The MRN Complex of Wheat

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Abstract: The MRN complex is formed by the interaction of the products of the *Mre11*, *Rad50* and *Nbs1* genes. This complex plays a central role on repair of double-strand breaks (DSBs) and acts in a great number of cellular processes. In this study we have performed the analysis of the MRN complex in diploid and polyploid species of wheat. The molecular characterization was carried out in the diploid *T. monococcum* (genome A) and *Ae. tauschii* (genome D) and in the tetraploid *T. turgidum* (genomes A and B). The results obtained showed that in all cases the genes presented the main characteristics previously described in other species. A modified FISH protocol was used to locate the *Rad50, Mre11* and the *Nbs1* genes on the homoeologous chromosomes 5, 2 and 1, respectively. Analysis of expression showed that the hexaploid *T. aestivum* was the species with the higher level of expression whereas the rest of the species analysed showed no relation with its ploidy. Also, quantification of the expression of each homoeologous gene in the polyploid species evidenced in some cases a process of silencing after polyploidization. The study of the interaction between the proteins demonstrated that the interaction of proteins was not restricted to each genome, detecting interaction between proteins belonging to different genomes.

Keywords: homoeologues; homologues; *Mre11*; MRN; *Nbs1*; *Rad50*

The MRN complex plays a central role on repair of double-strand breaks (DSBs) and acts in a great number of processes including cell-cycle checkpoint signaling, homologous recombination (HR), non homologous end joining (NHEJ), replication, meiosis and telomere maintenance. This complex is a hexamer (Figure 1) formed by the interaction of the products of the *Mre11*, *Rad50* and *Nbs1* (*Xrs2* in yeast) genes (Williams *et al.* 2009). The importance of these genes is revealed by the fact of that orthologous of the genes *Mre11* and *Rad50* have been identified in all taxonomic kingdoms whereas *Nbs1* is found only in eukaryotic cells. Further, disruption of any of these genes are lethal in mammals whereas hypomorphic mutations in humans lead to diseases like Nijmegen breakage syndrome (NBS), ataxia-telangiectasia-like disorder (ATLD) and NBS-like disorder (NBSLD) (for mutations in *Nbs1*, *Mre11* and *Rad50* respectively. The variety of MRN functions is owed to several enzymatic activities and structural characteristics of the members of the complex. Thus, the interaction of multiple MRN

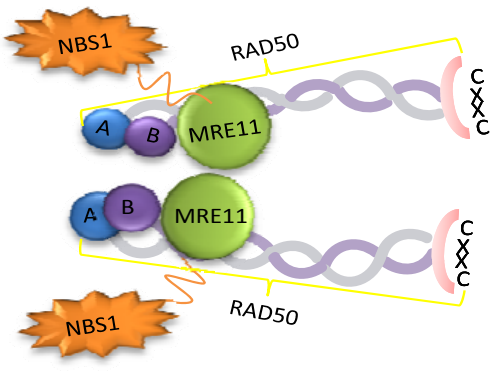

Figure 1. Structure of the MRN complex

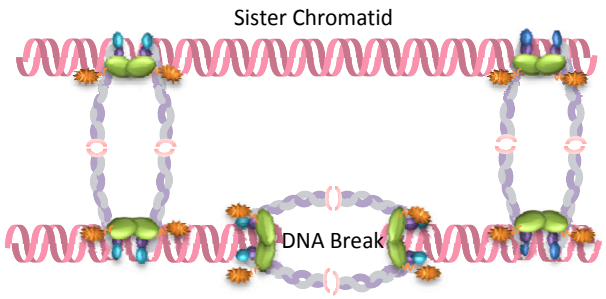

Figure 2. Structural function of the MRN complex tethering DNA breaks and sister chromatid

complexes functions as a scaffold bridge, tethering duplexed DNA not only to organizing DNA for repair but also to tether sister chromatids during homologous recombination (Figure 2).

In spite of the importance of this complex, most of the work has been done in yeast and human and only a few works on model plant species have been reported. In this work we present the characterization of the complex in diploid and polyploid species of wheat emphasizing the analysis of homologue and homoeologue expression and the study of interactions between the genes.

MATERIALS AND METHODS

MRN genes were characterized from diploid species *Triticum monococcum* L. (genome A) and *Aegilops tauschii* Coss. (genome D), and from the tetraploid species *Triticum turgidum* L. cv. Vitron (genomes A and B). Expression analyses were also extended to the hexaploid wheat *Triticum aestivum* L. cv. Chinese spring (genomes A, B and D). The assignation of genes to genomes was performed using nulli-tetrasomic lines of hexaploid wheat cv. Chinese spring. Molecular characterization was carried out by comparative genetic as previously described (De Bustos *et al.* 2007). FISH localization of genes was performed as described by Pérez *et al.* (2009). Expression analyses were carried out by QPCR using LNA probes (Roche) following the recommended protocol provided by the manufacturer. Analysis of interaction was performed using the Matchmaker LexA Two-hybrid System (Clontech).

