DNA recombination in plants

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Chapter 3

Arabidopsis RecQsim, a plant-specific member of the RecQ helicase family, can suppress the MMS hypersensitivity of the yeast *sgs1* mutant

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ABSTRACT

The Arabidopsis genome contains seven genes that belong to the *RecQ* family of ATP-dependent DNA helicases. *RecQ* members in *Saccharomyces cerevisiae* (*SGS1*) and human (*WRN*, *BLM* and *RecQ4*) are involved in DNA recombination, repair and genome stability maintenance, but little is known about the function of their plant counterparts. The *Arabidopsis thaliana RecQsim* gene is remarkably different from the other *RecQ*-like genes due to an insertion in its helicase domain. We isolated the *AtRecQsim* orthologues from rice and rape and established the presence of a similar insertion in their helicase domain, which suggests a plant specific function for the insert. The expression pattern of the *AtRecQsim* gene was compared with the other Arabidopsis *RecQ*-like members in different tissues and in response to stress. The transcripts of the *AtRecQsim* gene were found in all plant organs and its accumulation was higher in roots and seedlings, as compared to the other *AtRecQ*-like members. In contrast to most *AtRecQ*-like genes, the examined environmental cues did not have a detectable effect on the accumulation of the *AtRecQsim* transcripts. The budding yeast *sgs1* mutant, which is known to be hypersensitive to the DNA damaging drug MMS, was transformed with the *AtRecQsim* cDNA. The *AtRecQsim* gene suppressed the MMS hypersensitivity phenotype of the *sgs1* cells. We propose that the Arabidopsis *RecQsim* gene, despite its unusual structure, exhibits an evolutionary conserved function.

INTRODUCTION

The genetic content of living cells persistently suffers from damage caused by external and internal factors and is maintained by various DNA repair mechanisms. These repair pathways, commonly, require the involvement of DNA helicases. Most DNA helicases share a highly conserved helicase domain with seven signature motifs (Gorbalenya et al., 1989; Ellis et al., 1995) and utilize the energy derived from hydrolysis of ATP to perform essential roles in DNA repair, transcription and replication (reviewed in Matson et al., 1994; Villani and Tanguy, 2000). According to the amino acid sequences of these motifs, the super family of DNA helicases can be divided into several sub families, one of which is the RecQ family of ATP- dependent DNA helicases (DEXH box DNA helicases). The RecQ protein was originally described in *Escherichia coli*, as a component of the RecF recombination pathway (Nakayama et al., 1985). Thereafter, proteins with high homology to the *E. coli* RecQ protein have been grouped and, until now, more than 60 members of this sub family have been reported from different organisms, including bacteria (*E. coli*), fungi (*Saccharomyces cerevisiae*, *Schizosaccharomyces pombe*, *Neurospora crassa*), animals (*Caenorhabditis elegans*, *Drosophila melanogaster*, *Xenopus laevis*, *Mus musculus*), human and plants (*Arabidopsis thaliana*). In all cases studied, RecQ proteins have been shown to exhibit DNA helicase activity in a 3' to 5' direction (Karow et al., 1997; Suzuki, 1997; Sun et al., 1999; Neff et al., 1999) and they are involved in the maintenance of genome stability

(reviewed by Chakraverty and Hickson, 1999; van Brabant et al., 2000; Hu et al., 2001). The *E. coli RecQ* gene is involved in DNA recombination (Nakayama et al., 1985) and a *recQ* null mutant shows an increased level of illegitimate recombination (Hanada et al., 1997). *S. cerevisiae*, which lacks a functional *SGS1* gene, shows an increased level of both homologous and illegitimate recombination (Gangloff et al., 1994) and exhibits a reduced life span (Sinclair et al., 1997; Sinclair and Guarente, 1997). Furthermore, *sgs1* cells are hypersensitive to the DNA-damaging drug methyl methanesulfonate (MMS) (Mullen et al., 2000; Miyajima et al., 2000a, b) and the DNA synthesis inhibitor hydroxyurea (HU) (Yamagata et al., 1998; Frei and Gasser, 2000). In contrast to unicellular organisms, which contain only one *RecQ*-like gene, multicellular organisms contain several homologues. For example, the human genome contains at least five *RecQ*-like genes. It has been shown that a mutation in the *WRN* gene, which results in Werner's syndrome (WS), causes reciprocal chromosomal translocations and extensive genomic deletions (Fukuchi et al., 1989). Mutations in the *BLM* gene, found in patients with Bloom's syndrome (BS), cause an increase in sister chromatid exchanges, which leads to hyperrecombination (Imamura et al., 2001). Patients with a mutation in the *RecQ4* gene suffer from Rothmund-Thomson syndrome (RTS) and show enhanced chromosomal rearrangements and somatic mosaicism (Kitao et al., 1999; Lindor et al., 2000). In addition, WS, BS and RTS patients exhibit an increased risk of cancer, have a high degree of genome instability and in most cases suffer from early ageing. Interestingly, both *WRN* and *BLM* genes can partially compensate for the hyperrecombination phenotype of the yeast *sgs1* mutant, however, only the *BLM* gene is able to suppress the *sgs1* HU hypersensitivity (Yamagata et al. 1998) and early ageing phenotypes (Yamagata et al., 1998; Heo et al., 1999). The results obtained from complementation studies, together with the sequence similarity of the helicase domain, suggest that the RecQ proteins exhibit evolutionary conserved functions.

