Chapter 10

Genome Stability in *Drosophila*: Mismatch Repair and Genome Stability

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Chapter Outline

1. INTRODUCTION

Living organisms are subjected to a variety of endogenous and exogenous damages. Since DNA alterations caused by such factors are highly risky not only for individuals, but also for the continuity of species, many protective systems such as DNA repair are present to counter the effects of each damaging factor. Among these systems, mismatch repair (MMR) has been recognized to play a very important role in the preservation of genome stability. Historically, MMR was proposed to play a role in the accurate processing of genetic recombination during meiosis [1]. The importance of MMR was highlighted by the observation that MMR systems are well conserved from prokaryotes to higher eukaryotes [2,3]. To affect the repair of damaged DNA, MMR is thought to engage in crosstalk with other repair systems [4,5], and it has also been proposed that signaling cascades leading to cell cycle arrest and the induction of apoptosis might be regulated by MMR pathways depending on the damaging factors [6]. *Drosophila* also possesses a similar MMR system to *Escherichia coli* and mammals. MMR activity that is responsible for the repair of heteroduplex DNA containing mismatched base pairs has been demonstrated in extracts from cultured cells, embryos and adult flies [7]. The proteins and genes involved in MMR have been identified, and evidence that MMR systems play an important role in maintaining genome stability during both mitotic replication and meiotic recombination has accumulated.

2. MMR ACTIVITY IN *DROSOPHILA*

Holmes et al. demonstrated the strand-specific mismatch correction activity in *Drosophila Kc* cells [8]. They performed an in vitro MMR assay using nuclear extracts from cultured *Drosophila Kc* cells and human fibroblast HeLa cells. A similar MMR activity was detected in both *Kc* and HeLa cell nuclei, and the repair was almost limited to nicked strands of heteroduplex DNA containing mismatched base pairs. Mispairs were repaired with efficiencies in the order $G \cdot T > G \cdot G \simeq A \cdot C > C \cdot C$. On the other hand, Bhuki-Kaur et al. observed that MMR activity was higher in *Drosophila* tissue extracts than in HeLa cells, and that MMR activity was expressed continuously throughout the *Drosophila* life span, from the embryo to the adult fly [7]. They prepared cell extracts from wild-type Oregon-R embryos (after 0–18 h oviposition), young adults (4–5 days after eclosion), and aged senescent adults (35 days after eclosion) and measured the repair activity of each extract according to the methods described by Thomas et al. [9]. Heteroduplex DNA containing mismatched base pairs, and 1 and 5 bp loops were prepared from a replicative form of bacteriophage M13mp2. Following the incubation of the heteroduplex phage DNA with *Drosophila* tissue extracts, heteroduplex phage DNA was transfected into competent *E. coli* in an effort

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to identify which strand was repaired as determined by the observation of plaque phenotypes. It was discovered that MMR activity was constantly present, from the embryo to the adult fly, and it was at higher levels than in Hela cells. T·G and G·G mispairs were efficiently repaired in a nick-dependent manner consistent with the findings of a previous report [7], whereas the repair of A·A, C·C, C·T, T·T, C·A, G·A, and A·G mispairs and both loops was not nick dependent. The A·A mismatch was the most efficiently repaired, and the efficiency of repair was in the order as described earlier. *Drosophila* appears to require MMR activity throughout its life span, although the reasons are unclear. Bhui-Kaur et al. also observed that the nick-dependent repair was reduced in the extract of the *Drosophila mei-9* mutant which is defective in nucleotide excision repair (NER) in somatic cells [10] and defective in crossover during meiotic recombination [11]. These results suggested that MMR collaborates with other repair systems to complete an accurate repair in an effort to maintain genome stability and introduce the possibility that the Mei-9 protein acts as an endonuclease to incise the strand possessing misincorporated bases or loops, whereas the *mei-9* gene has been shown to encode the human XPF homolog-protein [10].

