Recombination and the maintenance of plant organelle genome stability

Summary
Like their nuclear counterpart, the plastid and mitochondrial genomes of plants have to be faithfully replicated and repaired to ensure the normal functioning of the plant. Inability to maintain organelle genome stability results in plastid and/or mitochondrial defects, which can lead to potentially detrimental phenotypes. Fortunately, plant organelles have developed multiple strategies to maintain the integrity of their genetic material. Of particular importance among these processes is the extensive use of DNA recombination. In fact, recombination has been implicated in both the replication and the repair of organelle genomes. Revealingly, deregulation of recombination in organelles results in genomic instability, often accompanied by adverse consequences for plant fitness. The recent identification of four families of proteins that prevent aberrant recombination of organelle DNA sheds much needed mechanistic light on this important process. What comes out of these investigations is a partial portrait of the recombination surveillance machinery in which plants have co-opted some proteins of prokaryotic origin but have also evolved whole new factors to keep their organelle genomes intact. These new features presumably optimized the protection of plastid and mitochondrial genomes against the particular genotoxic stresses they face.

I. Introduction
Maintenance of genome stability is an absolute requirement for cell growth and proliferation. Failure to maintain such stability results in the accumulation of mutations and genomic rearrangements that can quickly become deleterious. For example, genomic instability characterizes the majority of human cancers (Halazonetis et al., 2008). Thus, at the...
In this regard, plants provide a unique opportunity to study genomes located in the nucleus, mitochondria and plastids. and must therefore maintain the stability of three separate organelle genomes are preserved. and nuclear genomes, and thus little is known about how the systems that regulate genome stability in bacterial research effort so far has been directed towards unravelling the systems that regulate genome stability in bacterial and nuclear genomes, and thus little is known about how organelle genomes are preserved.

Plant cells contain two types of endosymbiotic organelles and must therefore maintain the stability of three separate genomes located in the nucleus, mitochondria and plastids. In this regard, plants provide a unique opportunity to study the strategies used by organelles to protect their genomes. The organelle genomes of flowering plants do not encode any of the RRR proteins required for the maintenance of DNA (Bock, 2007; O’Brien et al., 2009). Consequently, organelles have to import these factors to ensure that their genetic material remains functional and is accurately transmitted. Until recently, none of these factors was known, but a series of reports have now identified several families of nuclear-encoded proteins implicated in the maintenance of plant organelle genome stability (Abdelnoor et al., 2003; Zaegel et al., 2006; Shedge et al., 2007; Maréchal et al., 2009). A common theme among all these protein families is their involvement in the suppression of recombination between repeated DNA sequences. Indeed, elimination or mutation of these proteins results in large-scale rearrangements of organelle genomes that eventually perturb the function of plastids and/or mitochondria. This in turn is often accompanied by a variety of striking phenotypes, ranging from variegation to cytoplasmatic male sterility (CMS), underlining the importance of the small organelle genomes for plant development and function.

In this article, we summarize current knowledge concerning the relationship between the regulation of recombination and the maintenance of genome stability in plant

---

**Box 1 Glossary**

**Chromatids**: each of the two copies of DNA that constitute a replicated chromosome.

**Crossing-over**: reciprocal exchange of distal sections of two homologous DNA molecules that accompanies gene conversion of the region proximal to the double-stranded break.

**Cytoplasmic male sterility** (CMS): a maternally inherited trait that renders plants unable to produce viable pollen. CMS is frequently associated with rearrangements of the mitochondrial genome.

**Gene conversion**: nonreciprocal transfer of information from one DNA sequence to another. The damaged recipient sequence is converted to its intact donor homologue. Gene conversion events can be associated with crossing-over or not.

**Head-tail concatemers**: DNA concatemers contain multiple successive copies of the same sequence. In a head-tail concatemer, one end of a copy (the head) is linked to the opposite end of the following copy (the tail).

**Holliday junction**: cross-shaped mobile junction between four different strands of DNA.

**Homologous recombination** (HR): conservative repair process based on the exchange of genetic information between two quasi-identical DNA sequences.

**Homeologous**: homeologous DNA sequences are only partially homologous.

**Hypomorphic mutation**: Type of mutation where the modified gene product has a reduced activity.

**Loss of heterozygosity** (LOH): loss of an allelic difference between two homologous chromosomes. Following LOH, the cell becomes homozygous for a given allele or series of alleles.

**Microhomology**: region of homology shorter than 30 bp.

**Nonchromosomal stripe** (NCS): maternally inherited phenotype in which maize (*Zea mays*) plants exhibit poor growth, aberrant morphology and leaf striping as a result of chloroplast defects.

**Recombination**: formation of new alleles or combination of genes through any mechanism.

**Substoichiometric shifting** (SSS): rapid changes in the stoichiometry of rearranged DNA molecules present in plant mitochondrial genomes. These molecules can be either dramatically amplified or suppressed.

**Unequal crossing-over** (UCO): crossing-over between improperly aligned chromosomes. UCO results in nonreciprocal exchange of genetic material and generates chromosomes of different lengths.

**Variegation**: the presence of differently coloured sectors on various parts of the plant, usually the leaves or the stems. Variegation is often caused by a perturbation of chlorophyll biosynthesis by chloroplasts.
organelles. We discuss the roles of recombination-based mechanisms in the replication, repair and evolution of plant organelle DNA and also present the different protein families that modulate recombination in plastid and mitochondria genomes. This review shows that, while the basic recombination mechanisms are broadly conserved among nuclear, bacterial and organelle genomes, the recombination surveillance machinery of plant organelles comprises newly evolved protein families specific to plants working side by side with conserved factors of prokaryotic origin. Studying the interplay between these different proteins will surely yield surprising new insights into the maintenance of genome stability.

II. Roles of recombination in organelle genome stability

1. Homology-dependent DNA repair pathways

The capacity of cells to repair damage incurred by their DNA is a significant aspect of genome stability. In all organisms, lesions can be fixed by either conservative or error-prone repair pathways. The former accurately repairs damaged DNA while the latter can result in slight to substantial modifications of the genome (see Kimura & Sakaguchi (2006) for a comprehensive review of these processes in plants).

Homologous recombination (HR) is an important and evolutionarily conserved error-free homology-dependent DNA repair process used to eliminate potentially harmful lesions, particularly double-stranded breaks (DSBs). This type of lesion can result from the exposure of cells to a variety of genotoxic stresses including ionizing radiation, chemical agents and endonucleolytic digestion. DSBs are traditionally envisioned as double-ended breaks arising from a fracture in the duplex. However, recent research has shown that a significant portion of these lesions are actually single-ended DSBs produced when replication forks encounter single-stranded breaks in DNA, or as a consequence of replication fork stalling/collapse at particular base modifications or DNA sequences (Wyman & Kanaar, 2006). Homology-directed repair of these replication fork breakdowns and double-ended DSBs is critical for cell viability and relies on the exchange of genetic information between two DNA molecules that share a substantial length of quasi-identical sequence (Cox et al., 2000) (at least 50 to 100 bp of homology is required; Singer et al., 1982; Watt et al., 1985). During this process, the damaged recipient molecule obtains information to guide its repair from an intact donor duplex.

