ISSN 1479-2621
Plant Genetic Resources: Characterization and Utilization (2014) **12**(S1); S99–S103
doi:10.1017/S1479262114000367

Cloning and characterization of Ku70 and Ku80 homologues involved in DNA repair process in wheat (*Triticum aestivum* L.)

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Abstract

Error-prone repair of radiation-induced DNA double-strand breaks (DSBs) results in DNA mutation that is essential for mutation breeding. Non-homologous end joining might be the principal DSB repair mechanism in eukaryotes, which is mediated and activated by Ku protein, a heterodimer of 70 and 80 kDa subunits. In this study, on the basis of complementary DNA (cDNA), the genomic sequences of *TaKu70* and *TaKu80* genes in all the three genomes of wheat were characterized. Only single-nucleotide substitutions and no insertions or deletions were detected in the exons of *TaKu70* and *TaKu80* genes. The size of the introns exhibited a slight variation between the sequences. Yeast two-hybrid analysis demonstrated that TaKu70 and TaKu80 formed a heterodimer, and electrophoretic mobility shift assays revealed that this heterodimer bound to double-stranded DNA, but not to single-stranded DNA. The quantitative polymerase chain reaction analysis revealed that the expression of *TaKu70* and *TaKu80* genes was up-regulated under γ-ray irradiation in a dose-dependent manner in the seedlings of wheat. These results suggest that TaKu70 and TaKu80 form a functional heterodimer and are associated with the repair of the induced DSBs in wheat.

Keywords: DNA repair; DSB; Ku protein; wheat

Introduction

Wheat is one of the three most important crops in the world due to its value as a major food source and its unique suitability for bread production. Mutation breeding, in which ionizing radiations (IRs) are the most frequently used mutagens, has resulted in significant increases in the quality and yield of wheat during the past century (Ahloowalia and Maluszynski, 2001; Ahloowalia *et al.*, 2004; Liu *et al.*, 2004; http://mvgs.iaea.org/AboutMutantVarities.aspx). It is well known that IR cause clustered DNA damages, particularly double-strand breaks (DSBs) in the genome. Error-prone DSB repair processes have been inferred to play important roles in radiation-induced mutations in both mouse germ cells and mammalian somatic cells (Sankaranarayanan *et al.*, 2013).

Both radiation mutagenesis studies and radiation cytogenetic studies indicate that non-homologous end joining (NHEJ) might be the principal DSB repair mechanism that underlies the origin of mutations (Kanaar *et al.*, 1998; West *et al.*, 2004; Weterings and Chen, 2008). Ku70/80 heterodimer plays an important role in this process, which functions both as a DNA-binding protein and as an allosteric activator (Lieber *et al.*, 1997; Walker *et al.*, 2001; Mari *et al.*, 2006). The plant homologues of *Ku70* and *Ku80* were first cloned in *Arabidopsis thaliana* (Tamura *et al.*, 2002). The *atku80* mutant exhibited hypersensitivity to DNA-damaging agents (Friesner and Britt, 2003). We had cloned the complementary DNA sequences of *TaKu70*

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and *TaKu80* in wheat, the deduced amino-acid sequences of which shared high homology with Ku70 and Ku80 of other plants (Zhu *et al.*, 2009). In the present study, we extended the genomic characterization of *TaKu70* and *TaKu80* genes and demonstrated the function of the TaKu70/TaKu80 heterodimer *in vitro*. We also analysed the response of *TaKu70* and *TaKu80* genes to IR in several wheat cultivars. The results indicated that *TaKu70* and *TaKu80* genes are required for DSB repair in wheat.

Materials and methods

Materials

The characterization of *TaKu70* and *TaKu80* genes of hexaploid *Triticum aestivum* L. cv. Chinese spring (AABBDD), tetraploid *Triticum turgidum* L. (AABB), and diploid *Triticum monococcum* L. (AA) and *Aegilops Tauschii* Coss. (DD) was carried out.

Amplification and cloning of sequences

Genomic DNA was extracted using the cetyltriethylammonium bromide (CTAB) method. Universal primers (Table S1, available online) were designed to amplify the genomic sequences using the FastStart High Fidelity PCR System (Roche, Mannheim, Germany). The amplified fragments were cloned into pGEM®-T (Promega, Madison, WI, USA) and sequenced at Sangon Biotech Company (Beijing, China). Specific primers (Table S1, available online) were used to determine the genome types.