3. MMR GENES IN *DROSOPHILA*

The genes encoding proteins involved in MMR have been identified in *Drosophila*. In many eukaryotes, two sets of MMR initiation complexes, MSH2–MSH6 and MSH2–MSH3, bind to DNA lesions whose binding property corresponds to the type of the mismatch; for example, the human MSH2–MSH6 complex can bind to the mismatch region, and the MSH2–MSH3 complex binds to loops, but not to mismatched base pairs [2]. In *Drosophila*, the MSH2 ortholog is encoded by the *spellchecker1* gene referred to as *spel1* [12], and the MSH6 ortholog referred to as the *Msh6* gene was identified from the complete *Drosophila* genome sequence (reviewed in 13). The *spel1* gene is positioned at 35A4–35B1 on the left hand of the second chromosome [13], and the *Msh6* gene is at 71B6 on the left hand of the third chromosome [14]. However, in *Drosophila*, a homologous sequence of the gene encoding the MSH3 protein is absent [15]. Therefore, only the Spel1–MSH6 complex might be engaged in the recognition of mismatched heteroduplex DNA, including base–base mismatches, small loops, and possibly large loops. The presence of *E. coli* MutL orthologs is inferred from sequence homology where *Mlh1* and *Pms2* genes are positioned at 44B8 and 51F11, respectively, on the right hand of the second chromosome [16,17]. In *Drosophila*, a gene encoding the *E. coli* MutH homologous protein has not been identified as it has been found in other eukaryotes. However, it is estimated that two nucleases, *tos* and *mei-9* gene encoding products, might play important roles in MMR. The sequence analysis has revealed that the *tos* gene encodes a protein referred to as TOSCA which is highly related to the Exo1 protein and is a double-stranded DNA 5′–3′ exodeoxyribonuclease specifically induced in meiotic prophase I in *Schizosaccharomyces pombe*, and it is a member of the RAD2 protein family that plays a role in NER [18]. TOSCA is selectively expressed in *Drosophila* developing oocytes. Therefore, the *tos* gene may play an important role in the maintenance of genome stability by repairing mismatches that may occur during replication or recombination in oogenesis [19]. Mei-9, a product of the *mei-9* gene and an ortholog of mammalian XP-F, might act as a substitute for MutH and engage its incision activity during NER as mentioned earlier. It is considered that similar to TOSCA, Mei-9 plays a role in the repair of damaged DNA during both meiosis and mitosis.

4. MMR AND MICROSATELLITE INSTABILITY

MMR systems play important roles in maintaining the high fidelity of genomic DNA by the recognition and repair of mismatched base pairs during DNA replication [20]. It is well documented that a lack of MMR increases genomic instability and the risk of certain types of cancer such as hereditary non-polyposis colorectal cancer (HNPCC) [21,22]. Microsatellite instability (MSI), a typical genomic instability, caused, for example, by frameshift mutation leads to the mutation of various target genes and can lead to the development of cancer by the inactivation of responsible genes [23]. A deficiency in MMR leads to MSI manifested by the alteration of repeat lengths not only in mammals, but also in *Drosophila*. In the MutS-deficient mutant of *Drosophila* (*spel1−/−*) constructed by Flores and Engels, the rate of MSI in long runs of dinucleotide repeats increased [12]. They observed alterations in the repeat number of microsatellites after 10–12 fly generations in *spel1*-null offspring. From the results of seven loci of microsatellites, the length of dinuleotide microsatellite loci altered with a variation of 3.1–26.5%, but it was not scored in microsatellite loci comprising trinucleotide repeats. In wild-type *Drosophila*, the mutation rate of microsatellites is averaged as 6.3×10^{-6} with 24 loci, and is lower than in several mammalians in which the rates are estimated to be in the order of 10^{-3} to 10^{-5} [24–27]. This discrepancy is considered to be due to the shorter length of microsatellites in *Drosophila* compared to mammals. The highest mutation rate was shown in the longest microsatellite region (28 repeats of the CA dinucleotide), at a similar level to the mutation rate found in mammalians (3×10^{-4}) [25]. The frequency of microsatellite alteration depends on the repeat sequence and the expression of MMR [26]. The G·T repeat sequence was subjected to the highest alteration rate in the presence of MMR, while the alteration rate of the A·T repeat sequence was higher in the absence of MMR.