Most of what is known about HR is derived from observations made in bacteria and yeast models. Still, studies using more complex organisms have demonstrated that the fundamental mechanisms underlying this type of recombination are highly conserved (Pâques & Haber, 1999; Persky & Lovett, 2008). Multiple different mechanisms of HR that promote accurate DNA repair have been inferred from experimental results using a variety of reporter systems to monitor recombination (Fig. 1). The double-stranded break repair (DSBR) model, also known as the double Holliday junction model, can fix DNA by gene conversion that can be coupled with crossing-over (Szostak et al., 1983). Crossing-over during meiosis is an important source of genetic diversity and is well explained by the DSBR model although some species actually use a single Holliday junction to carry out meiotic crossing-over (Cromie et al., 2006). However, when crossing-over occurs between homologous chromosomes in somatic cells, it can lead to loss of heterozygosity if chromatids carrying the same alleles segregate together during mitosis, and this can have devastating consequences (Stark & Jasin, 2003). Moreover, if crossing-over occurs between nonallelic repeated sequences (unequal crossing-over), it can duplicate or delete the DNA located between the repeats, thereby producing genome rearrangements and effectively promoting genomic instability. Accordingly, in all organisms tested, mitotic recombination is not frequently associated with crossing-over. To explain this bias, variants of another model called synthesis-dependent strand annealing (SDSA) were postulated. In this type of HR, gene conversion occurs without any crossing-over (Nasmyth, 1982; Thaler & Stahl, 1988). Finally, because the late stages of DSBR and SDSA rely on the capture of the second end of the DSB, these models cannot readily explain the repair of single-ended DSBs (Fig. 1a). Fortunately, these frequently occurring lesions can be fixed by the break-induced replication (BIR) pathway of HR, also known as recombination-dependent replication (RDR). In contrast to the other two HR mechanisms, in which DNA polymerization occurs only on leading strands, BIR/RDR requires the formation of a full replication fork capable of both leading and lagging strand synthesis (Llorente et al., 2008). This HR pathway thus results in the repair of broken replication forks through gene conversion associated with nonreciprocal crossing-over (Fig. 1b). All of the different pathways of HR apparently coexist in a sort of hierarchical competition, which provides organisms with a flexible homology-directed DNA repair machinery that confers resistance to a wide variety of genotoxic stresses and maximizes genomic stability.

2. Homology-dependent DNA repair in plant organelles

Although knowledge concerning homology-dependent DNA repair has been mainly acquired through the study of nuclear and prokaryotic genomes, there is now ample evidence for an active system of recombination in both plant organelles and the mitochondria of several other taxa...
In the plastids of most flowering plants, a frequent and easily observable flip-flop recombination event occurs between the large inverted repeats and gives rise to two equimolar plastome isoforms which differ in the orientation of the two single copy regions (Palmer, 1983). This provides evidence (reviewed in Barr et al., 2005; Kmiec et al., 2006). In the plastids of most flowering plants, a frequent and easily observable flip-flop recombination event occurs between

---

**Fig. 1** Mechanisms of homologous recombination. Solid lines correspond to single strands of DNA, small arrowheads represent elongating/invading 3’-ends and dotted lines represent new DNA synthesis. Solid pointed arrowheads indicate single-stranded breaks. The damaged recipient duplex is coloured in blue whereas the intact donor duplex is shown in red. (a) Double-ended double-stranded breaks (DSBs) can be fixed by double-stranded break repair (DSBR) or synthesis-dependent strand annealing (SDSA) pathways. During DSBR, both 5’-ends of the DSB on the damaged recipient duplex are first resected to yield 3’ single-stranded overhangs. A free 3’-end can then invade a donor duplex at a homologous sequence, forming a D-loop. DNA synthesis can now proceed from the 3’-end past the position of the original DSB. The displaced donor strand can then anneal to the other side of the break on the recipient DNA molecule. DNA polymerization and ligation of the free ends yield two Holliday junctions that can be resolved in a variety of ways by cutting either the crossed (open arrowheads) or noncrossed strands (black arrowheads). If both junctions are resolved in the same orientation, gene conversion without crossing-over is achieved. Alternatively, if the junctions are resolved in opposite orientation, gene conversion is accompanied by reciprocal crossing-over. SDSA follows the same steps as DSBR until the extension of the invading 3’-end. Once the extension has gone past the original break site, the invading end dissociates from the donor duplex and re-associates with the recipient DNA by complementary base pairing. DNA synthesis can now fill in the gaps and ligation always yields a gene conversion event without crossing-over. (b) Single-ended DSBs produced by the demise of replication forks can be successfully repaired by the break-induced replication/recombination-dependent replication pathway (BIR/RDR). In the illustrated case, a single-stranded break on the lagging strand template leads to replication fork collapse. Then, as in DSBR and SDSA, resection of the 5’-end on the broken DNA frees a 3’-OH single-stranded overhang which can invade a homologous donor duplex. DNA synthesis starts and the extended 3’-end is unable to find a complementary second end. To synthesize the missing strand, a replication fork is established and polymerization proceeds to the end of the donor molecule. This results in gene conversion accompanied by nonreciprocal crossing-over. This figure is modified from Hastings et al. (2009b).
for a DSBR-like HR mechanism in plastids as reciprocal crossing-over is required for the orientation change of the single-copy regions in genome-sized circular DNA molecules. However, it is now recognized that most plastid DNA (ptDNA) exists as linear/concatemeric/highly branched complex molecules and these inversions were reinterpreted as resulting from a BIR-like, recombination-dependent replication mechanism between different linear copies of the plastome (Bendich, 2004; Oldenburg & Bendich, 2004) (see Section II.3 and Fig. 2). Additional and abundant proof for the presence of an efficient recombination apparatus in plastids comes from the production of transplastomic plants (Boynton et al., 1988; Svab et al., 1990; Koop et al., 2007). Integration of plasmid-borne foreign DNA within the plastid genome occurs by gene conversion with or without crossing-over, consistent with DSBR, and requires a substantial length of homology on both sides of the sequence to be integrated (≥ c. 150 bp) (Day & Madesis, 2007). Similarly, excision of intervening sequences between short DNA repeats introduced in the plastid genome constitutes a viable strategy to remove antibiotic resistance marker genes or even endogenous genes from transformed plastid genomes and also argues in favour of an active DSBR pathway in plastids (Fischer et al., 1996; Iamtham & Day, 2000; Klaus et al., 2004; Kode et al., 2006).

The mending of DNA damage through homology-directed DNA repair has been demonstrated in plastids. Induced DNA breaks in the plastid genome of the green alga Chlamydomonas reinhardtii using the homing endonuclease I-CreI were fixed by HR (Durrenberger et al., 1996). A more recent study suggests that the chloroplast of Chlamydomonas can use both the DSBR and SDSA pathways to fix double-stranded lesions (Odom et al., 2008). Recombination can also act as a repair mechanism in flowering plants as it was shown to be highly efficient at fixing point mutations in tobacco (Nicotiana tabacum) plastids (Khakhlova & Bock, 2006).

Recombination is also an active process in mitochondria and is responsible for the highly dynamic character of plant mitochondrial genomes (Mackenzie, 2007). Indeed, the mitochondrial genome of angiosperms (flowering plants) generally contains many fairly large repeated sequences (> 1 kbp) which can frequently recombine intra- or intermolecularly. These multiple HR events result in a multipartite highly redundant organization of the mitochondrial genome where subgenomes coexist in approximately equal stoichiometry (Palmer & Shields, 1984; Fauron et al., 1995). Homologous recombination also occurs sporadically between shorter repeats (> 100 bp, < 1 kbp) and produces rearranged DNA molecules called sublimons (substoichiometric genomes) that are generally present in low copy numbers in wild-type (WT) mitochondria (Small et al., 1987). These recombined molecules have been proposed to constitute important intermediates during the evolution of plant mitochondrial genomes (Small et al., 1989; Shedge et al., 2007).

Finally, a low frequency of illegitimate recombination between regions of microhomology has also been reported in both organelle genomes (Ogihara et al., 1988; Kanno et al., 1993; Hartmann et al., 1994; Moeykens et al., 1995; Maréchal et al., 2009). Thus, recombination definitely happens in plastids and mitochondria and appears to be limited to large repeats (> 100–200 bp) in normal genomes or to rare events involving microhomologous repeats that are easily observable only on an evolutionary timescale.

