Characterization of Arabidopsis thaliana ortholog of the human breast cancer susceptibility gene 1: AtBRCA1, strongly induced by gamma rays

S. Lafarge and M.-H. Montané*

CEA Cadarache, DSV-DEV, Laboratoire de Radiobiologie Végétale, Bat 185, F-13108 St Paul Lez Durance Cedex, France

Received November 18, 2002; Accepted December 5, 2002 DDBJ/EMBL/GenBank accession no. AF515728

ABSTRACT

hBRCA1 is involved in 20–45% of inherited breast cancer cases and is implicated in many mechanisms involved in response to DNA damage. To date, BRCA1 orthologs have been characterized in vertebrate genomes only. We have identified the ortholog of BRCA1 in Arabidopsis thaliana. AtBRCA1 is a 5.5 kb part of the locus At4g21070. The corresponding mRNA of 3.5 kb is composed of 14 exons and encodes a 941 amino acid protein (104 kDa). AtBRCA1, which has one N-terminal RING finger, two C-terminal BRCT and the p300/CBP interacting domain, shows a high similarity to hBRCA1 in these motifs and has the same characteristic molecular organization. We have also identified a putative ortholog in rice (OsBRCA1). With 941 and 968 amino acids, respectively, AtBRCA1 and OsBRCA1 are the shortest members of the BRCA1 family, and may represent a plant specificity. AtBRCA1 is expressed ubiquitously in plant tissues, at levels depending on organ type, with highest levels in flower buds and exponentially growing cell cultures. Increase of mRNA levels in all plant tissues 1 h after irradiation with the highest induction level of approximately 150 times for a 100 Gy dose is consistent with a putative role of AtBRCA1 in DNA repair and in cell-cycle control.

INTRODUCTION

DNA damage induced by gamma irradiation, or by other factors such as radio-mimetic drugs, leads to the enhancement of several pathways involved in DNA repair, cell-cycle control, transcriptional regulation and apoptosis. Two major mechanisms are responsible for the repair of double-strand breaks (DSB) generated by ionizing radiation (IR): homologous recombination (HR) and non-homologous end joining (NHEJ) (1–3). Several genes implicated in these pathways are remarkably conserved in different species and are characterized by specific domains (4,5). Genome sequencing of different organisms now allows us to find, by homology searches, new orthologs of genes described earlier.

Several Arabidopsis thaliana genes, identified previously in other organisms, are known to be involved in the repair of DSB. Among them, AtXrcc4 (6), AtLig4 (6), AtRad50 (7), AtKu70–80 (8) and AtMre11 (9) are mainly implicated in NHEJ, AtATM (10) and AtRAD51 (11) in HR. At date, orthologs of hBRCA1, implicated in either HR or NHEJ, and also in several other pathways, have been described only in vertebrates: in mammalian (12–14), Xenopus (15) and Gallus genomes (16).

hBRCA1 is located at 17q21 and is involved in 20–45% of inherited breast cancer cases and ~80% of families predisposed to breast and ovarian cancer (17). hBRCA1 is a 220 kDa nuclear phosphoprotein with functional domains: an N-terminal RING finger domain, and two BRCA1 C-terminal (BRCT) domains with a transactivating activity in the C-terminus (18). hBRCA1 is phosphorylated by kinases like ATM (19), chk2 (20) or ATR (21) after DNA damage that leads to delocalization of hBRCA1 and enhancement of transcription-coupled DNA repair (22). Phosphorylation of the transcriptional repressor CtIP by ATM after DNA damage releases hBRCA1 from its interaction with CtIP and allows its transcriptional activity (23,24).

hBRCA1 is involved in transcription regulation, by transactivating transcription through direct protein interactions. hBRCA1 stimulates p53 transcriptional activity on the p21waf1/cip1, mdm2 and bax promoters (25,26), but also in a p53-independent manner for p21waf1/cip1 and c-myc promoters (27,28). Harkin et al. (29) have shown that enhanced hBRCA1 expression led to the modulation of the expression of several genes, such as GADD45. Moreover, hBRCA1 is a component of the RNA polymerase II holoenzyme (30,31) and interacts with proteins involved in the regulation or the transcription machinery, including CtIP (32), CREB binding protein (CBP)/p300 (33) and RNA helicase A (34). A role in chromatin remodeling has been attributed to hBRCA1 through its association with the histone deacetylases HDAC1, HDAC2 (35) and the BRG1 subunit of the SWI/SNF complex (36). Recently, it has been shown that hBRCA1 can also bind directly to DNA, a property which may be associated with its roles in transcription and in DNA repair (37).