In conclusion, the lack of the MutS ortholog frequently increases the mutation rates of microsatellite loci even in *Drosophila*, which is consistent with previous reports pertaining to bacteria, yeast, and mammalians. Significant changes in microsatellite length also occurred during the repair of double-strand DNA breaks in the *spel1*-null mutant, where a greater than fivefold increase in the rate of repeat length changes was observed [12].

Numerous proteins other than MMR proteins involved in DNA repair should contribute to genome stability during somatic replication and trans-generation events. Velázquez and collaborators reported that in the *Drosophila* PCNA mutant (*mus209*) germline, genomic instability is induced through MSI at a lesser extent than that in the *spel1* mutant. The rate of MSI in *mus209* was higher in heterozygotes than in homozygotes with PCNA mutation [27,28]. On the other hand, the product of the *mus201* gene, a mammalian XPG ortholog essential for the excision repair of the global genome, is not associated with the MMR process [29].

5. THE ROLE OF MMR IN MEIOTIC RECOMBINATION

The functions of genes other than the *spel1* gene involved in MMR have yet to be delineated. Since 2000, it has been revealed that MMR proteins play an important role in an accurate crossover generated through meiotic recombination in yeast [30] and mice [31]. In *Drosophila*, Radford et al. reported the involvement of *Msh6* in meiotic recombination [32]. Crossovers between homologous chromosomes are indispensable for the accurate chromosome segregation during meiosis [33] and also in *Drosophila* [34]. In an effort to understand the processes involved in meiotic recombination including DNA double-strand breaks (DSBs), crossover and chromosome segregation during meiotic cell division, many studies have been performed using crossover-defective mutants. The undesirable postmeiotic segregation occurs when heteroduplex DNA formed during meiotic recombination is not repaired correctly at the first meiotic division. In the *Drosophila Msh6* mutant [32], the frequency of postmeiotic segregation is higher compared to the wild-type and *mei-9* mutant in which the frequency of crossovers is reduced due to the lack of nicking activity of Holliday junction formed during meiotic recombination [35]. Crown et al. proposed a new meiotic recombination model for *Drosophila*. When *Drosophila* is defective in canonical and short-patch MMR (the Msh6 mutant), the XPC homolog encoded by the *mus210* gene, a damage recognition factor in NER, is involved in the repair of mismatched heteroduplex DNA together with the Mei-9 protein [36]. MMR is speculated to repair not only mismatches during replication, but also heterogeneous DNA duplexes that result during meiotic recombination.

6. MMR AND SOMATIC CELL MUTATION

DNA repair by the MMR system is best investigated in *E. coli*, and the lack of MMR increases genomic instability by generating a mutator phenotype with the increased spontaneous and induced mutation rates, as previously mentioned and also observed in our investigations [37]. In mammals, MMR deficiency is responsible for an increased cancer risk and causes HNPCC that accompanies genome instability in humans [22,23]. Although it is widely recognized that cancers can develop by the gradual accumulation of somatic cell mutations, it remains to be revealed whether MMR deficiency can affect the frequency of somatic cell mutations, including chromosomal alterations. Flores and Engels cloned a *mutS* ortholog gene from *Drosophila* referred to as *spellchecker1* (*spel1*) and constructed two lines that possess a deletion of DNA tract including the *spel1* gene at different regions [12]. When they examined the sensitivity of *spel1*-null mutant flies to methyl methanesulfonate (a methylating agent) or γ-irradiation, the *spel1*-null mutant was insensitive to such genotoxic factors, although the mutant exhibited a significant MSI without treatment with damaging factors [12]. Williams et al. in 2011 revealed that the *spel1*-null mutant was hypermutable to diepoxybutane (a crosslinking agent) [38]; in this assay, the number of tumors caused by mutation of the tumor-suppressor gene (*lats*) served as the mutation frequency. They also demonstrated that a product of the Fanconi anemia (FA)-related gene in *Drosophila* plays an important role in the repair of DNA crosslinks, although the function of the FA-related gene and Spe11 gene products appeared to be epistatic [38].