Irradiation and growth conditions

Wheat seeds were subjected to γ-ray irradiation at doses of 100, 150 and 250 Gy (7.5 Gy/min) using a Co^{60} irradiator at Peking University (Beijing, China). The irradiated seeds were grown in a growth chamber at 21°C under a 16 h light–8 h dark cycle. The seedlings were harvested after 3 d for total RNA extraction.

Quantitative polymerase chain reaction (qPCR) analysis

Total RNA was extracted using TRIzol® Reagent (Life Technologies, Carlsbad, CA, USA). Real-time PCR and qPCR were carried out using the iScript™ cDNA Synthesis Kit and SsoFast™ EvaGreen Supermix (Bio-Rad,

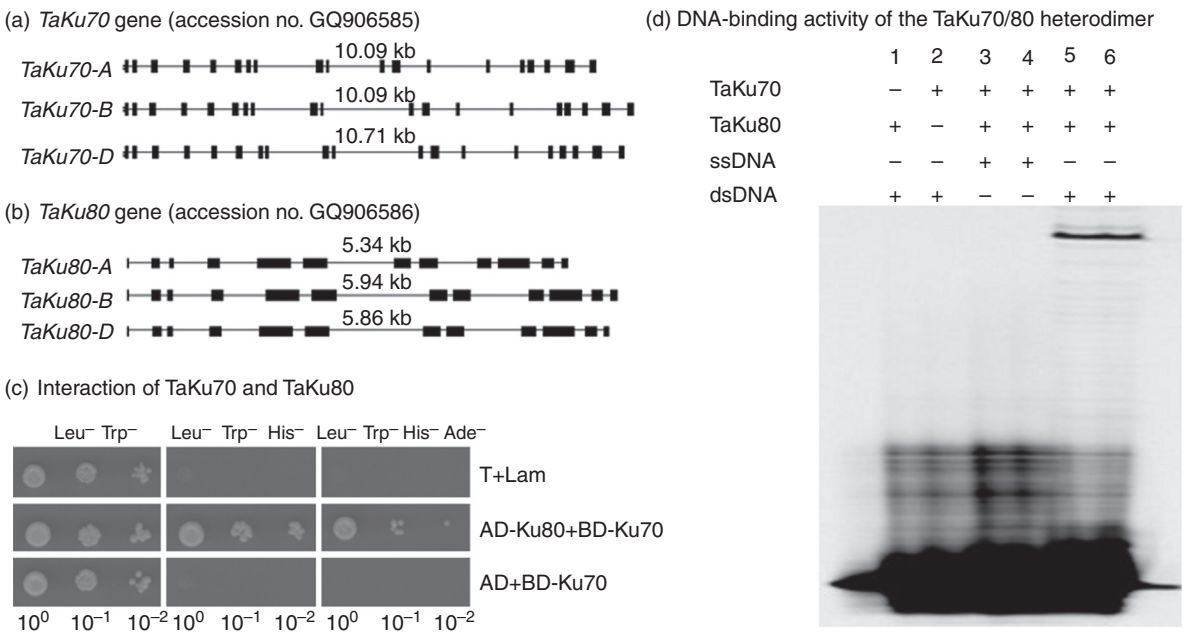

Fig. 1. Molecular characteristics of the Ku70 and Ku80 homologues of hexaploid wheat (*Triticum aestivum* L.). (a) and (b) Genomic organization of *TaKu70* and *TaKu80* genes. A total of 19 and 12 exons (black boxes) and 18 and 11 introns (lines) were identified for *TaKu70* and *TaKu80,* respectively. (c) Yeast two-hybrid assay. The growth of yeast cells harbouring the recombinant plasmids for both AD-TaKu80 and BD-TaKu70 revealed the protein–protein interaction between TaKu70 and TaKu80 proteins (AD-Ku80 + BD-Ku70). Neither negative control (T + Lam) nor BD-Ku70 (AD + BD-Ku70) could activate the reporter gene. (d) Electrophoretic mobility shift assay. Neither TaKu70 (lane 1) nor TaKu80 (lane 2) alone could affect the mobility of probes, suggesting that neither protein alone bound to DNA. The combination of TaKu70 and TaKu80 induced a substantial decrease in the mobility of the double-stranded DNA (dsDNA) probe (lanes 5 and 6), without affecting that of the single-stranded DNA (ssDNA) probe (lanes 3 and 4).

https://doi.org/10.1017/S1479262114000367 Published online by Cambridge University Press

Hercules, CA, USA). As multi-internal controls, *18S rRNA* (GenBank: JF489233) and *actin* (GenBank: AAW78915) were selected. The qPCR primers (Table S1, available online) were designed using Beacon Designer 7.9 (PREMIER, Palo Alto, CA, USA).