hBRCA1 is also involved in maintaining genome integrity in several ways. First, it interacts in nuclear foci with several
proteins involved in DNA repair such as hRad51 (38), BRCA2 (39,40), hRad50/hMre11/nibrin (41) and the BRCA1-associated genome surveillance complex (BASC) (42). Secondly, it is involved in controlling centrosome duplication by interaction with gamma-tubulin, a component essential for nucleation and mitotic spindle assembly (43). Xu et al. (44) have found that 30% of brca1

Gene structure prediction was identified using BLAST implemented on NCBI databases with the hBRCA1 sequence, using the SMART database (http://smart.embl-heidelberg.de/), alignment of sequences using Alignp, and phylogenetic analysis using PHYlogeny Inference Package on Infobiogen web page (http://www.infobiogen.fr). BLAST research on rice genome (Oryza sativa) was performed on the Beijing Genomics Institute database (http://btn.genomics.org.cn/rice) (52).

**RNA isolation and reverse transcription**

Total RNA extraction was done using Trizol® (Invitrogen) on ground frozen tissues according to the manufacturer’s protocol. RNA was quantified spectrophotometrically at 260 nm. One microgram of RNA was reverse transcribed using the first-strand cDNA synthesis kit (Amersham Pharmacia) with random hexamers at 37°C for 1 h.

Poly(A)⁺ mRNAs were obtained using the Dynabeads mRNA direct kit (Dynal) following the instructions of the manufacturer. Briefly, 200 μg of ground frozen tissue was lysed using 1.5 ml of lysis/binding buffer. mRNA isolation was performed on centrifuged crude lysate using 500 μl of Dynabeads oligo (dT)25. cDNA synthesis was performed directly on the beads using oligo (dT)25 primers. Reverse transcription was performed for 1 h at 37°C using 500 U M-MLV reverse transcriptase in 10 mM DTT, 500 μM dNTP, 50 mM Tris–HCl (pH 8.3), 75 mM KCl, 3 mM MgCl₂ (Invitrogen). The first-strand cDNA covalently linked to the beads was used for PCR amplification or 5’ rapid amplification of cDNA ends (RACE).

**cDNA sequencing**

To identify the 5’ end of AtBRCA1, 5’ RACE was done using poly(A)⁺ RNA as template and either poly(T) or a specific primer in the 3.5 kb predicted sequence for reverse transcription. A string of deoxyguanosine was added at the 3’ end of the cDNA by a DNA terminal transferase (10). PCR was done using (GA)₁₂GCTCAGTAGTC₁₄ as forward primer and AtBIq-R as reverse primer. PCR products were sequenced as described below.

Gene structure prediction allowed us to design oligonucleotides corresponding to the putative cDNA sequence. Amplification was performed in 25 μl containing 10 mM Tris–HCl (pH 8.3), 50 mM KCl, 0.001% gelatin, 400 nM primers (MWG-biotech), 200 μM dNTP, 2.5 mM MgCl₂, 1.5 U Taq polymerase (Sigma) and 5 ng of cDNA at 94°C for 5 min, followed by 35 cycles of 94°C for 30 s, 55°C for 30 s, 72°C for 1 min, and 10 min at 72°C. PCR products were purified on Qiaquick columns following the manufacturer’s instructions (Qiagen). Sequence reactions were done with the BigDye terminator kit (Applied Biosystems) on 30 ng of PCR product and analyzed on an ABI Prism 310 (Applied Biosystems).

**Northern blot analysis**

Poly(A)⁺ mRNA of cultured cells (5 μg/lane) were separated on gel under denaturing conditions and transferred to a nylon membrane (Hybond N⁺, Amersham). Gel was stained with ethidium bromide to ensure that equal amounts had been loaded. Hybridization was performed overnight at 50°C with digoxigenin (DIG)-labeled probes in buffer provided by the manufacturer (Roche). Labeling of probe (400 nt) was performed using the PCR DIG labeling kit (Roche) with two

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**MATERIALS AND METHODS**

**GenBank accession**

Arabidopsis thaliana BRCA1 (AtBRCA1) had the GenBank accession no. AF515728.