Most of our knowledge about the role of helicases, especially in the maintenance of genome stability, is based on the results obtained from *E. coli*, yeast and human. Recently, it became clear that plants contain a large number of genes belonging to different classes of helicases (Arabidopsis Genome Initiative, 2000). For example, the DEAD-box RNA helicase family in Arabidopsis contains 53 putative members, but little is known about their functions (Aubourg et al., 1999; Boudet et al., 2001). The tobacco *VDL* gene encodes a plastid DEAD-box RNA helicase which is known to be involved in early chloroplast differentiation and plant morphogenesis (Wang et al., 2000). The Arabidopsis *NIH* gene is a putative member of the DNA/RNA helicases that is localised to the nucleus. It has been suggested that its basic amino acid sequences represent the DNA-binding domains while its tandem repeats are involved in dimerisation of the protein (Isono et al., 1999). The Arabidopsis *CAF* gene encodes a putative protein containing an N-terminal RNA helicase domain attached to a C-terminal RNaseIII-like domain. A mutation in the *CAF* gene converts the floral meristem to an undetermined state, which is coupled to the absence of axillary inflorescence meristems, thus, CAF appears to suppress the cell division in floral meristems (Jacobsen et al., 1999). These results indicate that plant RNA helicases are involved in various developmental processes; however, our knowledge about this gene family in plants is still scarce.

Hartung et al. (2000) have reported on the gene structure of Arabidopsis *RecQ*-like DNA helicases (Accession number AJ421618). The Arabidopsis genome contains seven *RecQ*-like genes located on four different chromosomes. Six of them are named *AtRecQl1*, *2*, *3*, *4A*, *4B* and *5*, respectively. The seventh homologue is called *AtRecQsim* (sim for

'similar') rather than *RecQ*-like because of the presence of an insert inside its helicase domain (Hartung et al., 2000). Nevertheless, their biological functions remain unclear.

Here, we begin with the functional characterisation of the Arabidopsis *RecQsim* gene through isolation of its rape and rice orthologues. We compare the structural features and gene expression profiles of the *AtRecQsim* gene with those of the other *AtRecQ*-like genes. In addition, we show that the *AtRecQsim* gene, despite its uncommon structure, is able to suppress the MMS hypersensitivity of the yeast *sgs1* mutant.

RESULTS

The *RecQsim* gene is plant-specific

The Arabidopsis RecQsim protein contains an insertion of ca. 100 amino acids between motifs III and IV of the helicase domain, as compared to other RecQ helicases. Therefore, it has been suggested that this protein might not be a functional RecQ-like helicase (Hartung et al., 2000). The deduced protein sequence of the *AtRecQsim* gene was used in a BLAST search and out of more than 60 *RecQ*-like genes that were found; we observed a single rice genomic fragment, which potentially encoded a protein with homology to the RecQ helicase domain and the AtRecQsim insert. The presence of a putative orthologue in rice implied that the *AtRecQsim* gene is functional and widespread in flowering plants. The putative RecQsim orthologues from rice (*Oryza sativa*, *OsRecQsim*) and rape (*Brassica napus*, *BnRecQsim*) were cloned and further supported this idea. Figure 1 shows the alignment of the deduced amino acid sequences of the Arabidopsis, rape and rice RecQsim proteins. The overall sequence homology (45% identity and 70% similarity) between the deduced Arabidopsis and rice RecQsim proteins is higher than that between AtRecQsim and its closest Arabidopsis homologue AtRecQl1 (20% identity and 44% homology) indicating that these genes are true RecQsim orthologues. The RecQsim helicase domains share the highest level of homology, where the three proteins are 58% identical and 79% similar. The homology is lower within the insert, however, a conserved region of 11 amino acids is present (VDDXDVX-CGEF), which might be of functional significance. The amino acid sequences of the insertions were used in a BLAST search and did not show homology to any other known protein or protein domain either in Arabidopsis or other organisms. Finally, the proteins share an area of homology at the N-terminus and 3' of the helicase domain. Together, the results suggest that RecQsim is a functional plant-specific gene.

The RecQsim proteins have unique properties

The protein domain structure of the putative RecQsim orthologues were studied in more detail and compared with the other AtRecQ-like proteins. The amino acid sequences of all proteins were analysed by the ProfileScan and Motifscan programs as described in Materials and methods and the results are presented in Figure 2. The N-terminus of the RecQsim proteins showed significant homology to the ubiquitin-associated domain (UBA). The UBA domain is a motif found in a variety of proteins, some of which are associated with the