3. Recombination-dependent replication of plant organelle genomes

In addition to its roles in the repair of a wide variety of DNA lesions and in the dynamics and evolution of organelle genomes, recombination also appears to be implicated in the replication of organelle DNA. Studying the topology of a given DNA molecule provides insights into its replication mechanism. Despite a widely held assumption, plant organelle DNA is not present exclusively as genome-sized

---

**Fig. 2** Recombination-dependent replication produces both isomers of the plastid genome. A free 3′-OH end of one copy of the plastid genome invades a homologous head-tail concatemeric duplex inside an inverted repeat (IR) and initiates replication. (1) If BIR/RDR initiates at one repeat, the final product is a head-tail concateamer with both single copy regions present in the same orientation. (2) If initiation occurs at the other IR, the final product yields a head-tail concateamer where one pair of successive small and large single copies is arranged in opposite orientations. In maize, the use of pulsed field gel electrophoresis, which was not employed in the original study (Palmer, 1983) to examine the arrangement of chloroplast DNA, demonstrates that the previously observed inversion isomers are present only in the context of head-tail concateamers in vivo. This invalidates the flip-flop recombination model that used genome-sized circular DNA to produce the ptDNA isomers (Oldenburg & Bendich, 2004). The pointed arrows with dotted lines indicate the progression of replication forks. The short red and green blocky arrows represent the inverted repeats. The large and small single copy regions (LSC and SSC) are depicted as long blue and short black thin arrows, respectively.
circles in vivo. Restriction mapping data arising from plastid and plant mitochondrial genomes can indeed be interpreted as circular molecules but the actual structure of these genomes can only be confidently assigned after direct observation of DNA. Early electron microscopy observations of purified organelle DNA showed abundant linear DNA molecules but these were dismissed as probable degradation products of larger circular molecules. In both plastids and mitochondria, electron microscopy also revealed the presence of circular DNA molecules harbouring tails (η-like structures), suggesting that rolling-circle replication is involved in the maintenance of both organelle genomes (Kolodner & Tewari, 1975; Backert & Borner, 2000). Concomitant observation of displacement loops (D-loops) in purified mtDNA led to the proposal of a dual origin-dependent θ to rolling-circle replication model for the plastid genome (Kolodner & Tewari, 1975). However, these models strongly relied on the postulate that most organelle DNA exists as genome-sized circles.

With the advent of new techniques which prevent potential shearing, for example pulsed-field gel electrophoresis, it became possible to observe intact organelle DNA (reviewed in Bendich, 1993, 2004; Day & Madesis, 2007). It is now well established that plant organelle DNA is constituted by a mixture of monomers and head-tail concatamers of circular and linear molecules together with highly complex branched structures (Deng et al., 1989; Backert et al., 1996; Oldenburg & Bendich, 1996, 2001, 2004; Backert & Borner, 2000; Lilly et al., 2001). These observations allowed the proposal of an RDR mechanism similar to that found in bacteriophage T4 and in bacteria to explain the complex arrangement of DNA in plant organelles (Luder & Mosig, 1982; Asai et al., 1994; Oldenburg & Bendich, 1996, 2001, 2004; Backert & Borner, 2000). In this model, a free 3'-OH single-stranded DNA overhang from one copy of the genome can invade another copy at a homologous site (Fig. 1b; RDR starts at the D-loop formation step). This primes DNA synthesis on the leading strand and a full replication fork is eventually established. Invasion of a linear DNA molecule by another one produces a branched structure, while invasion of a circular molecule produces a tailed circle containing a bubble. BIR/RDR can also conveniently explain flip-flop recombination if the invasion step is mediated by the inverted repeats of the plastid genome. In this case, RDR results in gene conversion accompanied by non-reciprocal crossing-over, thereby generating the inversion of the single copy regions (Fig. 2). Thus, RDR constitutes the replication model that best accounts for the structural complexity of DNA in plant organelles (Oldenburg & Bendich, 1996, 2001, 2004; Backert & Borner, 2000; Manchekar et al., 2006). At the present time, the relationship between RDR and the previously proposed replication models as well as the relative importance of each mechanism in the actual replication of organelle DNA remain unclear.

III. Recombination surveillance machinery in plant organelles

1. Perturbations of the recombination surveillance systems

Compared with plastid genomes, plant mitochondrial genomes are typically rich in fairly large repeated sequences (> 50 bp), and strict control of HR is required to maintain genome stability in this organelle (Table 1). Indeed, because of these numerous repeats, runaway recombination of mitochondrial DNA (mtDNA) is relatively common and can lead to vast rearrangements of the genome with ensuing changes in the gene expression pattern of mitochondria (Sakamoto et al., 1996; Arrieta-Montiel et al., 2009; and reviewed in Mackenzie, 2007). These modifications can have profound adverse effects on the functionality of mitochondria.

One of the most studied consequences of mitochondrial dysfunction caused by genomic instability is CMS, an agronomically important trait that facilitates the large-scale production of high-yielding hybrid plants. It is now clear that mitochondrial genomes are the major genetic determinants of this maternally transmitted phenotype. Importantly, recombination-mediated rearrangements in mtDNA and important alterations in the relative stoichiometry of these modified DNA molecules have been associated with the inability to produce viable pollen and also with spontaneous cytoplasmic reversion of the plants to a fertile state (Levings et al., 2006). All plants listed here have a pair of large inverted repeats (IRs) in their plastid genome. These IRs are included in the total number of plastid repeats.

<table>
<thead>
<tr>
<th>Species</th>
<th>Number of repeats</th>
<th>Accession numbers</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arabidopsis thaliana</td>
<td>1</td>
<td>NC_000932</td>
</tr>
<tr>
<td>Carica papaya</td>
<td>1</td>
<td>NC_010323</td>
</tr>
<tr>
<td>Nicotiana tabacum</td>
<td>1</td>
<td>NC_001879</td>
</tr>
<tr>
<td>Vitis vinifera</td>
<td>2</td>
<td>NC_006581</td>
</tr>
<tr>
<td>Oryza sativa (indica)</td>
<td>3</td>
<td>NC_008155</td>
</tr>
<tr>
<td>Sorghum bicolor</td>
<td>4</td>
<td>NC_008602</td>
</tr>
<tr>
<td>Triticum aestivum</td>
<td>5</td>
<td>NC_002762</td>
</tr>
<tr>
<td>Zea mays</td>
<td>5</td>
<td>NC_007579</td>
</tr>
</tbody>
</table>

Table 1 Repeated sequences in the organelle genomes of angiosperms

1Direct and inverted perfectly identical repeated sequences with a size ≥ 50 bp were identified using the REPUTER program and the published organelle genome sequences of the indicated flowering plant species (Kurtz et al., 2001). All plants listed here have a pair of large inverted repeats (IRs) in their plastid genome. These IRs are included in the total number of plastid repeats.
Recombination in mitochondria is not limited to HR between rather large repeats; it can also, albeit less frequently, use microhomologous repeats to alter mtDNA. For example, microhomology-mediated recombination has been reported to promote reversion to fertility in CMS maize plants and pearl millet (*Pennisetum glaucum*) (Schardl *et al.*, 1985; Feng *et al.*, 2009). Many other cases of such microhomology-mediated rearrangements (involving repeats ranging from 6 to 31 bp) have been reported to induce the nonchromosomal stripe (NCS) phenotype in maize mutants (Newton *et al.*, 1990; Hunt & Newton, 1991).