There have been no systems presently at hand to investigate somatic cell mutations directly in MMR-deficient *Drosophila*. To examine the involvement of MMR in somatic cell mutations, we have generated a new *Drosophila* strain in which the *spel1* gene is heterozygotically deleted and *mwh* genes are homozygotically mutated. We have developed a mutation assay referred to as the somatic mutation and recombination test (SMART) [39], using the newly generated flies and wild-type flies. In SMART, the recessive *mwh* gene imparts a multiple wing hair phenotype on wings when chromosomal recombination, chromosomal non-disjunction, and gene mutations are induced during somatic cell division. We examined whether genomic instability was induced in the MMR-deficient *spel1*-null flies (*spel1*−/−) generated from a cross between a newly generated strain and another existing heterozygotic *spel1* mutant according to Flores and Engels [12]. Several microsatellite sequences were analyzed by PCR using each specific primer. The results showed that even after the fifth generation, microsatellite sequences were more frequently altered in MMR-deficient flies (*spel1*−/−) than in MMR-proficient flies (*spel1*+/−), as shown in Fig. 10.1 (Miyamoto: unpublished data).

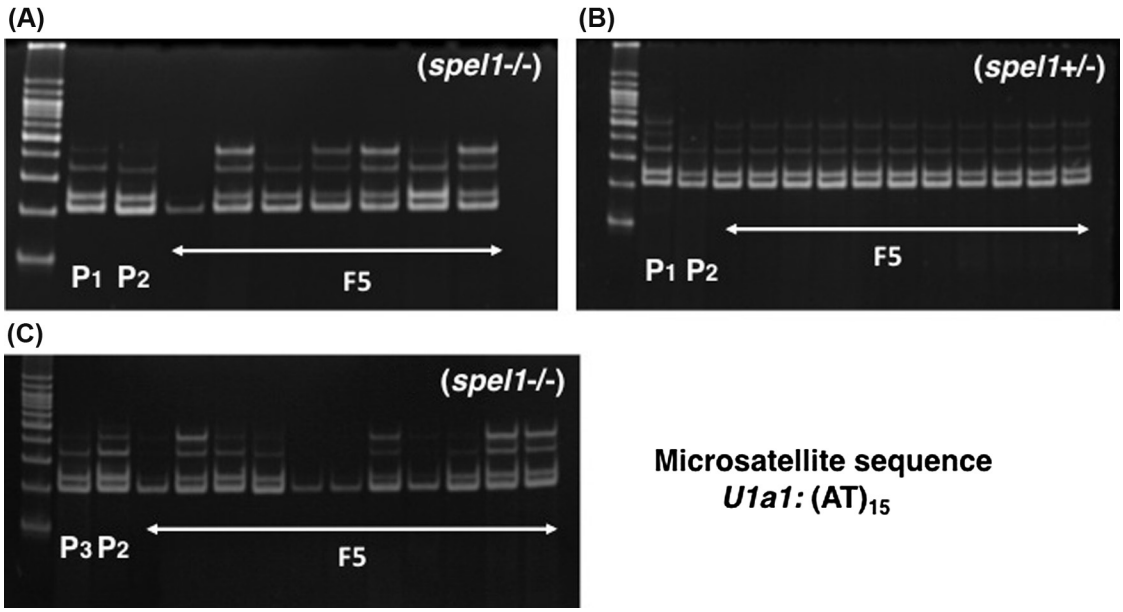

FIGURE 10.1 Alterations in microsatellite repeats detected by PCR using primers for the *U1a1* microsatellite sequence. (A) The fifth-generation flies (spel1−/−) from a cross between each existing heterozygote (*spel1*+/−) (P1 and P2). (B) Flies (*spel1*+/−) from a cross between each existing heterozygote (*spel1*+/−) (P1 and P2). (C) The fifth-generation flies (*spel1*−/−) from a cross between the existing heterozygote (*spel1*+/−) (P2) and the newly generated heterozygote (P3).