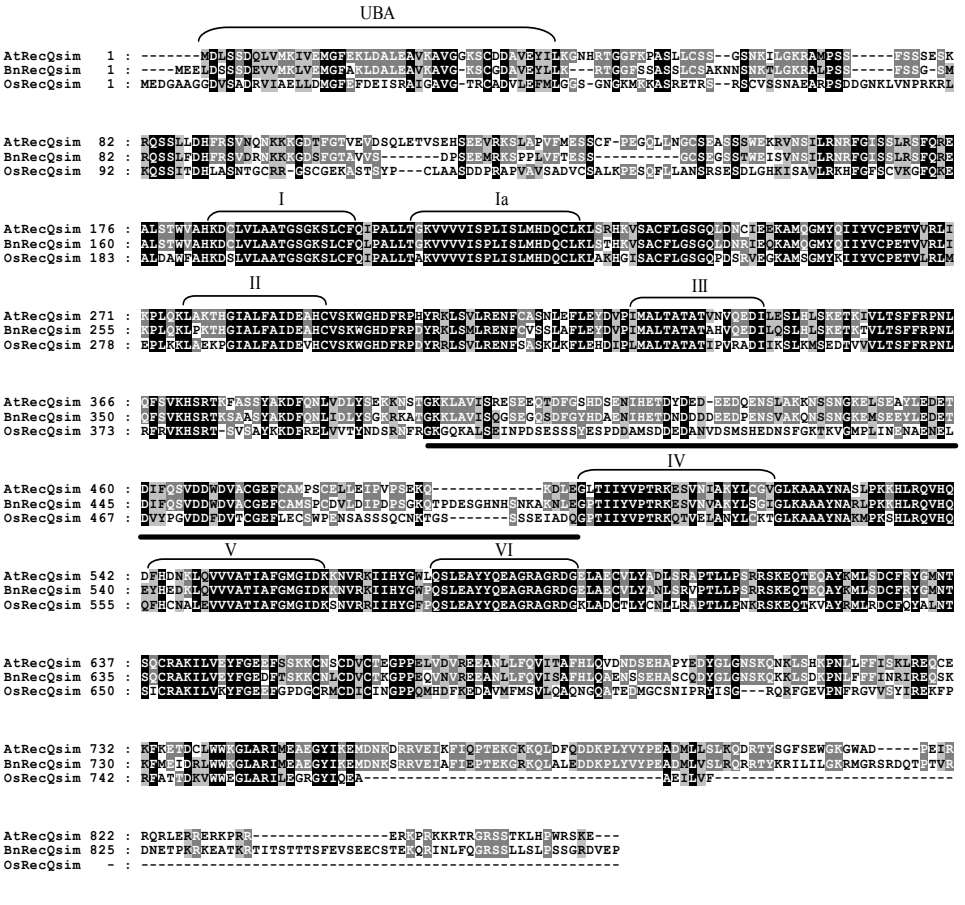

Figure 1. Amino acid sequence alignment of RecQsim proteins. The amino acid sequences of the predicted RecQsim proteins from Arabidopsis (AtRecQsim), rape (BnRecQsim) and rice (OsRecQsim) were compared using ClustalW, as described in Materials and methods. The seven helicase motifs and the ubiquitin-associated domain (UBA) are indicated. The insert is underlined.

ubiquitin-proteasome system (Bertolaet et al., 2001). Many RecQ members, including SGS1, WRN and BLM, have a helicase and RNaseD C-terminal (HRDC) domain. This domain was not found in the RecQsim proteins but is present in AtRecQl2, AtRecQl4A and AtRecQl4B. Further analysis revealed that the AtRecQl4A contains an EF-hand domain, which is postulated to be involved in cell signalling pathways (Michiels et al., 2002) and has not been reported in the RecQ helicases from other organisms. Remarkably, the AtRecQl4A and AtRecQl4B are highly homologous with 67% identity (83% similarity); however, only AtRecQl4A contains an EF-hand domain. The AtRecQl5 protein exhibits two distinct features. First, it has seven amino acid changes in the 100% conserved parts of the helicase domain, i.e., Y363F, G399S, D401N, L426M, D437A, R457N and R556G. Second, it contains an N-terminal proline-rich domain, which is postulated to be involved

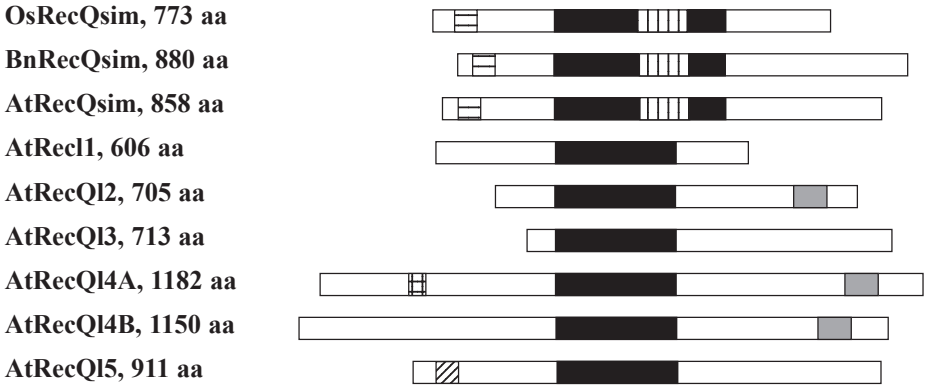

Figure 2. The RecQsim proteins are different from other AtRecQ-like members. The schematic structure of the Arabidopsis, rice and rape RecQsim proteins as compared with the other AtRecQ-like members. The proteins were analysed, using the ProfileScan program. The predicted domains are as follows: helicase domain (black), HRDC domain (grey), insert (vertically stippled), proline-rich region (diagonally striped), ubiquitin-associated domain (horizontally striped), EF-HAND domain (checked).

in non-sequence-specific DNA-binding properties of the Arabidopsis filamentous flower protein (Kanaya et al., 2002). These data confirm the notion that the RecQsim protein exhibits unique properties and suggests the same for some other AtRecQ-like proteins as well.

Expression pattern of the *RecQsim* genes in different organs of the plant

We compared the gene expression pattern of the *AtRecQsim* gene with the other *AtRecQ*-like genes in various organs of the plant at different stages of development. Preliminary results, obtained by northern analysis, revealed that the gene expression level of the *AtRecQ*-like genes is low (data not shown). Therefore, we exploited comparative RT-PCR to study their gene expression profile. Individual PCR reactions are inherently difficult to compare and therefore, several precautions were implemented to increase the validity of our comparison, as described in Materials and methods. Total RNA was isolated from 10 different tissues of the plant and used for the RT-PCR reactions. The result of this experiment (Figure 3A) indicated that the *AtRecQsim* gene has some expression characteristics in common with the other *AtRecQ*-like genes: (1) all seven homologues were expressed in all examined organs of the plant, (2) the amplification of transcripts of all seven homologues was higher in the tissues with high meristematic activity, and (3) as the organ aged (leaves and siliques) the mRNA accumulation of most of the genes dropped. Specific for *AtRecQsim* was the high level of RT-PCR product in roots and seedlings. *AtRecQl1* showed higher mRNA accumulation in siliques and flowers, while the transcripts of the *AtRecQl5* showed higher accumulation in the flowers but not in the siliques.