Apart from the large inverted repeats, there are very few repeated regions in the plastid genomes of flowering plants that could potentially serve as HR substrates (Table 1). Introduction of foreign repeated sequences by transformation of plastids often results in unintended secondary HR events (see Gray *et al.*, 2009 and references therein). This suggests that repeats large enough to recombine efficiently and induce rearrangements might be selected against as they have the potential to destabilize the plastid genome of flowering plants. Even though the number of large repeats in ptDNA is kept to a minimum, perturbations of plastid genome stability do occur and mainly result from illegitimate recombination events between microhomologous repeats. For example, in tobacco, *Nicotiana* plastid extrachromosomal element (NICE1), an extrachromosomal element formed during transformation, was found to excise from plastid DNA through recombination between two imperfect repeats of 16 bp (Staub & Maliga, 1994). Accumulation of head-to-tail concatemeric DNA has also been reported in a photosynthetically incompetent *C. reinhardtii* mutant that failed to express *atpB* RNA and had been transformed with a plasmid carrying a weak *atpB* construct. In some of the transformants, the defect in *atpB* expression was rescued by an over-amplification of the plasmid, which was found to be present as an episome of 20–30 copies per plastid genome and reorganized by recombination in a head-to-tail configuration (Kindle *et al.*, 1994).

Both mitochondrial and plastid genomes are more frequently subject to alterations under specific environmental conditions. In particular, long-term tissue/cell culture and albinism can strongly destabilize plant organelle genomes (Day & Ellis, 1984; Rode *et al.*, 1987; Vitart *et al.*, 1992; Kanazawa *et al.*, 1994; Kawata *et al.*, 1997; Cahoon *et al.*, 2003). Overall, the evidence shows that external stress (i.e. variation in the selective pressure for photosynthesis/respiration and transformation of foreign DNA in plastids) placed on the recombination machinery of plant organelles can render it more error-prone. Some of these conditions could also favour specific rare (substoichiometric) rearranged DNA molecules that would normally be corrected by the DNA repair machinery but are conserved because of the useful traits they confer on organelles. Alternatively, these rearranged molecules might become more abundant when selective pressure is relaxed simply because they replicate better.

Early on, nuclear mutations were also recognized as a major cause of genomic instability in plant organelles. For example, in maize, the WF9 nuclear genome is particularly prone to mitochondrial genome rearrangements which produce an NCS phenotype, implying that some particular genes in this background destabilize mtDNA (Newton & Coe, 1986). Other recently reported alleles in the maize P2 line dramatically increase the abundance of modified mtDNA molecules, which result in leaf striping patterns of different colours (Kuzmin *et al.*, 2005). In plastids, mutation of the *plastome mutator* (*PM*) nuclear locus produces variegated *Oenothera* plants as a result of a strongly increased rate of deletion, duplication and point mutations in the plastid genome (Chiu *et al.*, 1990; Chang *et al.*, 1996; Stoice & Sears, 1998). Although the specific alleles responsible for most cases of organelle genome instability have not yet been identified, a number of genes that actively prevent deleterious recombination activity in organelle genomes have recently been characterized. Interestingly, these genes often encode proteins that are targeted to both organelles or are part of families comprising mitochondrial- and plastid-localized members, suggesting that at least some genome maintenance strategies are shared by plastids and mitochondria. These studies provide us with a preliminary portrait of the recombination surveillance machinery in plant organelles. So far these proteins include MutS homologue 1 (MSH1), RecA-like recombinases and two families of plant-specific single-stranded DNA (ssDNA)-binding proteins: the organellar ssDNA-binding proteins (OSBs) and the Whirleys.

2. MSH1

Mutation of the nuclear locus *chloroplast mutator* (*CHM/MSH1*) in Arabidopsis yields variegated plants bearing sectored green/white/yellow leaves symptomatic of dysfunctional chloroplasts. This phenotype is inherited in a non-Mendelian fashion which led its original discoverer and Arabidopsis research pioneer Georges Rédei to propose
that the variegation was caused by mutations in the plastid genome (Rédei, 1973). Nearly 20 yr later, a surprising correlation between variegation in chm plants and rearrangements of the mitochondrial genome through recombination was established (Martinez-Zapater et al., 1992). Moreover, despite extensive investigation, no modifications of the plastid genome could be detected, indicating that genomic instability in mitochondria probably leads to the observed chloroplast defects in chm plants (Martinez-Zapater et al., 1992; Mourad & White, 1992). A subsequent study described the molecular arrangement of two novel mtDNA species present in the progeny of a cross between chm1–3 (ecotype Landsberg erecta (Ler)) and WT (ecotype Columbia (Col-0)) plants. One of the rearrangements was ascribed to an illegitimate recombination event mediated by microhomologous regions of 11 bp (Sakamoto et al., 1996). However, as this analysis was based on preliminary mitochondrial genome sequences from Arabidopsis ecotype C24, which differs from the other ecotypes in the regions implicated in the rearrangements, the recombination events were later reinterpreted as resulting from HR between larger dispersed repeats (> 200 bp) (Unseld et al., 1997; Forner et al., 2005; Ziegler et al., 2006; Shedge et al., 2007; Arriesta-Montiel et al., 2009). Further examination of mtDNA rearrangements showed that every repeated sequence of the mitochondrial genome between 108 and 560 bp was subject to asymmetric recombination in chm plants. The efficiency of recombination correlated with the length and extent of sequence similarity between individual repeats (Arriesta-Montiel et al., 2009). The mtDNA rearrangements found in these plants are also often accompanied by altered mitochondrial transcript profiles (Sakamoto et al., 1996; Arriesta-Montiel et al., 2009).

Cloning of the CHM gene revealed that it has significant sequence similarity with MutS, a bacterial gene with roles in DNA recombination and mismatch repair (MMR) (Abdelnoor et al., 2003). Similarly to MSH1 in yeast, this MutS homologue is targeted to mitochondria. In Saccharomyces cerevisiae, msh1 mutations cause a petite phenotype indicative of defective respiration that is accompanied by large-scale rearrangements and point mutations in the mitochondrial genome (Reenan & Kolodner, 1992). Consequently, the Arabidopsis CHM gene was renamed MSH1 (Abdelnoor et al., 2003). So far, mitochondrial MutS homologues have only been found in yeast, plants and corals (Culligan et al., 2000). Because msh1 plants do not accumulate point mutations in the mitochondrial genome and their fitness remains constant in time, plant MSH1 homologues seem to have specialized in the regulation of recombination instead of being required for accurate mtDNA MMR (Abdelnoor et al., 2003). In agreement with the established role of MSH1 in Arabidopsis, a down-regulation of MSH1 expression in tomato (Solanum lycopersicum) and tobacco was shown to destabilize the mitochondrial genome. Remarkably, in these two plant species the mtDNA rearrangements seem able to induce a stable (nonreversible) CMS phenotype, suggesting that this strategy could be used to obtain nontransgenic male-sterile lines in a variety of crops (Sandhu et al., 2007).