X-ray irradiation induces DNA DSBs and oxidative damage resulting in somatic cell mutations. We observed that chromosomal recombination was accounted for over 70% of mutations induced by X-ray irradiation in *Drosophila* (Toyoshima-Sasatani, unpublished data) in agreement with the previous report in which chromosomal recombination was mainly accounted for the mutation detected in SMART [39]. When we examined mutations induced by X-ray irradiation, the mutagenicity of X-rays was unexpectedly found to be lower in MMR-deficient flies than in MMR-proficient flies, as shown in Table 10.1 (Miyamoto, unpublished data).

A lack of MMR is presently thought to induce a mutator phenotype. However, *Drosophila* appears to lose its mutator phenotype since the spontaneous mutation rate remains unchanged in *spel1*-null and *spel1*+/− flies. During somatic cell division of *Drosophila*, the pairing of each homologous chromosome occurs, and daughter cells obtain the assortment of paternal and maternal chromosomes. When DNA is subjected to the damaging factors such as X-rays, chromosomal recombination occurs between maternal and paternal chromosomes paired at damaged sites or in the neighborhood through DNA strand breaks [39]. The mutagenicity in this assay is assessed according to the extent of chromosomal recombination, and the mutagenicity decreases if recombination is blocked. Our results suggested that MMR is also required for homologous recombination through strand breaks induced by DNA damage.

Alkylated DNA bases are well-known lesions that can induce mutations followed by carcinogenesis. MMR is involved in the repair of base pairs consisting of alkylated and normal bases, following the recognition of the mismatched base pair by MutS or MutS homolog proteins. The efficiency of recognition appears to be dependent on the alkyl group [40]. When we examined the mutation rate of *N*-nitrosodimethylamine (NDMA) and *N*-nitrosodiethylamine (NDEA) using the *Drosophila* wing spot test (SMART), NDMA was found to be more recombinogenic than NDEA [41]. When we performed mutation assays using the newly generated flies, the mutagenicity of NDMA was found to be significantly lower in the MMR-deficient flies (*spel1*−/−) than in the MMR-proficient flies (*spel1*+/−). The converse was observed in the case of NDEA. These results suggested that the MutS protein recognizes DNA methylated lesions more frequently than ethylated lesions even in *Drosophila*, and that the MutS homolog protein functions to induce chromosomal recombination following DNA strand breaks and gene mutations in *Drosophila*. These findings were unexpected and contrast the results of the *E. coli* mutation assays where the mutation rate of alkylating agents was markedly higher compared to wild type, as shown by many investigations including those performed in our laboratory [37]. Zhang et al. examined human fibroblast mutants and suggested that the MNNG-induced homologous recombination requires functional MMR [42]. In their experiments, the MNNG-induced recombination decreased, although the MNNG-induced gene mutations at the *hprt* gene were elevated. The elevation in gene mutations can be accounted for by considering the canonical function of MMR in which the methylated guanine or thymine residues are targets of repair, and MMR deficiency results in the absence of mismatched base-pair

TABLE 10.1 Mutagenicity of X-ray Irradiation in MMR-deficient and -Proficient *Drosophila* as Determined by the Wing Spot Test

	spel1−/−		*spel1+/−*	
X-ray Dose (Gy)	Survival (%)[a]	Mutagenicity[b]	Survival (%)[a]	Mutagenicity[b]
0	100	0.25	100	0.24
5	107	1.96[c]	96	2.67
10	130	3.58[c]	89	5.76
15	117	3.31[c]	92	8.82
20	92	5.31[c]	95	9.16

[a]*Survival (%) = the number of flies from nontreated larvae/the number of flies from irradiated larvae × 100.*
[b]*Mutagenicity is represented by the number of* mwh *mutant cell colonies per wing.*
[c]*P < .01, a significant difference from the corresponding* spel1+/− *flies.*