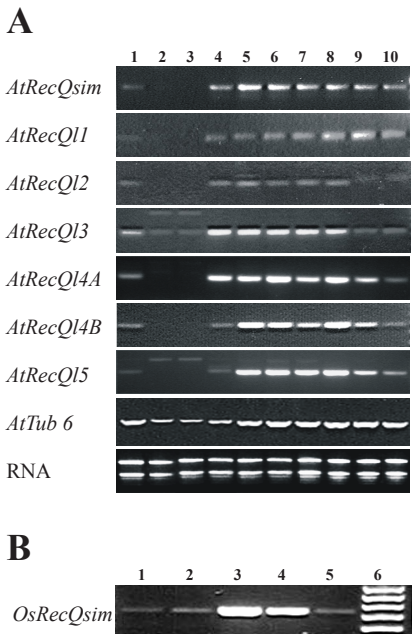

Figure 3. Expression pattern of the *AtRecQsim* gene as compared to other *RecQ*-like genes. **(A)** Total RNA was isolated from different plant tissues and used in comparative RT-PCR, as described in Materials and methods. 1, premature leaves; 2, fully expanded green leaves; 3, mature leaves with 20–30% yellow surface; 4, inflorescence; 5, roots; 6, seedlings; 7, top meristem; 8, flowers; 9, young siliques (less than 3 mm long); 10, mature green siliques (more than 7 mm long). The genes amplified in the RT-PCR are indicated. The *AtTub6* gene was used as a control. RNA, visual confirmation of RNA amount used in the RT-PCR reactions. All RT-PCR reactions were repeated at least three times with similar results. **(B)** Expression of the rice *OsRecQsim* gene. Total RNA was isolated from different tissues and used in RT-PCR. 1, root (mature plant); 2, young root (three-leaf stage plant); 3, flower (pre-anthesis); 4, young leaf (three-leaf stage plant); 5, mature leaf blade; 6, marker.

The expression pattern of the *OsRecQsim* gene in different organs of the plant was analysed by RT-PCR. The result of this experiment shows that the *OsRecQSim* transcripts are present in all examined organs, similar to that of the *AtRecQsim* gene (Figure 3B). In summary, the results suggest that the *AtRecQsim* gene has both common and specific expression characteristics, as compared to the other *AtRecQ*-like genes.

The gene expression profile of the *AtRecQ*-like genes under stress conditions

It has been shown that members of the RecQ family of helicases in yeast and human are involved in DNA repair via recombination (Chakraverty and Hickson, 1999; van Brabant et al., 2000; Hu et al., 2001). Several studies have revealed that, in Arabidopsis, stress can cause DNA damage (Ries et al., 2000) and an increase in the rate of homologous recombination (Lebel et al., 1993; Lucht et al., 2002). Therefore, the effect of stress on *AtRecQsim* gene expression was analysed and compared with the other *AtRecQ*-like genes. Total RNA was isolated from the aerial tissues of the plants grown under UV-light, NaCl, drought, cold and ABA stress conditions and was used in comparative RT-PCR reactions, using the same precautions as described above. A representative picture of the results is shown in Figure 4. Because every stress treatment had its own control condition, the rates of amplification in any stress condition can solely be compared with its own control treatment. The average intensity of the amplified products obtained from three replicates was quantified, as described in Materials and methods. According to this quantification, the expression level of the *AtRecQsim* gene was not affected in response to the examined

stress factors. The *AtRecQl3* gene showed a similar insensitivity, however, mRNA accumulation of the other *AtRecQ*-like genes responded to at least one stress condition. Upon cold treatment, the accumulation of both *AtRecQl1* and *AtRecQl4A* mRNAs was increased 3-fold, while that of the *AtRecQl4B* gene was decreased to the same extent. The NaCl and UV treatments did not have an obvious effect, while the ABA and drought treatments caused suppression in the mRNA accumulation of some of the *AtRecQ*-like genes. Most notably, drought treatment caused a 5-fold decrease in the *AtRecQl5* PCR product. Thus, the *AtRecQsim* gene expression did not respond to stress and this is different from several other *AtRecQ*-like genes.

Arabidopsis RecQsim complements the MMS hypersensitivity of the yeast *sgs1* mutant

Budding yeast (*S. cerevisiae*) contains one *RecQ*-like gene, called *SGS1*. The *sgs1* mutant shows hypersensitivity to the DNA synthesis inhibitor, HU and the DNA damaging compound, MMS. Recently, the MMS hypersensitivity has been widely used as a sensitive marker in complementation studies of the yeast *sgs1* mutant (Mullen et al., 2000; Miyajima et al., 2000a; Mullen et al., 2001; Fricke et al., 2001; Mankouri et al., 2002). Thus, we examined whether the *AtRecQsim* gene can complement this phenotype. We cloned the *AtRecQsim* cDNA 3' of the *GAL1* promoter and transformed *sgs1* cells with the resulting construct. The MMS hypersensitivity of the transformants was measured and compared with that of the yeast *sgs1* mutant and the corresponding wild type. The extent of the complementation was quantified by measuring the growth rate of the cells, as described in Materials and methods. The results represented in Figure 5A and 5B show that the *AtRecQsim* gene can suppress the MMS hypersensitivity of the yeast *sgs1* mutant.