In the bacterial MMR system, MutS homodimers bind preferentially to mispaired or looped-out DNA in order to direct repair of mismatched heteroduplexes and prevent recombination between divergent sequences. They perform their task with the help of the adaptor protein MutL and the endonuclease MutH (Rayssiguier et al., 1989 and reviewed in Jun et al., 2006). Plant MSH1 proteins contain three recognizable domains in addition to three other conserved domains of unknown function (Abdelnoor et al., 2006). At the N-terminus, following the transit peptide, there is a domain similar to the MutS-I DNA-binding and mismatch recognition motif. Plant MSH1 homologues also contain an ATPase domain conserved in all MutS-like proteins and a unique GIY-YIG endonuclease domain found in a number of other nucleases such as the excinuclease component uvrC and the homing endonuclease I-TevI (Fig. 3a) (Abdelnoor et al., 2003, 2006). Point mutations in both the ATPase and endonuclease domains of MSH1 induce variegation and reorganization of mtDNA, highlighting the functional importance of these domains. There are apparently no plant MutL or MutH homologues predicted to localize to organelles. This, together with the peculiar domain architecture of MSH1, suggests that this protein represents a compact system with mismatch recognition and endonuclease functions bundled together. The role of MutS family proteins in preventing recombination between homologous DNA regions is broadly conserved in bacteria, yeast, humans and plants (Rayssiguier et al., 1989; Worth et al., 1994; Datta et al., 1997; Elliott & Jasin, 2001; Emmanuel et al., 2006). Although the molecular details remain fuzzy, combined evidence raises the possibility that plant MSH1 homologues may function in the early steps of recombination by favouring heteroduplex rejection, thereby stabilizing the mitochondrial genome. Interestingly, MSH1 proteins from Arabidopsis and tomato can localize to both organelles whereas their counterparts from maize and soybean (Glycine max) are targeted solely to mitochondria (Christensen et al., 2005; Abdelnoor et al., 2006). As no plastid DNA rearrangements have been observed to date in msh1 mutants, it is possible that MSH1 proteins could play additional roles in the plastids of some plant species.

3. RecA homologues

The eubacterial recombinase RecA and its eukaryotic homologues of the Rad51 family are essential proteins for the central steps of HR (reviewed in Cox, 2007). They are key factors for the accurate pairing of homologous DNA sequences, the promotion of strand invasion and the migration of branches during the recombination process. All the
different pathways of HR (DSBR, SDSA and BIR/RDR) begin by a strand-exchange reaction mediated by RecA family proteins, making RecA and its homologues crucial for homology-directed DNA repair of a variety of genome-stabilizing lesions, including DSBs and stalled replication forks (reviewed in Cox et al., 2000).

Very few genes of the RecA family have been found outside prokaryotes. The discovery of a RecA homologue encoding a protein that localizes to the stroma of pea (Pisum sativum) chloroplasts was thus somewhat surprising (Cerutti et al., 1992). The presence of RecA-like strand-exchange activity in pea chloroplasts and soybean mitochondria also supports the existence of functional RecA homologues in plant organelles (Cerutti & Jagendorf, 1993; Manchekar et al., 2006). Complete sequencing of the nuclear genome of Arabidopsis revealed that it harbours at least three active RecA homologues: RecA1, RecA2 and RecA3 (Table 2). A fourth RecA-like gene exists but as it encodes a heavily truncated protein, it is probably a pseudogene (Khazi et al., 2003; Sedge et al., 2007). Fusions between GFP and the first 80 amino acids of these proteins established that RecA1 localizes to plastids, RecA3 to mitochondria and RecA2 to both organelles.

Mutation of the mitochondrion-targeted RecA3 leads to large-scale rearrangements of mtDNA. These rearrangements are caused by HR between repeated sequences (> 150 bp). The effect of RecA3 mutation on mtDNA recombination is different from that observed in msh1 plants. Indeed, the affected repeats appear to be a subset of those perturbed in MSH1 mutant plants (Sedge et al., 2007; Arrieta-Montiel et al., 2009). These modifications are partially reversible as reintroducing a functional copy of RecA3 can result in the loss of aberrant DNA molecules in the majority of the progeny. Strikingly, recA3 single mutants have no phenotype that could distinguish them from WT plants, indicating that mitochondria can cope with a certain level of rearrangements. The other two RecA homologues are apparently essential for viability as null

---

**Fig. 3** Protein families of the organelle recombination surveillance machinery. (a) Rectangular representation of proteins with established roles in preventing aberrant recombination of plant organelle genomes. Arabidopsis MutS homologue 1 (MSH1) is a 120-kDa MutS homologue fused with a GIY-YIG endonuclease domain. Organellar RecA homologues are 40–45-kDa proteins containing a well-conserved bacteria-like RecA-fold. Organellar single-stranded DNA (ssDNA)-binding proteins (OSBs) are 30–50-kDa proteins which harbour an oligonucleotide/oligosaccharide binding (OB)-fold-like domain putatively involved in oligomerization and a variable number of ssDNA-binding PDF domains in their C-terminal region. Depicted here is the Arabidopsis OSB1 protein with a single PDF domain. Whirleys are c. 24-kDa proteins that bind single-stranded DNA and RNA through their highly-conserved central Whirly domain. TP, transit peptide. (b) Crystallographic three-dimensional structure of Solanum tuberosum Whirly 1 (StWhy1). Left, cartoon representation of an StWhy1 protomer with β-strands coloured in yellow, α-helices in red and loops in green. Right, cartoon representation of an StWhy1 tetramer showing its whirligig-like appearance (adapted from Desveaux et al., 2002). These representations were generated using PrMOL (DeLano, 2002).
alleles could not be recovered, suggesting that they play crucial roles in plant organelles (Shedge et al., 2007).

A similar phenomenon was reported in disruptants of the mitochondrion-targeted RecA homologue \textit{PpRecA1} in the moss \textit{Physcomitrella patens}. In this case the rearrangements involved shorter repeats (> 40 bp, < 100 bp) as there are no quasi-identical repeats larger than 90 bp in the mitochondrial genome of \textit{P. patens} (Terasawa et al., 2007; Odahara et al., 2009). Recombination between shorter repeats in Arabidopsis \textit{recA3} mutants cannot be ruled out, as the mtDNA of these plants has not been systematically assessed for recombination among sequences < 100 bp. Additionally, the \textit{PpRecA1} gene appears to be important for the accurate and efficient repair of damaged mtDNA as it is transcriptionally activated when moss is grown on media containing genotoxic agents such as mitomycin C and bleomycin. MtDNA repair is also slowed down in \textit{PpRecA1} mutants following treatment with the alkylating agent methyl methane sulphonate (Oda\-hara et al., 2007). It is thus clearly established that organelle-targeted RecA homologues in plants have important roles in the prevention of recombination between short repeated sequences and in the promotion of DNA repair.

The biochemical properties and the molecular details underlying the biological roles of RecA recombinases have been studied extensively (reviewed in Bell, 2005). The strand-exchange reaction begins with the polymerization of RecA onto ssDNA overhangs present at lesion sites, thereby forming a presynaptic filament in the presence of ATP. A homology search step allows the alignment of the presynaptic RecA-ssDNA complex with an intact homologous donor double-helix. The presynaptic complex is then able to destabilize the donor duplex and catalyse strand exchange which forms a D-loop. All participants are subsequently dissociated upon ATP hydrolysis (Chen et al., 2008). The central RecA-fold, which also contains the ATP-binding and hydrolysis domains, is generally well conserved in plant homologues, suggesting a similar mode of action for bacterial and plant RecA recombinases (Fig. 3a; Shedge et al., 2007). Interestingly, the RecA3 protein differs from RecA1 and RecA2 in that it lacks the acidic C-terminal domain also found in prokaryotic RecA proteins. C-terminal deletions in bacterial recombinases enhances most \textit{in vitro} activities of RecA, supporting an autoregulatory role for this portion of the protein (Cox, 2007). \textit{In vitro} assays using purified bacterial proteins have also shown that the antirecombination activity of MutS is a result of the inhibition of RecA-mediated strand-transfer activity between ssDNA molecules and homologous or damaged DNA duplexes (Worth et al., 1994; Calmann & Marinus, 2004). This suggests that MSH1 might have a similar regulatory role in the strand-exchange activity of RecA homologues in plant organelles. In accordance with the demonstrated role of mitochondrial RecA proteins in plants, bacterial RecA by itself also prevents strand-exchange reactions involving substantially diverged sequences (Hahn et al., 1988).

