Arabidopsis DNA double-strand break repair pathways

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Abstract

DSBs (double-strand breaks) are one of the most serious forms of DNA damage that can occur in a cell’s genome. DNA replication in cells containing DSBs, or following incorrect repair, may result in the loss of large amounts of genetic material, aneuploid daughter cells and cell death. There are two major pathways for DSB repair: HR (homologous recombination) uses an intact copy of the damaged region as a template for repair, whereas NHEJ (non-homologous end-joining) rejoins DNA ends independently of DNA sequence. In most plants, NHEJ is the predominant DSB repair pathway. Previously, the Arabidopsis NHEJ mutant atku80 was isolated and found to display hypersensitivity to bleomycin, a drug that causes DSBs in DNA. In the present study, the transcript profiles of wild-type and atku80 mutant plants grown in the presence and absence of bleomycin are determined by microarray analysis. Several genes displayed very strong transcriptional induction specifically in response to DNA damage, including the characterized DSB repair genes AtRAD51 and AtBRCA1. These results identify novel candidate genes that encode components of the DSB repair pathways active in NHEJ mutant plants.

Introduction

Plants, as sessile organisms which require sunlight for energy, are continuously exposed to a range of environmental mutagenic agents including UV irradiation, decay of naturally occurring radioisotopes and soil pollutants such as heavy metals. DNA damage also arises from endogenous factors including reactive oxygen species [1]. One of the most serious forms of DNA damage is chromosomal breakage, where a DSB (double-strand break) occurs in the DNA duplex. Failure to repair correctly even one DSB can result in the loss of large amounts of genetic information and ultimately may result in cell death. This extreme cytotoxicity of DSBs has led to the evolution of a powerful and extensive array of rapid cellular responses that lead to cell-cycle arrest, recruitment of DSB repair proteins to the site of the DSB and repair of DNA damage [2].

DSB repair is mediated by two basic recombination mechanisms. HR (homologous recombination) is catalysed by proteins of the RAD52 epistasis group [RAD51, RAD52, RAD54, RAD55, RAD57 and the MRN (MRE11–RAD50–NBS1) complex]. An intact copy of the damaged region, which may be a sister chromatid, homologous chromosome or other identical/near-identical sequence, is used as a template to repair the break. Yeast and bacteria usually employ HR to repair DSBs if sufficient sequence homology is available, whereas organisms with larger genomes, including mammals and higher plants, usually repair DSBs by NHEJ (non-homologous end joining). In NHEJ, DSBs are simply rejoined, end-to-end and thus largely independent of DNA sequence. In this process, Ku70 and Ku80 proteins bind to DNA ends at sites of DSBs in the DNA. The DNA ends may then be processed by the MRN complex to make them suitable substrates for joining by the DNA ligase IV (Lig4)–XRCC4 complex. NHEJ and HR pathways are highly conserved throughout eukaryotic evolution. However, the relative activities of the two pathways differ depending on species, cell type and cell-cycle stage.

Analysis of Arabidopsis NHEJ mutants

Previous studies of the model plant Arabidopsis thaliana have identified mutants in the NHEJ pathway, including atku80, atku70, atlig4, atmre11 and atrad50 [3–6]. The latter two mutants are sterile, consistent with a role of the Arabidopsis MRN complex in meiotic HR. In contrast, mutations in atlig4 and the atku70/atku80 genes result in phenotypically normal plants under standard growth conditions, although telomere defects are present in atku mutants. However, these mutants display hypersensitivity to agents that induce DSBs including γ-irradiation, bleomycin and oxidizing agents, indicating the requirement for these genes under genotoxic stress. Despite hypersensitivity to bleomycin, NHEJ mutants can still grow in the presence of the drug at concentrations known to induce DNA DSBs [7]. This suggests that alternative DSB repair pathways may operate in these mutant backgrounds, which can at least partially compensate for the absence of NHEJ activity. Expression of components of these pathways may be up-regulated in the NHEJ mutant backgrounds and/or under conditions of genotoxic stress. These putative backup DSB repair pathways may play a greater role in the plants.
than in animals, where NHEJ mutations lead to severe developmental abnormalities (Ku70−/−, Ku80−/−) or lethality (Lig4−/−, Rad50−/−).

In the present study, a transcriptomics approach is used to identify genes transcriptionally up-regulated in response to the induction of DSBs in wild-type and the atku80 mutant backgrounds using Affymetrix whole genome arrays.