DISCUSSION

The Arabidopsis genome contains seven members of the RecQ family of helicases, which is relatively high. Prokaryotes and unicellular eukaryotes possess a single RecQ-like gene, whereas the *C. elegans* and human genomes contain four and at least five members, respectively (reviewed in Chakraverty and Hickson, 1999). It appears that, in general, the number of *RecQ* genes expressed in each organism is related to the complexity of that organism (Chakraverty and Hickson, 1999). This may explain why the number of the Arabidopsis *RecQ*-like homologues is more than that of unicellular organisms but it cannot explain why Arabidopsis has more *RecQ*-like genes than *C. elegans* and human. Arabidopsis has additional homologues of many genes involved in repair and recombination (RAR), as compared to other organisms (Arabidopsis Genome Initiative, 2000). This might be related, partly, to the sessile nature of plants in escaping from harsh genotoxic conditions and the late differentiation of their germ cells, which can easily pass on mutations to the next generation. In addition, evolutionary analysis revealed that some RAR genes in Arabidopsis originated through relatively recent gene duplication, as seems to be the case for the *AtRecQl4A* and *AtRecQl4B* genes (Hartung et al., 2000), and through gene transfer from the organellar genomes to the nucleus (Arabidopsis Genome Initiative, 2000). However, at present it is not clear whether any of *AtRecQ*-like gene(s) might be of organellar descent.

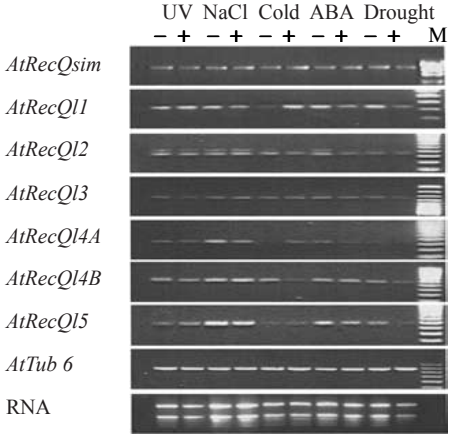

Figure 4. The effect of stress on the expression pattern of *AtRecQ*-like genes. Total RNA was isolated from the seedlings that were treated with (+) or without (-) the indicated stresses and used in comparative RT-PCR, as described in Materials and methods. The genes amplified in the RT-PCR are indicated. M, marker. The *AtTub6* gene was used as a control. RNA, visual confirmation of RNA amount used in the RT-PCR reactions. The RT-PCR experiment was repeated three times with similar results.

Among the Arabidopsis *RecQ*-like genes, *AtRecQsim* is a distinct member of the family, which as presented here, contains an insert of ca. 100 aa between motifs III and IV of its helicase domain. Our data show that the predicted rice and rape RecQsim proteins also contain a similar insert in the same position. The homology between the inserts of the RecQsim proteins indicates that this part of the gene has been introduced in their common ancestral gene before divergence of monocotyledons and dicotyledons. This suggests that the RecQsim gene may have a plant specific function, in which the insert plays a role. Although a similar-sized insert was not found in any other RecQ-like protein in a BLAST search, the homology between the RecQ members around the position of this insert is lower than at other parts of the helicase domain. Indeed, the *C. elegans* RecQ-like protein, E03A3.2, contains an insertion of ca. 10 amino acids in this region (Kusano et al., 1999), which indicates that some degree of variation is possible at this position. This is in agreement with the observation that the *AtRecQsim* gene can suppress the MMS hypersensitivity of the yeast *sgs1* mutant. The partial complementation, which is not unexpected in a heterologous system, can be further explained by at least two hypotheses. First, several studies have shown that a functional helicase domain is necessary for a plasmid-born SGS1 protein to suppress the MMS hypersensitivity of the yeast *sgs1* mutant (Onoda et al., 2000; Mullen et al., 2000; Saffi et al., 2000; Frei and Gasser, 2000; Miyajima et al., 2000a, b; Ui et al., 2001) and the 100 aa insertion may compromise the helicase activity of the AtRecQsim protein in yeast *sgs1* cells. Moreover, Ui et al. (2001) have shown that the physical and functional interaction between the SGS1 and topoisomerase III (TOP3) proteins is essential for a recombinant SGS1 protein to complement the HU/MMS sensitivity and hyper recombination phenotypes of a *sgs1* mutant. It has been suggested that the interaction between RecQ proteins and topoisomerases is conserved among all eukaryotes (Chakraverty and Hickson, 1999). This is in agreement with more recent reports showing that human BLM genetically interacts with yeast TOP3 (Wu et al., 2000) and that purified human TOPOIIIك is able to interact with yeast SGS1 (Ng et al., 1999). Although, up to date, there

is no direct evidence available demonstrating that plant RecQ members can bind to topoisomerases, our data showing that the AtRecQsim protein suppresses the MMS hypersensitivity of the *sgs1* mutant imply that this protein might be able to bind to yeast TOP3. The interaction properties may result in the observed partial complementation. Together, the results suggest that, although *AtRecQsim* is a unique gene among all known *RecQ* members, it might be a functional homologue of the yeast *SGS1* gene. However, we cannot exclude the possibility that *AtRecQsim* acts in an *SGS1*-independent pathway to suppress the MMS hypersensitivity of the yeast *sgs1* mutant.