Yeast two-hybrid analysis

Two plasmids, pAD-TaKu80 (*TaKu80-B*) and pBD-TaKu70 (*TaKu70-A*), were constructed and transformed into *Saccharomyces cerevisiae* strain HF7c. Yeast strains transformed with the corresponding plasmid were examined on a histidine-deficient plate.

Electrophoretic mobility shift assays (EMSAs)

TaKu70 and TaKu80 glutatione S-transferase (GST) fusion proteins were prepared and analysed by EMSAs as described by Tamura *et al.* (2002). IRDye800-labelled M_{13} oligonucleotide (LI-COR, Lincoln, NE, USA) was used as a single-stranded DNA (ssDNA) probe and that annealed with an antisense sequence was used as a double-stranded DNA (dsDNA) probe. EMSAs were carried out using the LI-COR 4300 system (LI-COR).

Results and discussion

Three types of TaKu70 *and* TaKu80 *genes are present in hexaploid wheat (*T. aestivum *L.)*

Based on previously published cDNA sequences of *TaKu70* and *TaKu80* genes (Zhu *et al.*, 2009), four and three sets of universal primers were designed to amplify genomic sequences in hexaploid *T. aestivum* L. cv. Chinese spring (genomes AABBDD). Three types of sequences were found for *TaKu70* and *TaKu80* genes, and it was assumed that each belonged to a different genome. Using specific primers designed based on the differences to amplify target fragments in the genomic DNA of *T. monococcum*, *T. turgidum*, *Ae. tauschii* and *T. aestivum*, the genome types of different sequences were determined. The sizes of *TaKu70* genes ranged from 10,089 bp for genome A (*TaKu70A*) and 10,712 bp