**Analysis of transcript profiles**

The transcript profile of four treatments were investigated: (i) control Arabidopsis Wassilewskija (Ws-2) plants, (ii) atku80 mutant plants, (iii) Ws-2 plants grown in the presence of bleomycin (1 µg·ml−1 for 3 days) and (iv) atku80 plants grown in the presence of bleomycin (1 µg·ml−1 for 3 days). Hybridization was performed through GARNET by the Nottingham Arabidopsis Stock Centre Affymetrix service (NASCArray) and the results from this experiment and approx. 1000 other arrays are available at http://affymetrix.arabidopsis.info [8,9]. Over the four arrays, approx. 12,000 genes gave significant signal and these were ranked in accordance with transcripts that displayed highest induction averaged over the three treatments relative to the Ws-2 control. Bleomycin treatment resulted in the induction of a wide range of genes involved in plant defence and stress. To identify genes putatively involved in the recognition, repair or signalling of DSBs, expression patterns of highly induced genes were analysed further using the publicly available NASCArray microarray data. The ‘spot history’ analysis program indicated that most bleomycin-induced genes were also up-regulated by a wide range of stresses including wounding and infection by pathogens. However, several highly induced genes showed very specific induction by bleomycin with low expression levels in the other experiments in the database (Figure 1). Three previously uncharacterized genes were chosen for further analysis by QPCR (quantitative PCR) (Figure 2) and are potential candidates for novel protein involved in plant DSB sensing, signalling or repair.

**Bleomycin inducible genes**

A previous study of 3000–4200 genes identified transcripts induced by incubating Arabidopsis seedlings for 6 h with bleomycin (1.5 µg·ml−1) and mitomycin C (66.7 µM) [10]. Transcripts that showed significant induction included thymidine kinase, MYB-like protein, RAD51, PARP-2 [poly(ADP-ribose) polymerase-2], RNR2 [ribonucleotide reductase 2] and the G2/mitotic-specific cyclin B1. These genes also displayed high levels of induction in the present study (Figure 2). In addition, the DNA repair gene BRCA1 showed a 34-fold induction after bleomycin treatment of the atku80 mutant, consistent with previous findings indicating BRCA1 induction after γ-irradiation [11]. The present study also identified RPA (replication factor A), a TBPIP (TATA-binding protein-interacting protein) orthologue, a NAM (no apical meristem) family protein and the cohesin SYN2 as strongly inducible by genotoxic stress. In addition, three previously uncharacterized genes showed

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**Figure 1** ‘Spot history’ showing expression levels of At5g48720 in all experiments of the NASCArray database

Expression signal values are grouped into 15 ranges and the frequency of experiments in which signal values fall into each range is plotted. Expression levels for At5g48720 are below 100 in most experiments, but specific induction by bleomycin results in expression levels above 500.

**Figure 2** Transcript induction for selected genes

Induction of transcript levels relative to wild-type untreated plants as determined by microarray analysis is shown for atku80 mutants (white bars), bleomycin-treated (1 µg·ml−1 for 3 days) wild-type plants (grey bars) and bleomycin-treated atku80 mutants (black bars). QPCR results are given in parentheses: a, bleomycin-treated atku80 mutants (±S.D.), b, QPCR data from [10] indicating the induction levels after the seedlings were given a 6 h bleomycin (1.5 µg·ml−1)/mitomycin C (66.7 µM) treatment.
very specific induction by bleomycin and these results were confirmed by QPCR analysis (Figure 2).

**Novel genes with putative roles in the maintenance of genome integrity**

Three novel genes displayed large increases in expression levels, specifically in response to bleomycin treatment. At5g48720 showed a 42-fold induction in transcript level, measured by QPCR, and analysis of the predicted protein sequence revealed a bipartite nuclear localization signal but no similarity to characterized proteins in the database. At2g45460, which displayed a 3.3-fold induction, encodes a predicted protein with a forked head associated domain. This motif is involved in the interaction of phosphorylated residues on proteins associated with nuclear signalling pathways including NBS1, CHK1 and MDC1. At4g22960, which has a domain (DUF544, pfam04424) with sequence similarity to yeast and mammalian proteins of unknown function, demonstrated up to a 42-fold increase in expression. Further characterization is required to determine whether the novel genes identified in the present study are directly involved in the response of the plant to genotoxic stresses, which induce DSBs.

**Conclusion**

These results have confirmed that transcriptomics is a powerful method to identify putative DSB repair/DNA damage signalling genes. Fourteen genes were readily identified by their very strong, specific induction in response to bleomycin treatment, including several well-characterized DNA repair genes and three novel genes. Further analysis of Arabidopsis transcript profiles in response to different genotoxic stresses and in different mutant backgrounds will allow the use of clustering tools to identify co-ordinately regulated genes. These results will allow the identification of DNA repair and damage signalling components, which may operate in novel pathways for maintaining genome integrity in plants.

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**References**


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