4. The organellar single-stranded DNA-binding proteins

The OSBs form a recently discovered family of plant-specific proteins that all share a particular affinity for ssDNA (Vermel et al., 2002; Zaegel et al., 2006). There are four different OSBs in Arabidopsis, which localize to mitochondria (OSB1), chloroplasts (OSB2) or both organelles

\begin{table}[h]
\centering
\begin{tabular}{|l|l|l|l|l|}
\hline
\textbf{Gene name} & \textbf{TAIR code} & \textbf{Function} & \textbf{Localization} & \textbf{Evidence for a role in organelle RRR} & \textbf{References} \\
\hline
\hline
\hline
\textit{Why} & A2:g02740 & ssDNA-binding proteins & Plastid & & Maréchal et al. (2009) \\
\hline
\textit{OSB1} & A1:g47720 & Plant-specific ssDNA-binding proteins & Mitochondrion & Deletion of \textit{OSB1} increases recombination between short homologous sequences in mtDNA & Zaegel et al. (2006) \\
\hline
\textit{OSB2} & A4:20010 & ssDNA-binding proteins & Plastid & & Pfalz et al. (2006) \\
\hline
\textit{OSB3} & A5:44785 & ssDNA-binding proteins & Plastid/mitochondrion & & \\
\hline
\textit{OSB4} & A1:g31010 & & Unknown & & \\
\hline
\textit{RecA1} & A1:g79050 & RecA homologues & Plastid & Deletion of \textit{RecA3} in \textit{Arabidopsis thaliana} or \textit{RecA1} in \textit{Physcomitrella patens} increases recombination between short homologous sequences in mtDNA & Shedge et al. (2007) \\
\hline
\textit{RecA2} & A2:g19490 & & Mitochondrion & & Odahara et al. (2009) \\
\hline
\textit{RecA3} & A3:g10140 & & Mitochondrion & & \\
\hline
\textit{RecA4} & A3:g32920 & & Pseudogene? & & \\
\hline
\textit{Msh1} & A3:24320 & MutS homologue & Plastid/mitochondrion & Mutation of \textit{Msh1} increases recombination between short homologous sequences in mtDNA & Abdelnoor et al. (2003) \\
\hline
\end{tabular}
\caption{Characterized protein families of the recombination surveillance machinery of plant organelle genomes}
\end{table}
(OSB3) as determined using GFP fusion proteins. Although expressed sequence tags have been reported for OSB4, the cDNA has not yet been cloned and its precise location and function have not been determined (Table 2). T-DNA insertion mutants of the OSB1, OSB2 and OSB3 genes were obtained but a phenotype was observed only in the progeny of OSB1 mutants. Indeed, some osb1 plants of the T4 and T5 generations develop variegated and distorted leaves. The affected plants exhibit rearrangements in multiple regions of the mitochondrial genome. These rearrangements were shown to result from HR between short repeated sequences (> 200 bp). In some of the plants, the accumulation of aberrant mtDNA molecules in osb1 plants is reversible by reintroduction of a WT OSB1 allele. However, when the rearranged DNA becomes too abundant, the phenotype is irreversible, presumably because the rearranged genome has become the principal component of mtDNA and compensating recombination cannot happen to any great extent. This is similar to the early and late msb1 mutant plants where prolonged propagation of msb1 plants by selfing results in a gradual accumulation of rearrangements (Arrieta-Montiel et al., 2009). Thus, like Msh1 and RecA3, Osb1 is part of the mitochondrial recombination surveillance machinery in Arabidopsis and acts by limiting the occurrence of HR between short repeated sequences.

Limited structure–function analysis of the OSBs has been performed (Zaegel et al., 2006). All OSBs have a transit peptide at their N-terminus to ensure their proper targeting to organelles. Although not extremely well conserved (30–50% similarity) in terms of amino acid residues, a domain structurally similar to the oligonucleotide/oligosaccharide binding fold (OB-fold), a hallmark of many ssDNA-binding proteins, is found in the middle part of OSBs. Additionally, the C-terminal section of these proteins contains a variable number of PDF motifs which are specific to plants (Fig. 3a). As their name implies, these 50 amino acid domains contain consecutive highly conserved PDF residues. In vitro nucleic acid binding assays revealed that Arabidopsis OSB1 and OSB2 bind preferentially to ssDNA with a high affinity ($K_d \approx 2 \text{ nM}$ for OSB2). Interestingly, the PDF motif is required for the ssDNA-binding activity of OSB1 while the OB-fold-like motif is dispensable. The residues required for high-affinity ssDNA binding in canonical OB-fold domains are not found in plant OSBs. It is possible that the OB-fold-like domain of these proteins has been transformed to fulfill other roles perhaps in oligomerization as the structural motifs required for this function of the OB-fold are still present.

5. The Whirlies

Whirlies form another small family of ssDNA-binding proteins that is present mainly in the plant kingdom (reviewed in Desveaux et al., 2005). The prototypical Whirly, Solanum tuberosum Whirly 1 (StWhy1), was first characterized as a nuclear transcriptional regulator of the pathogenesis-related gene PR-10a in potato (Matton et al., 1993; Despréts et al., 1995; Desveaux et al., 2000, 2004). Whirlies also possess active transit peptides at their N-terminus which can target these proteins either to the plastids or to the mitochondria. In fact, every completely sequenced angiosperm has at least one mitochondrion- and one plastid-targeted Whirly (mtWhirlies and ptWhirlies), suggesting that they play important roles in both organelles (Krause et al., 2005; Table 2). Recently, the dual nuclear and plastid localization of barley (Hordeum vulgare) Whirly 1 (H-Why1) was demonstrated by immunolocalization and cellular subfractionation experiments (Grabowski et al., 2008). The mechanisms and conditions which might permit and regulate the dual localization of Whirlies remain poorly characterized at present but investigations are ongoing (Krause & Krupinska, 2009).

In agreement with the multiple localizations of Whirly proteins, evidence abounds showing that these proteins have functions related to DNA metabolism in both organelles and the nucleus. Thus, members of the Whirly family act as transcriptional regulators for a number of nuclear genes and are important for optimal resistance to the biotrophic oomycete Hyaloperonospora parasitica (Desveaux et al., 2000, 2004; Gonzalez-Lamothe et al., 2008; Xiong et al., 2009). Whirlies have also been implicated in the maintenance of telomere length in nuclear chromosomes (Yoo et al., 2007). Moreover, there is ample evidence that Whirlies associate with plastid and mitochondrial genomes in a non-sequence-specific fashion, supporting a function of these proteins in the DNA metabolism of plant organelles. The Arabidopsis ptWhirlies AtWhy1 and AtWhy3 have been purified in association with the transcriptionally active chromosome of chloroplasts (Pfalz et al., 2006). In maize, the plastid-targeted Whirly, ZmWhy1, localizes to the stroma of chloroplasts and is tethered to thylakoid membranes via its interaction with ptDNA (Prikryl et al., 2008). In Arabidopsis, DNA immunoprecipitation experiments revealed that the mitochondrial AtWhy2 binds to many different regions of mtDNA without any obvious sequence consensus (Maréchal et al., 2008). Similar experiments showed that the chloroplast-targeted AtWhy1 and AtWhy3 interact with every ptDNA region tested (Maréchal et al., 2009). This nonspecific binding is also found in other plant species as ZmWhy1 also seemingly interacts with every region of ptDNA (Prikryl et al., 2008).