The *in silico* structural analysis of the Arabidopsis RecQ members indicates that, in addition to the *AtRecQsim* gene, some of the other Arabidopsis *RecQ* members show distinct features as well. Only AtRecQl2, 4A and 4B proteins carry the helicase and RNaseD C-terminal (HRDC) domain in their C-terminus. This domain, which is present in most RecQ family members studied, represents a structural scaffold that resembles auxiliary domains in proteins involved in nucleic acid metabolism and has been suggested to have a role in the functional differences between WRN and BLM proteins (Liu et al., 1999). In agreement with this notion, Moser et al. (1999) reported that 6 of 19 mutations found in WS patients were mapped within the exons encoding the HRDC domain. In addition to RecQ members, this domain is present in the human PM-Scl autoantigen protein, which is an RNase D homologue, and patients carrying a mutation in this gene are affected by polymyositis and scleroderma (Ge et al., 1992). Surprisingly, the HRDC domain of the SGS1 protein is, evidently, dispensable for the complementation of MMS sensitivity and hyper recombination phenotypes of the yeast *sgs1* mutant (Miyajima et al., 2000a, b; Ui et al., 2001). Therefore, the actual role of this domain in RecQ-like proteins remains unclear. AtRecQl4A, in contrast to AtRecQl4B, contains an EF-hand domain, which has not been reported to be present in other RecQ members. The EF-Hand domain is a Ca^{2+} binding domain, which is found in proteins that are involved in cell signal transduction (Michiels et al., 2002; Haeseleer et al., 2002). The AtRecQl5 protein is remarkably different from the other RecQ members. This protein shows alterations in seven positions inside the helicase domain where all other studied RecQ members contain identical amino acids. This suggests that this protein may have specific biochemical properties. However, it has been shown that an alteration in three of the 7 mentioned positions (D819, D853, R970 in SGS1 protein that correspond to the AtRecQl5 protein as N401, A437 and G556, respectively) did not affect the ability of an altered recombinant SGS1 protein to complement the MMS/HU sensitivities of the *sgs1* mutant (Ui et al., 2001). Therefore, the effect of amino acid changes in the AtRecQl5 protein on the helicase activity of the protein remains unclear. Together, the presence of the mentioned additional domains at the N- or C-terminal regions of the AtRecQ-like proteins suggests that they exhibit specialised functions. This notion is supported by data obtained from other RecQ members studied. Three human RecQ members studied, WRN, BLM and RecQ4, contain variation in their N and C-terminal regions (Lu et al., 1996; Karow et al., 1997; Sun et al., 1999; Neff et al., 1999). The WRN protein contains an N-terminal exonuclease domain and a C-terminal nuclear localisation signal (Kitao et al., 1999). RecQ4 contains an N-terminal ARG-rich domain, while BLM protein carries an ASP-rich domain in its N-terminal region. The fact that a mutation in any of these genes causes a different disease (WS, BS and RTS, respectively), supports the notion that these genes fulfil specialised roles in cellular metabolism. Furthermore, von Kobbe et al., (2002) showed that WRN and BLM proteins physically and functionally interact with each other. These authors indicated

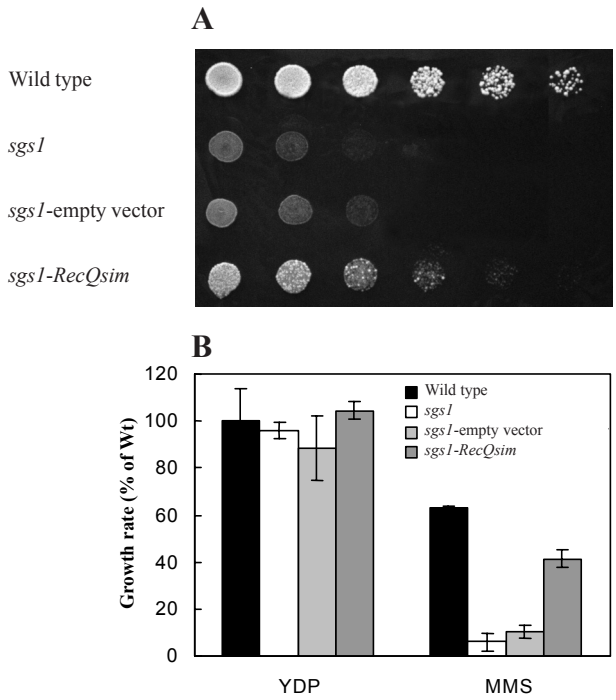

Figure 5. Partial complementation of the MMS hypersensitivity of the yeast *sgs1* mutant by the *AtRecQsim* gene. The *sgs1* mutant strain (KY12) was transformed with either the empty vector or a plasmid containing the *AtRecQsim* gene. Transformants were cultured on SC medium at 30 °C. **(A)** Exponentially growing cultures of the wild-type, *sgs1* and *sgs1* cells transformed with either the *AtRecQsim* cDNA or empty vector were serially diluted 5-fold (left to right) and each sample was spotted on YDP/GAL plates supplemented with 0.01% MMS and photographed after 3 days of growth at 30 °C, as described in Materials and methods. Four independent colonies of transformants were examined with similar results. **(B)** Liquid YDP/GAL mediums supplemented with or without 0.01% MMS were inoculated with overnight growing cultures of indicated cells. The growth rate of the colonies was measured after 20 h of growth at 30 °C and reported as percentage of the wild type in the absence of MMS. The error bars represent the standard deviation of the means.

that the exonuclease domain of the WRN protein participates in this interaction and, as the result of the interaction, the BLM protein can suppress the exonuclease activity of the WRN protein (von Kobbe et al., 2002). This interesting finding confirms the importance of the N-/C-terminal domains of the RecQ proteins and is in agreement with the idea that the N and C-terminal domains of the Arabidopsis RecQ-like proteins may play crucial roles.