**Materials**

Arabidopsis thaliana (var. Columbia, ecotype Col-0) seeds were grown in vitro for 4 or 20 days on modified Somerville medium in a chamber with a dark/light cycle of 10/14 h (49,50). Cell suspensions were cultivated at 25°C in an orbital shaker at 120 r.p.m. (Multitron II, Infors, Germany) under continuous light (60 μmol photons/m²/s) in MS medium as described earlier (51). Plants or cells were irradiated with a ⁶⁰⁵ source at a dose rate of 22 Gy min⁻¹, maintained for 1 h under respective standard growth conditions and then frozen in liquid nitrogen and ground for 5 min at 300 r.p.m. in the planetary ball mill PM400 (Retsch, Germany). Ground tissues harvested from independent experiments were pooled before RNA extraction.

**Computational analysis**

AtBRCA1 was identified using BLAST implemented on NCBI and TAIR databases with the hBRCA1 sequence, using the default parameter settings. Gene structure prediction was done on software implemented on the Softberry web page (http://www.softberry.com/), analysis of protein domains using the SMART database (http://smart.embl-heidelberg.de/), alignment of sequences using Alignp, and phylogenetic analysis using PHYlogeny Inference Package on Infobiogen web page (http://www.infobiogen.fr).
primers in the BRCA1 coding sequence (GAGAGATT-CGAGGTGCTCCA and GGTGCTCATCCAAACGAAT). Washing and detection were done according to the manufacturer’s protocol. Filters were exposed for 1 h to chemiluminescent detection film (Amersham).

**PCR amplification of cDNA coding sequence**

Amplification of cDNA coding sequence was obtained by PCR in 25 µl of buffer containing 20 mM Tris–HCl (pH 8.3), 10 mM KCl, 2 mM MgSO4, 10 mM (NH4)2SO4, 0.1% Triton X-100, 0.1 mg/ml BSA, 400 nM primers (AtB1-5‘-F ATCCGAAATGCGGAGCAGTA, AtB1-5‘-R CAACTC- TCCATAATCTCTAAACAAT), 200 µM dNTP, 1 mM MgCl2, 1.5 U Pfu Turbo polymerase (Stratagene) and 200 nM primers, 1 mM MgCl2, 1.5 U Pfu Turbo polymerase (Stratagene) for double-stranded DNA. The measure of the fluorescence emitted by SYBR Green associated with PCR products is directly proportional to the number of amplicons produced during each cycle. The cycle threshold (Ct) is the number of cycles needed to reach the fluorescence detection threshold and depends on the number of DNA templates at the start of the PCR. 18S ribosomal RNA was used as a reference gene provided a control for PCR quantification. Quantifications were done using the comparative Ct method. The ΔCt, which is the subtraction of the average 18S Ct value from the average Ct value of the target gene, is sample-specific and can be compared to the ΔCt of a calibration sample (for example the unirradiated sample). The amount of target, determined by normalization to the endogenous reference (18S) and relative to the calibrator, is 2−ΔΔCt with ΔΔCt being the subtraction of the calibrator ΔCt from the ΔCt of the sample, assuming that the efficiency of both PCRs is close to one (53). Primers were used were AtB1q-F CCATGTTATTTTTCATGCTG, AtB1q-R TGTTGGACACCTGCCATCTCT, AtRAD51q-F CGAGG- AAGGTATCTCTTGAG, AtRAD51-R GCACCTAGTGAA- CCCAGAG, 18S-F CGGACTACCACTCAAGGAA and 18S-R GCTGGAATTCGCGGCT. Amplicon length was near 100 bp. Amplifications were done in triplicate and performed in 25 µl containing 12.5 µl of 2× platinum quantitative supermix-UDG (Invitrogen), 200 nM primers, 0.2× SYBR Green and 5 ng of cDNA (50°C, 2 min for uracil-N-glycosylase activation; 95°C for 10 min and 40 cycles at 95°C for 15 s; 60°C for 1 min).

**RESULTS**

**Characterization and sequence of AtBRCA1 cDNA**

BRCA1 orthologs have been defined by the presence of one RING and two BRCT domains, respectively, at the N- and C-termini (12–16). We looked for the Arabidopsis putative ortholog by first considering the typical hBRCA1 structural organization of these three domains. The human BRCA1 protein sequence matched on Arabidopsis chromosome 4 at the At4g21070 predicted locus, which encoded a putative protein fragment of 1495 amino acids with strong similarities at the N- and C-termini.