Recently, a role for ZmWhy1 in chloroplast biogenesis was discovered. Hypomorphic mutants of this gene have pale green to completely white leaves depending on the severity of the Zmwhy1 allele, indicating that chloroplasts become defective following ZmWhy1 abrogation (Prikryl et al., 2008). The authors of this study isolated ZmWhy1 by co-immunoprecipitation with the splicing factor CRS1, which is implicated in the maturation of the atpF RNA

© The Authors (2010)
Journal compilation © New Phytologist Trust (2010)
(Jenkins et al., 1997). RNA immunoprecipitation experiments also revealed a specific interaction between ZmWhy1 and the atpF RNA. In accordance with these results, the splicing efficiency of the atpF intron was diminished in ZmWhy1 mutants, albeit not as drastically as in CRS1 mutant plants, suggesting an accessory role of ZmWhy1 in the maturation of this RNA. Additional analysis of these plants revealed that their plastid ribosomal RNA content was greatly diminished or even nonexistent in the most severe mutant. Exactly how ZmWhy1 is involved in the loss of maize plastid ribosomes remains unclear at present as no interaction occurs between ZmWhy1 and individual ribosomal RNAs.

To better understand the role of plastid-targeted Whirleys (ptWhirleys), Arabidopsis single and double knock-out (KO) lines of the AtWhy1 and AtWhy3 genes were recently characterized (Maréchal et al., 2009). Some of the progeny of KO1/3 plants (c. 5%) have sectored green/white/yellow leaves containing dysfunctional chloroplasts. This maternally inheritable variegation phenotype was shown to result from increased instability of the plastid genome in the absence of ptWhirleys. Plastids of KO1/3 plants accumulate aberrant ptDNA molecules which correspond to deletion, duplication and/or circularization events resulting from illegitimate recombination between microhomologous repeats. Most of these rearranged molecules are undetectable in WT plants, suggesting that they arise de novo in Whirly mutant plants. Very strong amplification of some of the rearranged DNA molecules correlates with the appearance of defective chloroplasts. This phenomenon is not unique to Arabidopsis, as similarly rearranged DNA molecules also accumulate in the plastids of maize lines with reduced levels of ZmWhy1 (Maréchal et al., 2009). The absence of a defect in rRNA maturation and the relatively benign phenotype of KO1/3 plants compared with ZmWhy1 mutants suggest that Whirleys form a flexible family of single-stranded nucleic acid proteins that can serve different purposes depending on cellular conditions and/or plant species. Thus, ptWhirleys prevent the accumulation of abnormal ptDNA molecules produced by microhomology-mediated recombination and have a conserved role in the maintenance of plastid genome stability.

A good amount of information is available on the various domains that compose individual Whirly proteins. Following their N-terminal transit peptides, some Whirleys have a putative transactivation domain. In StWhy1, this region contains a stretch of several glutamine residues, and the transactivation function of this domain has been demonstrated (Desveaux et al., 2004). Polyserine, polyglutamine and polyproline regions found in a number of transcriptional activators are also present in other members of the Whirly family but their transactivation potential has not been assessed yet (Triezenberg, 1995; Desveaux et al., 2005). As these domains are often involved in the recruitment of coactivators, they might function generally as protein–protein interaction modules depending on the cellular context. A highly conserved central domain forms the structural core of Whirly proteins and is necessary for their high-affinity ssDNA-binding activity (Kd ≈ 3 nM; L. Cappadocia and N. Brisson, unpublished results). Gel filtration analysis of Whirly–DNA complexes and X-ray crystallography have established that these proteins adopt a tetrameric quaternary structure (Desveaux et al., 2002). Finally, the C-terminus of Whirly proteins in flowering plants comprises a conserved short acidic/aromatic domain which has been proposed to modulate the ssDNA-binding capacity of Whirleys (Desveaux et al., 2005; Fig. 3a).

Resolution of the three-dimensional structure of the Whirly domain revealed a novel ssDNA-binding fold (Desveaux et al., 2002). Each protomer is composed of two anti-parallel β-sheets packed perpendicularly against each other along with three α-helices. In the tetramer, the β-sheets form blade-like extensions radiating from an α-helical core. The general impression of this peculiar quaternary structure is that of a whirligig, which suggested the name Whirly for this family of plant ssDNA-binding proteins (Fig. 3b).

6. Recombination mechanisms involved in organelle genome rearrangements

Recombination between large repeated sequences of the mitochondrial genome results in gene conversion accompanied by reciprocal crossing-over, which is the predicted product of the classical DSBR pathway (Palmer & Shields, 1984; Fauron et al., 1995) (Fig. 1a). The rare HR events between shorter repeats in mtDNA (> 50 bp, < 1 kbp) produce a very low number of rearranged molecules (sublimons) which are thus usually substoichiometric relative to the more abundant normal subgenomes (Small et al., 1987). As described above, environmental conditions or nuclear mutations in the recombination surveillance machinery of mitochondria can increase the frequency of recombination between these shorter repeats and can also induce rapid amplification of pre-existing rare rearranged molecules in a process called substoichiometric shifting (SSS) (see for instance (Arrieta-Montiel et al., 2001; Woloszynska & Trojanowski, 2009; and review by Mackenzie, 2007). Recombination events between short mitochondrial repeats almost always result in the accumulation of only one of the recombination products predicted by the DSBR model. It has often been argued that the other predicted recombination product did not accumulate substantially because of its putative inability to replicate efficiently. However, the fact that a single recombination product is the rule rather than the exception indicates that the recombination pathway that generates this product is asymmetric.

It is thus likely that short repeat-mediated recombination of the mitochondrial genome occurs via the BIR/RDR pathway of HR (Shedge et al., 2007; Fig. 1b). Indeed, the
The final product of BIR/RDR is gene conversion with non-reciprocal crossing-over, which corresponds to what is observed in short repeat-mediated mtDNA rearrangements. The use of such an asymmetric HR mechanism is also more parsimonious than the postulated general instability of one of the recombination products resulting from the symmetric DSBR pathway. BIR/RDR can produce a plethora of genome rearrangements including duplications, deletions, and inversions. Additionally, if strand invasion during BIR/RDR occurs on the same DNA molecule at a gapped site upstream of the invading single-stranded 3'-OH end, it can prime rolling-circle replication (McEachern & Haber, 2006; Hastings et al., 2009a). In this way, a single recombination event could rapidly amplify a given rearranged molecule and be important for SSS. A low level of HR using the DSBR pathway could also produce the reciprocal recombi-

**Fig. 4** Microhomology-mediated break-induced replication. (a) Single-ended double-stranded breaks (DSBs) produced by replication fork collapse can be nonconservatively repaired by the microhomology-mediated break-induced replication pathway (MMBIR). In the illustrated case, a single-stranded break on the lagging-strand template leads to replication fork collapse. Then, resection of the 5'-end on the broken DNA frees a 3'-OH single-stranded overhang which can invade a donor duplex at a microhomologous repeat. A replication fork is then established and DNA synthesis starts. The replication forks resulting from template switching often have low processivity and can ‘jump’ onto another template using multiple different repeats. Replication stops when polymerization reaches the end of the donor molecule. MMBIR can produce a wide variety of rearrangements which depend on the position of the template switch relative to the position of the replication fork collapse. If the switch occurs on a different DNA molecule behind the position of the break, a duplication is produced. Template switching on the same or a different DNA molecule ahead of the break will produce a deletion. Inversions can also be formed if the switch occurs in the wrong orientation on the same or a different molecule. A switch on the same molecule but behind the break at a single-stranded gap can produce a rolling circle which can rapidly amplify the rearranged DNA as illustrated in panel (b). This particular type of microhomology-mediated invasion can occur, for example, between two Okazaki fragments on the lagging strand of a stalled replication fork. Following invasion, a ligation step allows the formation of a circular molecule and rolling-circle replication ensues. This permits the rapid amplification of the DNA region between the two microhomologous repeats. Breakage of the circle reforms a single-ended DSB which can then recombine with another copy of the plastid genome through homologous recombination (HR) or microhomology-mediated recombination. The final product is a pDNA molecule containing concatemeric tandem repeats particularly prone to HR. This further recombination may create extrachromosomal double-stranded circular molecules. In this figure, the coloured rectangles indicate microhomologous repeats and junctions. (b) Modified from Hastings (2007).
nation products which are often observed at low levels in plant mitochondria (Wołoszynska et al., 2001; Zaegel et al., 2006; Wołoszynska & Trojanowski, 2009). However, SSS requires preferential replication of one of these two products and this is more readily explained through a BIR/RDR pathway. Alternatively or additionally, particular recombination products might be specifically amplified following mtDNA sorting during plant development.