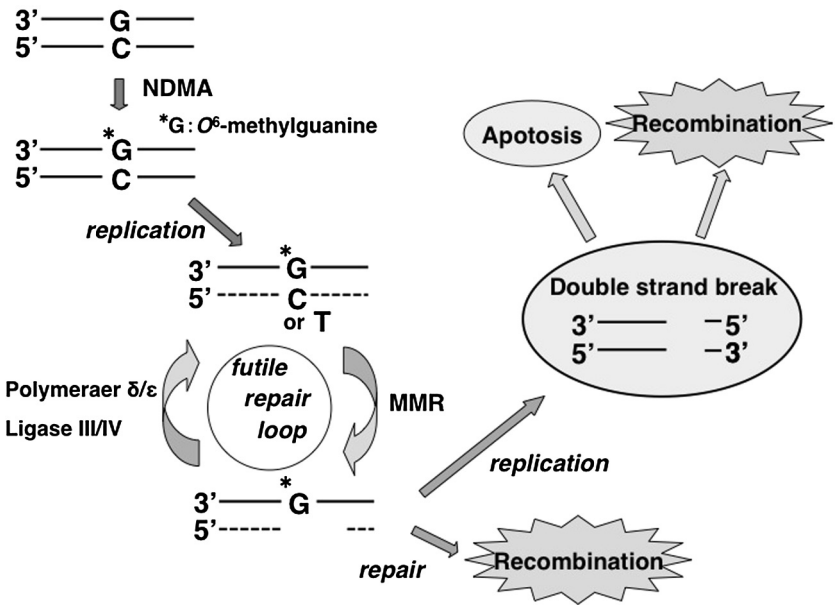

FIGURE 10.2 **Scheme outlining the involvement of MMR in chromosomal recombination induced by the methylating agent.** C or T is incorporated at the opposite site of methylated G during the first replication. Both normal bases are recognized as mismatches and removed from the newly synthesized strand. If the same event occurs next time, a so-called futile repair cycle is induced, and the strand possessing the methylated G is sustained in the single strand. This unstable state of DNA leads to double-strand breaks, and the recombination or apoptosis is then induced.

repairs, thereby leading to mutations. On the other hand, MMR can induce strand breaks during the repair process, and MMR might be required to facilitate chromosomal recombination through strand breaks. As shown in Fig. 10.2, the methyl-G·T pair is recognized as a base-pair mismatch by MutS or its homolog (Spel1·Msh6 heterodimer in *Drosophila*), and the methyl-G·C pair is also recognized as a mismatch, although at a lower frequency [40]. This repair step is known as a futile repair loop that occurs in the presence of functional MMR; its activity results in continuous strand breaks leading to recombination or apoptosis. Although further investigations are required, we speculate that the role of MMR in the somatic mutation recombination is as follows: if MMR is deficient, futile repair loops may be absent and thus are not activating chromosomal recombination. As a result, the recombination rate in somatic cells might decrease.

7. CONCLUSION

Living organisms continue efforts to maintain their genome by employing various devices. The induced and spontaneous DNA damage represents severe risks to genome stability. Therefore, organisms possess many countermeasures such as

repair systems to guard against DNA damage. Among these systems, MMR plays an indispensable role in both somatic and germ cells, and *Drosophila* is no exception. In this section, the characteristics of MMR in *Drosophila* for maintaining genome stability have been addressed. Genes involved in MMR and diverse functions are conserved in *Drosophila*, and while only one recognition complex is present in *Drosophila*, almost all other eukaryotes possess two sets of complexes. It has been revealed that MMR does not work alone but collaborates with other repair systems such as NER during meiotic and mitotic recombination. A lack of MMR induces genome instability and is generally represented by changes in microsatellite repeats. However, there is the possibility that the requirement of MMR in chromosomal recombination might facilitate mutation and chromosomal recombination in the damaged DNA.

GLOSSARY

Eclosion The emergence of an adult from the pupa.
Futile repair loop The MMR-dependent repair loop involved in the induction of strand breaks and the activation of recombination.
Oviposition Laying eggs.

LIST OF ABBREVIATIONS

FA Fanconi anemia
HNPCC Hereditary non-polyposis colorectal cancer
MMR Mismatch repair
MSI Microsatellite instability
NDEA *N*-nitrosodiethylamine
NDMA *N*-nitrosodimethylamine
NER Nucleotide excision repair
SMART Somatic mutation and recombination test

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