The gene structure of At4g21070 was determined with three gene structure prediction software packages (Softberry, GenScan, Grail). Two different types of results were given. One type predicted a single mRNA of >5.2 kb which encoded a protein with three PPR motifs, one RING domain and two BRCT domains. The other one predicted two distinct mRNA of 1.7 and 3.5 kb, respectively. The first was intron-less and encoded a protein containing a PPR domain which is never present in BRCA1 orthologs. The second has a predicted TATA box located 310 bp before the initiation codon and was made of 14 exons followed by a 3′ untranslated region (UTR) of 610 bp. The corresponding predicted protein has a RING and two BRCT domains at the N- and C-termini, respectively, the typical organization of the BRCA1 orthologs. To resolve this ambiguity in intron–exon prediction, we postulated the presence of two genes given by Softberry prediction software and performed northern blotting and 5′ RACE to characterize the structural organization of the At4g21070 locus. Northern hybridization of poly(A)+ mRNAs from cells with a probe corresponding to a part of the predicted 3.5 kb mRNA revealed a single band for a transcript of an overall size of 3.5 kb (Fig. 1A). The absence of signal in non-irradiated cells indicates a quite low abundance of the transcript in control conditions. Determination of 5′ UTR of the 3.5 kb transcript by 5′ RACE using either poly(T) or a specific primer for reverse transcription gave in each case a sequence of 50 bp before the translation initiation codon. We sequenced the cDNA of the BRCA1 domain-containing sequence of 3.5 kb that we named the putative AtBRCA1 gene. The AtBRCA1 mRNA is composed of 14 exons with a 2826 bp coding sequence (Table 1) located downstream of a 5′ UTR of 50 bp and upstream of a predicted 3′ UTR of 650 bp. It gave a mRNA length of 3.5 kb in accordance with the northern hybridization result (Fig. 1A). The corresponding AtBRCA1 genomic sequence from the TATA box to the polyadenylation signal is then 5.5 kb long. PCR amplification of the full-length coding sequence (Fig. 1B) gave a unique band as in northern blot analysis. This indicated the absence of splicing variants in the studied mRNA population. These results gave evidence of a wrong electronic annotation in this genomic area and confirmed the presence of two genes at the At4g21070 genomic locus. At this stage, they indicated that only one putative ortholog of BRCA1 was present in the Arabidopsis genome.

**AtBRCA1: protein motifs and homologies with BRCA1 orthologs**

The putative AtBRCA1 cDNA encoded a protein of 941 amino acids with a predicted molecular weight of 104 kDa. Analysis of domains confirmed the presence of one RING domain between amino acids 16 and 53 (E-value 9.74e–08) and two BRCT domains between amino acids 727 and 809, and 842 and 935 (E-values 2.91e–15 and 6.95e–13, respectively) (see Supplementary Material, Figure S1A and B). Alignment of AtBRCA1 and hBRCA1 showed 34% identity–61% similarity in the RING domain, and 28% identity–61% similarity in the BRCT region (see Figure S1C and D). In the SMART database, four protein sequences contained both RING and BRCT domains in the Arabidopsis proteome. One of them
carried a single BRCT domain in N-terminal and one RING in its central part. The wrong number and location of the domains does not correspond to the organization of BRCA1 orthologs and this candidate gene was discarded in our study. Among the three other sequences predicted from three sequencing projects of the At4g21070 locus only one (ID no. Q8RXD4) corresponded to the protein translated from the putative AtBRCA1 cDNA we have sequenced. Thus, only one protein in the A.thaliana proteome, at the At4g21070 locus, carried a RING and two BRCT domains located at the N- and C- termini, respectively, and was also twice as short as other orthologs. In order to see if this shorter length allowed a meaningful comparison between the tissues. Even the 18S rRNA expression levels were both enhanced 160-fold by IR. In adult flowering plants, a gradient of expression of AtBRCA1 transcript was observed from the bottom to the top of the control plants, with the highest value in flower buds, being 10 times higher than in rosette leaves (Table 2B). As in plantlets, AtBRCA1 was strongly induced in all tissues after IR and expression levels reached the same maximal value whatever the basal levels, strongly induced in all tissues after IR and expression levels reached the same maximal value whatever the basal levels. Inference Package with kitch-Fitch-Margoliash and least squares methods with evolutionary clock (58) clearly showed the distribution of sequences between vertebrates versus plants and in vertebrates, mammalian versus non-mammalian (Fig. 2B).

With all these elements, we proposed that the 3.5 kb mRNA, which is transcribed at the locus At4g21070, is the Arabidopsis ortholog of BRCA1, and suggested that plant orthologs might always be twice as short as vertebrate ones.