The extensive illegitimate recombination that is triggered in the plastid genome following removal of ptWhirlies is also best explained by a special nonconservative BIR-like pathway called microhomology-mediated break-induced replication (MMBIR) (Maréchal et al., 2009; Fig. 4). In this case, the microhomologous repeats (<30 bp) involved in recombination of the plastid genome are too short to serve as HR substrates. Actually, efficient strand exchange catalysed by bacterial RecA and essential for HR requires approx. 50 bp of perfect homology (Watt et al., 1985; Lovett et al., 2002). The MMBIR pathway functions independently of RecA and can account for the observation of both duplication/circularization and deletion events in plants lacking ptWhirlies, whereas other asymmetrical pathways such as microhomology-mediated end-joining, single-strand annealing and nonhomologous end-joining only produce deletion events (Slack et al., 2006; McVey & Lee, 2008; and review by Hastings et al., 2009a). Similarly, MMBIR might also create some of the numerous microhomology-mediated rearrangements previously reported in plastids and mitochondria. Following replication fork collapse and production of a single-ended DSB, microhomology-mediated template switching occurs and primes DNA synthesis at a different location, allowing completion of DNA replication and concomitant production of rearranged DNA molecules.

Interestingly, MMBIR is a highly conserved error-prone recombination mechanism that is an important source of genomic rearrangements in humans, yeast and bacteria. In humans, MMBIR can generate large-scale modifications of the genome and copy-number variation that are associated with the development of several diseases (Lee et al., 2007; Hastings et al., 2009b; Zhang et al., 2009). This type of microhomology-mediated recombination is also favoured by DNA-related stresses, notably in association with DSBs, leading to the hypothesis that MMBIR might function as a back-up repair mechanism when the homologous recombination machinery is overwhelmed (Arlt et al., 2009; Hastings et al., 2009a). Such a repair mechanism could provide plant organelles with an additional layer of protection against highly deleterious genotoxic stresses. Additionally, as MMBIR enables DNA rearrangements and generates copy-number variation, it is able to rapidly modify the genome. Some of the incurred changes might improve the survival rate of plants exposed to unfavourable environmental conditions. A similar mechanism, called stress-induced mutation, is present in bacteria and also produces fast genomic changes through the error-prone repair of DSBs (Ponder et al., 2005). As described above, certain plants maintain microhomology-mediated DNA rearrangements in their organellar genomes, supporting the idea that some of these modifications might prove beneficial (Ogihara et al., 1988; Hiratsuka et al., 1989; Kanno et al., 1993; Moeykens et al., 1995).

The interior of organelles is teeming with reactive by-products of the electron transport machineries which are potent inducers of DNA damage. Unexpectedly, plant organellar genomes actually have lower synonymous-substitution rates than nuclear genomes, suggesting that they mutate at a slower rate (Wolfe et al., 1987; Drouin et al., 2008). One possible explanation for this is that organelles are particularly efficient at repairing their genomes through conservative pathways including HR. Plastids and mitochondria contain a large number of genome-equivalents and these multiple genome copies are brought together in ribonucleoprotein particles called nucleoids. This closeness might favour error-free DNA repair through intermolecular recombination. The common use of BIR-like mechanisms during replication and repair of DNA could enhance the homogeneity of genomes in individual plant organelles. Indeed, gene conversion by BIR can extend from the break site to the end of the donor duplex. This is much larger than the small regions surrounding the breaks that are converted during DSBR or SDSA (Fig. 1). Hence, BIR/RDR would facilitate the conservation of sequence over long segments of DNA, thereby contributing to the lower synonymous-substitution rate of plant organellar genomes and overall genome stability.

IV. Conclusion and perspectives

With its documented roles in replication as well as in the accurate and error-prone repair of plastid and mitochondrial DNA, recombination truly represents a cornerstone of plant organelle genome stability. The large number of repeats and highly unusual organization and structure of the plant mitochondrial genome make it appear quite chaotic compared with the plastid genome, which is remarkably uniform when examined using classical techniques such as DNA gel blots. However, when ptDNA is scrutinized using more sensitive techniques, one uncovers previously invisible layers of complexity. Indeed, fluorescence in situ hybridization (FISH) shows that reorganized molecules represent from 0.8 to 2% of all ptDNA (Lilly et al., 2001). It seems plausible that some of the atypical molecules detected by FISH represent the low level of MMBIR that was revealed by PCR in WT Arabidopsis plants (Maréchal et al., 2009). It is possible that these molecules constitute unavoidable by-products of essential recombination processes which are
required for the proper replication and repair of ptDNA. Thus, under normal conditions, both plastid and mitochondrial genomes are subject to spurious recombination events that generate a certain amount of variability. For both plant organelles, some of this variation is preserved during evolution. The highly dynamic nature of the mitochondrial genome has been proposed to result from selective pressure for frequent spontaneous CMS which favours outcrossing while preserving the highly efficient self-fertilization in the population (Mackenzie, 2005). By contrast, evolution seems to counterselect against the presence of large repeats in the plastid genome, presumably because of the deleterious effect of ptDNA instability on plant fitness.

Contrasting with the drastic differences in the plasticity of their genomes, both organelles use similar mechanisms of recombination and share some components of the recombination surveillance machinery, supporting a convergence in the strategies used by plastids and mitochondria to protect and propagate their genetic material. The precise function of these factors will be the subject of future investigation. Although one can easily imagine that MSH1 and RecA proteins in plant organelles might function similarly to their bacterial homologues, the precise roles of the plant-specific ssDNA-binding OSBs and Whirlies in the maintenance of organelle genome stability remain shrouded in mystery. Although often envisioned as simple DNA-coating proteins, bacterial and mammalian ssDNA-binding proteins are surprisingly versatile and dynamic and recent studies now depict them as crucial organizers and mobilizers of genome maintenance complexes (Richard et al., 2008; Shereda et al., 2008; Sakaguchi et al., 2009). Studying the interplay between the various organellar ssDNA-binding proteins and the rest of the recombination surveillance machinery of plant organelles will certainly prove highly interesting. Our understanding of the processes that preserve plant organelle genomes has progressed considerably in the last decade with the identification of the first few organelle DNA RRR proteins. This provides a basis for ongoing work that will unravel the detailed mechanisms of recombination, replication and repair in the small but crucial genomes of plastids and mitochondria.

Acknowledgements

We thank Laurent Cappadocia for his help with the analysis of organelle genome repeats. We thank members of the Brisson lab for inspiring discussions and critical reading of the manuscript. A.M. was supported by scholarships from the Natural Science and Engineering Research Council of Canada (NSERC) and the Fonds Québécois de la Recherche sur la Nature et les Technologies. Research in the Brisson lab is supported by a grant from NSERC.

References


Wise RP, Pring DR, Gengenbach BG. 1987. Mutation to male fertility and toxin insensitivity in tobacco (*t*)-cytoplasm male maize is associated with a


