanogens of archaea (Figure 7). Our phylogenetic studies showed that MutS1 and MutL genes in methanogens were likely acquired from Firmicutes by HGT. The conservation of the unusual neighboring MutS1 and MutL in both Firmicutes and archaea not only supports the HGT from Firmicutes to archaea, but also suggests that ancient methanogens acquired both MutS1 and MutL genes via a single HGT event. In halophile archauna, one of MutS1 genes, MutS1B, is also closely linked to MutL. Considering that the best hits of halophilic MutS1 and MutL are from Firmicutes in bacteria, and halophile MutS1B and MutL are also neighboring genes, we postulate that halophile MutS1 and MutL were also simultaneously transferred from Firmicutes, although this hypothesis lacks support from phylogenetic analysis (Figure 3).

Our analysis indicated that MutS1 and MutL are absent in most archaea species. The mutation rates of genomes could significantly increase without an efficient repair mechanism, especially for those archaea that live in harsh environments. However, the mutation rates are not
enhanced in some archaeal species without the MutS1/MutL-dependent MMR pathway (62). Therefore, alternative repair pathways should exist in these archaeal species to correct replication errors. Previous genomic context analyses have shown that there is a putative DNA repair system specific for thermophilic archaea and bacteria (55). This putative repair mechanism is not fully analogous to the MMR system, so the understanding of the full extent of DNA mismatch repair pathways awaits future investigations.

Molecular co-evolution of the MMR duo

We have observed co-occurrence patterns of MutS1 and MutL homologs in cellular organisms, suggesting that a loss of one gene could subsequently lead to the loss of the other gene in a genome. Study on the origins of archaeal MutS1 and MutL genes suggests co-acquisition of these two genes from bacteria. Phylogenetic analysis further indicates that these two gene families share very similar evolution profiles. For example, both gene families have experienced multiple gene duplication events during early evolution of eukaryotes. As a result of gene duplication, different mismatch repair heterodimers and meiosis-specific proteins appeared in both the families. These observations strongly suggest that, as physically interacting duo in MMR, a heritable change in one gene could become selective force for a complementary change in the other one. Therefore, the MutS1 and MutL gene families have evolved in a correlated fashion. Co-evolution at molecular level has been commonly observed between host and parasites, and between ligands and receptors (63–67). However, it has not been reported on DNA metabolic genes to our knowledge. Our study provides a prominent example, suggesting that co-evolution might also play important roles in the gene network of DNA metabolism.

CONCLUSIONS

This study provides an overall picture of the evolutionary history of MutS and MutL gene families that play crucial roles in maintaining genome stability. We identified three new subfamilies in the MutS family, and showed that the MutS gene family has experienced many gene duplication, loss and HGT events during early evolution. Our data suggest that the archaeal MutS1 and MutL genes were originated from bacteria by HGT. The eukaryotic MutS and MutL homologs were also originated from bacteria, indicating that bacteria could be an important source for eukaryotic informational genes. The results provide direct evidence that genomes are highly dynamic, in part, because they can acquire genes from even very distant organisms. We also showed that the MutS1 and MutL genes display a pattern of strict co-presence and co-absence, indicating that they have evolved in a correlated way. Our results about the origins of MutL and MutS1 homologs of eukaryotes and archaea further support the co-evolution between the MutL and MutS1 genes during a long-term evolutionary history. In summary, our phylogenetic results have established an evolutionary foundation for future studies on the contributions of MutS and MutL genes to genome stability and the functions of the newly recognized MutS subfamilies.

SUPPLEMENTARY DATA

Supplementary Data are available at NAR Online.

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