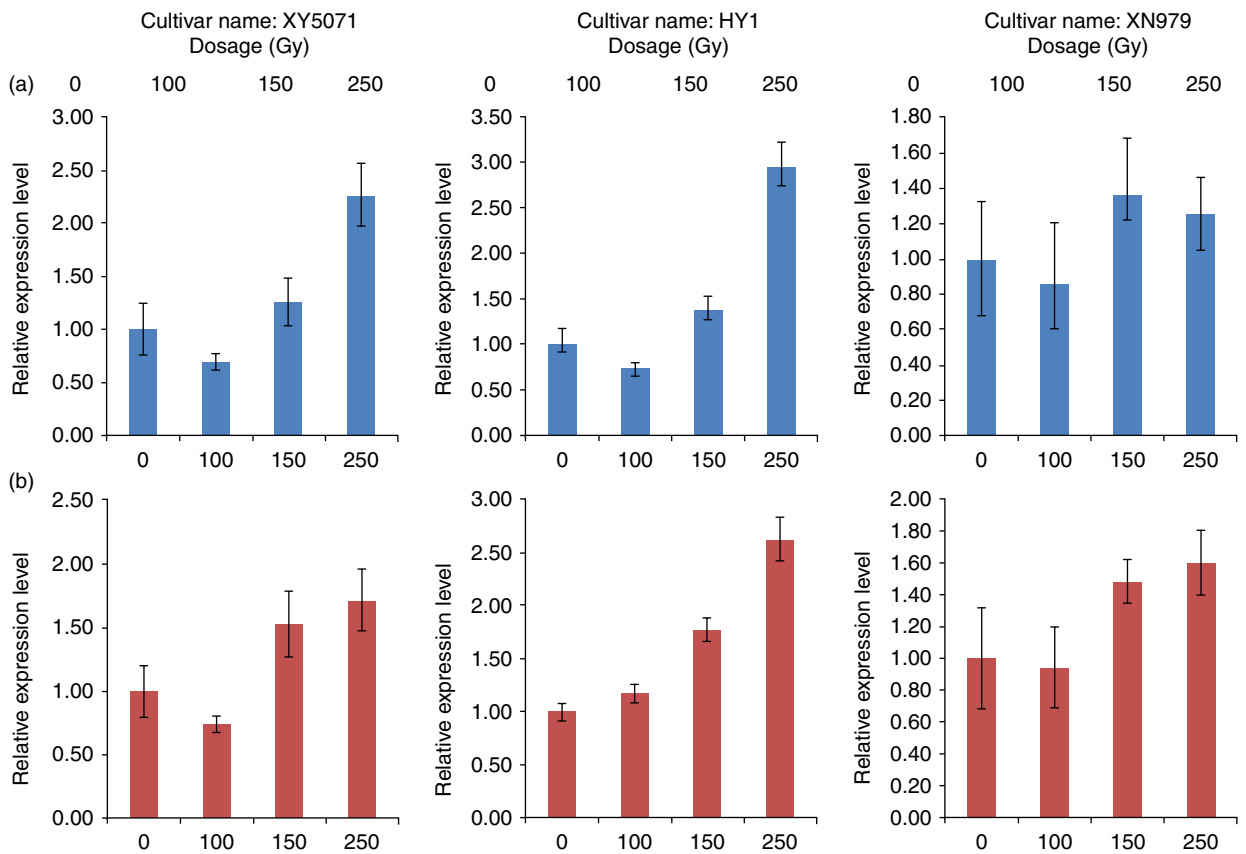

Fig. 2. Transcriptional activation of *TaKu70* and *TaKu80* genes in response to ionizing radiation (IR). (a) and (b) Transcriptional levels of *TaKu70* and *TaKu80* genes were up-regulated by IR in a dose-dependent manner in different wheat cultivars.

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for genome D (*TaKu70D*) to 10,899 bp for genome B (*TaKu70B*). Comparison between genomic and cDNA sequences confirmed the presence of 19 exons and 18 introns in all the genomes (Fig. 1(a)). The sizes of *TaKu80* genes ranged from 5337 bp for genome A (*TaKu80A*) and 5856 bp for genome D (*TaKu80D*) to 5936 bp for genome B (*TaKu80B*). Comparison between genomic and cDNA sequences confirmed the presence of 12 exons and 11 introns in all the genomes (Fig. 1(b)). Differences found between the exons of *TaKu70* and *TaKu80* genes were due to single-nucleotide substitutions, and no insertions or deletions were detected. The size of the introns exhibited a slight variation between the sequences due to single-nucleotide substitutions and insertions/deletions.

TaKu70 and TaKu80 form a functional heterodimer in vitro

Ku protein has been thought to always exist and function as a heterodimer, which is essential for DSB repair process (Liang *et al.*, 1996; Jin and Weaver, 1997; Gell and Jackson, 1999). In the yeast two-hybrid system, only the yeast strain transformed with both pAD-TaKu80 and pBD-TaKu70 grew on the histidine-deficient medium (Fig. 1(c)). In EMSAs, neither TaKu70 nor TaKu80 alone could visibly affect the mobility of the ssDNA and dsDNA probes. By contrast, the combination of TaKu70 and TaKu80 led to a significant decrease in the mobility of the dsDNA probe, but not in that of the ssDNA probe (Fig. 1(d)). These results indicate that TaKu70 and TaKu80 form a functional heterodimer that exhibits the ability to bind to dsDNA *in vitro*.

Expression of TaKu70 *and* TaKu80 *genes is induced by IR*

To determine whether the expression of *TaKu70* and *TaKu80* genes is regulated by induced DSBs, seeds of several wheat cultivars were treated with γ-ray irradiation. Different doses of IR (100–250 Gy) caused a marked arrest in seedling growth in a dose-dependent manner. The expression of *TaKu70* and *TaKu80* genes was detected in the seedlings of the IR-irradiated seeds and their controls by qPCR. Tanscriptional up-regulation of both *TaKu70* and *TaKu80* genes occurred under IR treatment in a dose-dependent manner in all the examined cultivars (Fig. 2(a) and (b)). Consistent with the role of the Ku70/80 heterodimer in NHEJ (Walker *et al.*, 2001), *TaKu70* and *TaKu80* genes appeared to be associated with the repair of the IR-induced DSBs during wheat seed germination.

Supplementary material

To view supplementary material for this article, please visit http://dx.doi.org/10.1017/S1479262114000367

Acknowledgements

This study was supported by the National Natural Science Foundation Research Program (grant no. 11305261), the National 973 Program (grant no. 2014CB138101), and the National 863 Program (grant no. 2012AA101202) and the International Atomic Energy Agency project (CRP15651 and RAS5056).

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