The results presented in this article suggest that the expression pattern of most *AtRecQ*-like genes is age-dependent. Kawabe et al. (2002) also reported that the expression of human WRN, BLM, RecQ4 and RecQ1 proteins are differentially upregulated in cells that are actively proliferating, and their expression levels are modulated in different ways during the cell cycle. Their results have been confirmed by Gee et al. (2002) who showed that the

expression of the rat *WRN* gene was strongest in the embryonic rat brain and significantly decreased in adult brain cells. In addition, a strong correlation was found between the expression of *BLM* and the proliferation status of cells, as this gene is expressed at higher levels in tumours than in normal quiescent tissues (Turley et al., 2001). Altogether, it seems that the higher expression level of the *AtRecQ*-like genes in tissues with higher meristematic activity is similar to that of the *WRN* and *BLM* genes in human cells. The presented data indicating that the *AtRecQ*-like genes are expressed at low levels and their transcripts are detectable in all organs of the plant is, in general, in agreement with previously published results (Hartung et al., 2000). Furthermore, as shown here, the expression of most Arabidopsis *RecQ*-like genes in response to various environmental cues is differentially regulated. Thus, it seems likely that the expression characteristics contribute to the specific functions of the *AtRecQ*-like genes.

In conclusion, we have shown that the Arabidopsis *RecQ*-like genes exhibit different gene expression profiles and encode proteins with distinct structural features. The *RecQsim* gene appears to be unique, but widespread in the flowering plants. Despite its unusual helicase structure, the function of RecQsim is evolutionary conserved as judged by its ability to suppress the MMS hypersensitivity of the yeast *sgs1* mutant. Further analysis of the complementation ability of the other *AtRecQ*-like genes in yeast *sgs1* mutant, together with the study of *AtRecQ*-like knockout mutants may help to understand the function of the Arabidopsis *RecQ*-like genes in plant metabolism.

MATERIALS AND METHODS

Plant materials and growth conditions

All experiments with Arabidopsis were performed with *Arabidopsis thaliana* accession Columbia (Col-0). Seeds were surface-sterilized and sown on growth medium (GM) as described (Valvekens et al., 1991). Plates were kept at 4 °C in the dark for 4 days, then transferred to a climate room at 21 °C and 65% relative humidity with 16 h light (60 µmol $m^{-2}s^{-1}$)/8 h dark cycles. Alternatively, seeds were sown directly on soil and after four days of cold treatment at 4 °C in the dark, were transferred to the same climate room.

Salinity treatment: seeds were surface-sterilized and sown on square GM plates, as described above. The plates were placed vertically to allow the roots to grow on the surface of the medium. After 10 days, about 15 seedlings were transferred onto Whatman 3MM papers soaked in GM liquid medium with or without 200 mM NaCl and kept in the climate room for 5 h.

ABA treatment: 10-day old seedlings grown on GM plates were sprayed with 10 ml of 100 µM ABA/0.1% v/v methanol or H_20/0.1% v/v methanol as a control and were kept in the climate room for 4 h.

UV treatment: surface-sterilized seeds were grown vertically on GM plates. After 10 days of growth, plates were exposed to a mixture of white (160 µmol $m^{-2}s^{-1}$) and UV-B (1 kJ $m^{-2}h^{-1}$) lights for 3 days. Four tubes of type TLD58W/84 and two tubes of type TLD58W/83 provided the white light. The UV light was provided by two fluorescent tubes, type TL40W/12 (Philips, Netherlands). The light was either filtered through a cellulose diacetate

foil (CA, Tamboer, Netherlands) to eliminate UV-C or a polyester foil (Mylar type D, Du Pont de Nemours, Luxembourg) to eliminate all UV radiation for the control plants.

Cold treatment: GM plates containing 10-day old seedlings were covered by aluminium foil and either kept on ice or at 21 °C in the dark for 48 h.

Drought treatment: 14-day old seedlings grown on GM plates were either transferred onto three pieces of dry Whatman 3MM paper for 3 h or transferred to water-soaked Whatman 3MM papers.

After the treatments, the plants were harvested in liquid nitrogen and stored at -80 °C until used for RNA extraction.

RNA extraction

Total RNA from Arabidopsis young leaves (10 days old) and roots (20 days old) was extracted from plants grown on GM medium. Total RNA from mature green leaves and the leaves with 20–30% yellow surface was extracted from plants grown for 30 days on soil. Total RNA from flower buds, young siliques (less than 3 mm long), mature siliques (more than 7 mm long), and top meristem (2 mm of the inflorescence meristem without the floral buds) was isolated from plants grown on soil at different stages of growth. To isolate total RNA from the inflorescence, the top meristem and the flower buds were harvested together from plants grown on soil. Harvested plant tissues were frozen in liquid nitrogen and were stored at -80 °C until used for RNA isolation as described (Sambrook and Russell, 2001). Rice RNA samples were generously provided by Annemarie Meijer (Ouwerkerk et al., 2001). Total RNA from *Brassica napus* L. cv. Jet Neuf (rape) roots and embryo (3 weeks old) was isolated with TRIzol reagent (BRL Life Technologies). Poly(A)$^+$ mRNA was isolated with the mRNA Separator Kit (Clontech), according to the instructions of the supplier.