Expression of AtBRCA1 mRNA in plant organs and after gamma irradiation

First, AtBRCA1 mRNAs were quantified in whole plantlet tissues after gamma irradiation with doses ranging from 1 to 300 Gy (Fig. 3A). Real-time quantitative PCR was used due to the hard detection conditions by northern analysis without genotoxic stress. A maximal induction of 140 times was observed for a dose close to 100 Gy, but a more than 20-fold induction was already reached at the low doses of 1–3 Gy. Expression levels of transcripts in plantlet leaves versus roots were hardly different within the control tissues (Table 2A) but were both enhanced 160-fold by IR. In adult flowering plants, a gradient of expression of AtBRCA1 transcript was observed from the bottom to the top of the control plants, with the highest value in flower buds, being 10 times higher than in rosette leaves (Table 2B). As in plantlets, AtBRCA1 was strongly induced in all tissues after IR and expression levels reached the same maximal value whatever the basal levels, except for cauline leaves. We can underline the highest value in flower buds, being 10 times higher than in rosette leaves. In siliques, the relative proportion of 18S rRNA levels was too low compared to other tissues to allow a meaningful comparison between the tissues. Even the siliques presumably showed a reduced translational activity compared to the highly dividing tissues, the induction of AtBRCA1 was still more than 10 times (Table 2C).

In human cell cultures, hBRCA1 has been found to interact with the DSB repair protein hRAD51. So, we looked for the

Table 1. Structure organization of AtBRCA1 mRNA

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Figure 1. Analysis of AtBRCA1 expression. (A) Northern blot analysis of AtBRCA1 in control (C) and 25 Gy irradiated (IR) Arabidopsis cells. (B) Gel electrophoresis of AtBRCA1 coding sequence obtained by PCR amplification using cDNA population from cells.
co-induction of the *Arabidopsis* transcripts of these two genes after IR in cell suspensions and in plant organs. Basal levels of *AtRAD51* and *AtBRCA1* mRNA were 10-fold higher in exponentially growing cell cultures than in entire plants (data not shown). Interestingly, transcripts were co-induced in the same relative proportion in *Arabidopsis* cell cultures as well as in entire plantlets (Fig. 3B). The fold induction value of *AtRAD51* mRNA was between 10 and 80 times and was, in all cases, 50–70% of the *AtBRCA1* induction factor. But since *AtRAD51* was expressed twice more than *AtBRCA1* in both models without IR (data not shown), the same final stoichiometry was finally reached. Taken together, these results demonstrated the ubiquity of *AtBRCA1* transcripts in all *Arabidopsis* tissues and its strong transcriptional induction after DNA damage by IR in the same way as *AtRAD51*.

**DISCUSSION**

Identification of *AtBRCA1* was done by similarity search and the gene predicted in databases differed from that experimentally isolated. Errors in some exon/intron boundaries were found and some programs merged the sequence of the *AtBRCA1* gene with a second gene. During this study, this fusion gene, which does not exist, containing three PPR motifs, one RING and two BRCT domains, was given as a potential *Arabidopsis* *BRCA1/BARD1* ortholog in an article on *BRCA1* and *BARD1* identification in the *Xenopus laevis* genome (15). Thus, we have characterized the *Arabidopsis* ortholog of *BRCA1*, which was located on chromosome 4 and gave a 3.5 kb transcript that encoded a 941 amino acid protein (104 kDa) with one N-terminal RING and two C-terminal BRCT domains as hBRCA1. In the RIKEN *Arabidopsis* full-length complementary (RAFL) cDNA collection (59), we have found the clone corresponding to *AtBRCA1* cDNA sequence (RAFL09-61-E20), which confirmed our results. This showed the difficulty of precise gene prediction, and also that orthologs can still be identified between distantly related species. To date, *AtBRCA1* is the first non-vertebrate *BRCA1* ortholog.

The known functions of hBRCA1 are nearly all linked to RING and BRCT domains with an E3 ubiquitin autoligase activity and interaction with several proteins in the RING domain, while the BRCT domains are involved in transcriptional transactivation and are also required for interaction with several proteins. This BRCT domain is a signature of proteins involved in DNA repair and is present in numerous species, including vertebrates, yeast and plants, suggesting a conserved role for this domain. As shown by Joukov et al. (15), the *Xenopus* structural ortholog of BRCA1 was also reported as a functional ortholog. So, conserved domains strongly indicated that this structural organization is the signature of BRCA1 orthologs, and are linked to conserved activities. In addition to these two kinds of functional domains, *AtBRCA1* shared an additional similarity between amino acids 181 and 206 of...
hBRCA1, a region of interacting domains with c-myc (60) and p300/CBP (61), proteins which are endowed with acetyltransferase activity and interact with transcription factors. Since such orthologs have been described in Arabidopsis (62), it may be possible that these two Arabidopsis proteins also interact.