RESULTS AND DISCUSSION

The results of molecular characterization are presented in Table 1. Homoeologous genes of the three components of MRN complex showed a high level of homology between them, and in phylogenetic studies, they were also preferentially grouped with the genes belonging to plant species *Arabidopsis* and rice, showing more evolutive distance with those of the human and yeast genes. TyrFISH technique allowed the location of *Mre11* on the long arm of homology group two, *Rad50* on

Table 1. Results of molecular characterization of the MRN genes in the wheat species analysed

MRN genes (species)	Size in pb of gDNA (databank accession)	Size in bases of mRNA (databank accession)	Protein (aminoacids)
Mre11A (*T. monococcum*)	4678 (AM049169)	2457 (AM049175)	699
Mre11A (*T. turgidum*)	4662 (AM049171)	2510 (AM049174)	699
Mre11B (*T. turgidum*)	4719 (AM049172)	2440 (AM049173)	699
Mre11D (*Ae. tauschii*)	4766 (AM049170)	2456 (AM049176)	699
Rad50A (*T. monococcum*)	17006 (EU159421)	4508 (EU159417)	1316
Rad50A (*T. turgidum*)	16093 (EU159423)	4466 (EU159419)	1316
Rad50B (*T. turgidum*)	16942 (EU159424)	4473 (EU159420)	1316
Rad50D (*Ae. tauschii*)	16470 (EU159422)	4387 (EU159418)	1316
Nbs1A (*T. monococcum*)	3772 (EU561339)	1832 (EU561338)	575
Nbs1A (*T. turgidum*)	3772 (EU561343)	1834 (EU561342)	575
Nbs1B (*T. turgidum*)	3799 (EU561345)	1842 (EU561344)	575
Nbs1D (*Ae. tauschii*)	3790 (EU561341)	1830 (EU561340)	575

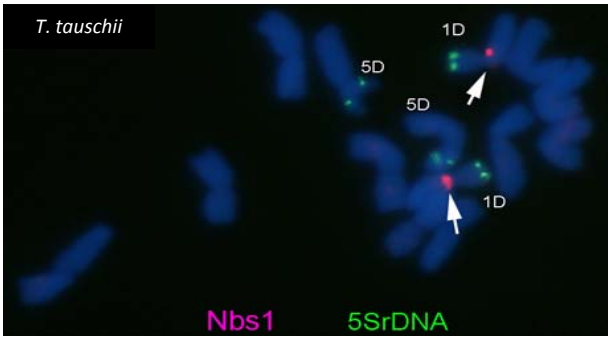

Figure 3. Chromosome location of *Nbs1* gene by Tyr-FISH in the wheat species *T. tauschii*; probe 5s rDNA identify chromosomes 1 and 5 of wheat; the *Nbs1* gene is located on the long arm of chromosome 1

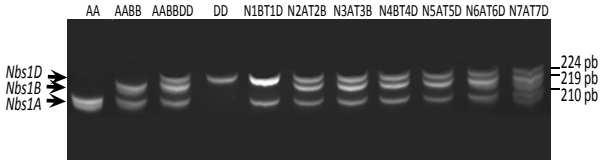

Figure 4. Assignment of *Nbs1* genes to wheat genomes using the nullitetrasomic lines of wheat

the short arm of homology group five and *Nbs1* on long arm of homology group one (Figure 3). The genes characterised were assigned to genomes using nullitetrasomic lines (Figure 4).

The results of the expression analyses of the *Nbs1* gene (Figure 5) showed the lower values in the wheat species analysed respect to the other two genes. Expression of *Mre11* and *Rad50* was similar in *T. monococcum* and *T. turgidum* accordingly with the equimolar ratio observed for their proteins in the MRN complex (De Jager *et al.* 2002). However, the expression observed for these two genes was different in the other two species, mainly for *T. aestivum* where *Rad50* showed the highest value of expression obtained in the study.

The relative expression of each of the three homoeologs (A, B and D) of the MRN genes was analysed in the two polyploid species of wheat (Figure 6; data showed only for *Nbs1*). In the three genes, a clear biased expression was detected between homoeologs (Table 2). The biased expression of B homoeologs might indicate ongoing silencing of one or both other homoeologues in polyploid wheat (De Bustos *et al.* 2007). The interactions between the products of individual homoeologues was analysed by two-hybrid to determine whether the protein binding required to form the MRN complex is restricted to the products of genes carried by the same genome or whether proteins encoded by different genes, independent of the genome to which they belong, are involved. The results obtained indicate the inexistence of specificity in the protein interactions. This would guarantee the formation of an MRN complex in wheat.

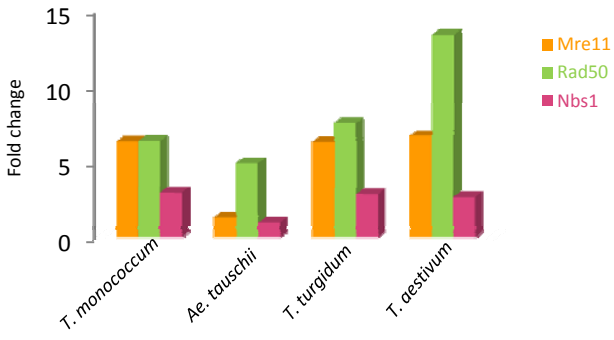

Figure 5. Analyses of expression of the MRN genes in the wheat species; the expression of *Nbs1* in the species *Ae. tauschii* was used as calibrator

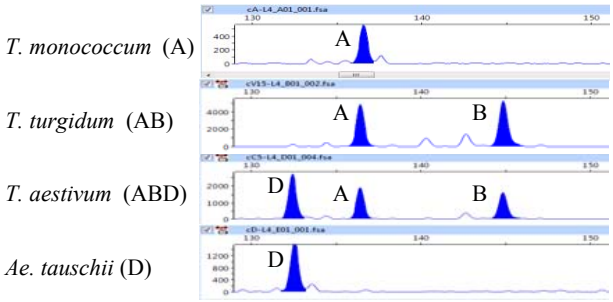

Figure 6. Expression of *Nbs1* homoeologous genes in the wheat species assessed by SSCP

Table 2. Expression of homoeologous MRN genes in the polyploid wheat species (in %)

Genes	Species	Homologs		
		A	B	D
Mre11	*T. turgidum*	69	31	–
	T. aestivum	42	18	40
Rad50	*T. turgidum*	30	70	–
	T. aestivum	25	60	15
Nbs1	*T. turgidum*	73	27	–
	T. aestivum	71	24	5

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