Molecular cloning and sequencing of the *RecQsim* cDNAs

The cDNA sequence of the rice *OsRecQsim* gene (Accession number AY180331) was determined by cloning and sequencing of internal cDNA fragments and the 5'/3' ends, with gene-specific primers and the 5'/3' RACE kit (Roche). To obtain the full-length cDNA sequence of the rape (*BnRecQsim*) gene (Accession number AY180332), a rape root cDNA library was constructed in the vector λTriplEx2 with the SMART cDNA Library Construction Kit (Clontech). Screening of 1.5×10^5 recombinant clones with a 3' *Nco*I-*Sca*I *AtRecQsim* cDNA fragment resulted in the isolation of a 1744 bp cDNA fragment, corresponding to the 3' part of the *BnRecQsim* gene. Gene specific primers, designed according to the sequence of this fragment, were used in the Marathon cDNA Amplification kit (Clontech) following the instructions of the manufacturer.

Comparative RT-PCR

The reverse transcription (RT) reactions were performed in a total volume of 20 µl, with 2 µg of total RNA and 0.2 µM oligo-dT primers, using the Omniscript RT kit (Qiagen, Germany), following the instructions of the supplier. The RT enzyme was subsequently heat inactivated at 95 °C for 5 min. and the RT reactions were diluted 4 times with water. A

single RT reaction of each tissue/stress condition was used for comparative RT-PCR reactions of all *AtRecQ*-like genes. The PCR reactions contained 0.2 mM of each dNTPs, 0.2 µM of each primer, 4 µl of the diluted RT reaction, 1× Taq polymerase buffer and 1 unit of Taq DNA polymerase (Roche Diagnostics, Germany). The following primers, which were designed based on the sequences of the splicing sites to inhibit the competitive effect of the genomic DNA amplification, were used in the comparative RT-PCR reactions. *RecQl1*, 5'AAGAGCCATCACAGGAACACGG, 5'ACAAGAACTTAGAAGAGATAATC *RecQl2*, 5'AACACTGCTCACAAATTTGACG, 5'ATTCAGGATCAGGTTATGGGTT *RecQl3*, 5'TGAAAGAAGACTTGAGGACCAA, 5'TTAAGAACAAGATCCATGAAGAC *RecQl4A*, 5'CTTCTTTCACACTGGCTGTTGC, 5'TAATGAACCTATTGCAGGCCAA *RecQl4B*, 5'GCTTATTGATTCCCCCATTCCA, 5'GTGGCAAAAGTGAATCCCTTTT *RecQl5*, 5'GTATCAGAATGGTCTCATAACT, 5'CATGCCGAATGCTACAGTTGCA *RecQsim*, 5'TGGTTCAGGCCAACTTGATAAT, 5'ACTATGTTTAACAGAAAATTGTAG *Tubulinβ6*, 5'TGCGACTGTCTTCAAGGTTTCC, 5'GCTCTCAGCTTCAGTAAACTCCA *OsRecQsim*, 5'GCATTGACTGCTACTGCTACCATC, 5'GACATTTGATTTGTCAATTCC CAT.

A cDNA fragment of the *Tubulinβ6* gene (Accession number AF360260) was used as the control of the efficiency of RT reactions and to confirm the equality of the amounts and integrity of RNA used in the RT reactions. In all cases, the RT-PCR reactions were performed in a total volume of 50 µl and samples of 10 µl of the PCR reactions were analysed electrophoretically after 20, 25, 30 and 35 cycles to find the linear range of amplification for each gene. All RT-PCR experiments have been repeated three times and the intensity of the amplified products was determined with Sigma Scan Pro software (SPSS, USA).

Computer analysis

The BLAST searches were performed with the TAIR and NCBI BLAST programs at http:/ /arabidopsis.org/ Blast/ and http://www.ncbi.nlm.nih.gov/BLAST/, respectively. The deduced amino acid sequences of the proteins were analysed for their domains with the ProfileScan program at http://www.ebi.ac.uk/interpro/scan .html and the Motifscan at http:/ /hits.isb-sib.ch. The alignment of the Arabidopsis, rape and rice *RecQsim* genes was performed using the ClustalW program at http://npsa-pbil.ibcp.fr/.

Yeast complementation assay

The wild-type DH6.61D (*MATa trp1 ura3 his3 leu2 can1 cyh2*) and *sgs1*, KY12 (*MATa his3 leu2 ura3 can1 cyh2*) strains used in this study were kind gifts from Hideo Ikeda (Yamagata et al., 1998). An *AtRecQsim* cDNA fragment containing the complete ORF was amplified by reverse transcription-PCR with primers 5'ATTACTGGTACCAATTCGATGG ATTTGTCTTCTGAT and 5'GAATAAGAAGGTACCTCTTTGCTTCTCCATGGATGT, containing the *Kpn*I site (underlined) and cloned in pGEMT-Easy (Promega). Sequencing of the cloned fragment confirmed that the cDNA was error free. The *Kpn*I fragment of this plasmid was further cloned in the yeast expression vector pYES2/CT (Invitrogen), following the instructions of the supplier. The yeast *sgs1* competent cells were transformed by electroporation and positive clones were isolated on Ura$^-$ plates. Subsequently, exponentially

growing cultures were serially diluted by 5-fold, starting from cultures at the same OD_{600} and 5µl samples were spotted onto YPD/Gal plates supplemented with 0.01% v/v MMS, essentially as described by Ui et al. (2001) and Yamagata et al. (1998). The plates were incubated at 30 °C for 3 days and photographed. The extent of the complementation was quantified by inoculation of liquid YDP/GAL medium, with or without 0.01% v/v MMS, with overnight growing cultures of similar OD_{600}. The growth rate of the colonies was measured after 20 h of growth at 30 °C.

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