To date, with 941 and 968 amino acids, respectively, AtBRCA1 and OsBRCA1 are the shortest members of the BRCA1 family, except the alternative splicing variant hBRCA1ΔII, which still contains the two kinds of functional domains and has a molecular weight of 110 kDa. Analysis of other orthologs showed that sequence conservation is particularly important in the RING and BRCT domains but less significant between them. This may suggest that the BRCA1 central region is species-specific. We also notice the reduced length in the predicted sequences of the Arabidopsis and rice putative orthologs of BRCA2 (63), an interacting partner of hBRCA1 and hRAD51 in human cells. If we looked at orthologs of other genes implicated in DNA repair between Arabidopsis and human, some of them are different in length and/or in structural composition. For instance, in AtLig IV (1219 amino acids), there is only one BRCT domain and two in hLig IV (911 amino acids). AtATM (3856 amino acids) is
longer than human ortholog (3056 amino acids) and presents additional domains, while AtRAD51 is closely related to the human ortholog which shares the same domains and length (342 versus 339 amino acids for hRAD51). So, there are slight differences between *Arabidopsis* and human orthologs, but domains linked to protein activities are always conserved within the same structural organization.

*AtBRCA1* is expressed ubiquitously but seemed to be elevated in proliferating tissues like in flower buds and in cell cultures. This may be linked to a higher rate of division and suggests a similar role in cell-cycle control of *AtBRCA1* as shown for hBRCA1. *AtBRCA1* mRNA levels are highly increased after gamma irradiation; this could be due to an enhanced transcription and/or a higher mRNA stability. We observed maximal induction near 100 Gy, a non-lethal dose generally used to screen mutants hypersensitive to gamma irradiation, but *AtBRCA1* was also significantly up-regulated at doses between 1 and 20 Gy. Then, this increase seems to be dose-related from 1 to 100 Gy suggesting a DNA lesion amount dependency. Basal *AtBRCA1* expression in different plant tissues varied, but was quantitatively the same after gamma irradiation, showing a higher induction factor in tissues with low basal levels and a plateau response level of *AtBRCA1* mRNA. More strikingly, a lower induction observed in cell suspensions compared to plantlets whatever the irradiation dose, may be linked to a higher basal mRNA level. This could be related to homogeneity of cultured cells growing in exponential phase compared to heterogeneity of plantlets, which are a mosaic of meristems and differentiated tissues. The strong correlation between fold induction and dose suggests a highly sensitive and precise regulation system linked to DNA repair and/or to cell-cycle control, which is consistent with the role of hBRCA1. However, there are some discrepancies in the regulation of mRNA levels after DNA damage among species. Indeed, *hbRCA1* mRNA is induced by irradiation but much less than *AtBRCA1* (64), and *hRAD51* is not induced after IR, whereas levels of *AtRAD51* mRNA were found to be increased by irradiation, confirming a previous study (11). In our study, fold induction of both genes seemed to be correlated whatever the tissues or irradiation doses, suggesting a common regulation of transcript amount.

Considering molecular organization, transcript behaviour after IR of *AtBRCA1* and the large conservation of DNA repair functions and associated proteins among eucaryotes strongly argue in favor of the fact that *AtBRCA1* is indeed the ortholog of hBRCA1. The presence of RING, BRCT and p300/CBP interacting domains in *AtBRCA1* may guide us in determining the function of this plant ortholog, using our knowledge of the human gene. As several partners of hBRCA1 are present in *Arabidopsis*, including *AtRAD51*, *AtATM*, *AtBRCA2* and p300/CBP, there may be conserved functional interactions between them. But we have also to find out its functions specific to plant physiology. For instance, absence of *Arabidopsis* orthologs of genes such as BARD1 or p53, which are key elements in the DNA damage response of human cells and partners of hBRCA1, may help in answering the question of species specificity of conserved functions.

**SUPPLEMENTARY MATERIAL**

 Supplementary Material is available at NAR Online.

**ACKNOWLEDGEMENTS**

We thank Elena Marin-Nussaume, V. Garcia, A. Tissier and C. Triantaphylides for support and advice, and N. Uhrhammer for critically reading the manuscript.

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