BIOLOGIA PLANTARUM 46 (3): 363-368, 2003

The 26S proteasome of the resurrection plant *Tortula ruralis*: cloning and characterization of the TrRPT2 subunit

X. CHEN and A.J. WOOD*

Department of Plant Biology, Southern Illinois University - Carbondale, Carbondale, IL 62901, USA

Abstract

Tortula ruralis is an important experimental system for the study of plant desiccation tolerance. EST gene discovery efforts utilizing desiccated gametophytes have identified a cDNA *TrRpt2* encoding a predicted polypeptide with significant similarity to the 26S proteasome regulatory subunit IV. TrRPT2, the 446 amino acid deduced polypeptide, has a predicted molecular mass of 49.6 kDa, and a predicted pI of 8.15. Phylogenetic analysis demonstrated that previously characterized RPT2 polypeptide sequences could be reproducibly grouped into 3 major clades and that TrRPT2 forms a discrete evolutionary group. RNA blot hybridizations were used to analyze *TrRpt2* expression in response to: *1*) desiccation and rehydration, *2*) abscisic acid-treatment, *3*) increased NaCl concentration, and *4*) NaCl-shock. *TrRpt2* steady-state mRNA transcript levels are unchanged in response to all treatments and the gene is constitutively expressed.

Additional key words: ABA, bryophytes, desiccation, moss, salinity.

Introduction

In eukaryotic cells, most proteins in the cytosol and nucleus are degraded via the ubiquitin-proteasome pathway (Coux *et al.* 1996, Voges *et al.* 1999). The 26S proteasome (EC 3.4.99.46) is a multisubunit, multicatalytic endopeptidase complex that catalyzes the ATP-dependent degradation of ubiquitinated polypeptides (Rechsteiner 1998). In plants, this degradative pathway is involved in pathogen resistance (Becker *et al.* 1993, Homma *et al.* 1994), senescence (Homma *et al.* 1994), hormone response (Ruegger *et al.* 1998), wound-signalling (Ito *et al.* 1999) and progress through the cell cycle (Genschik *et al.* 1994, 1998).

The 26S proteasome (> 2.5 MDa) consists of a barrel-shaped proteolytic core complex (the 20S proteasome), capped at one or both ends by the 19S regulatory complex. The 20S proteasome consists of four stacked rings of seven related α- and seven related β-subunits in a twofold symmetric (α7β7β7α7) configuration (Groll *et al.* 1997). The 19S regulatory complex is made up of at least 18 subunits and can be dissociated into two subcomplexes, the distal 'lid' and proximal 'base'. The lid complex is essential for the recognition and binding of the of ubiquitinated substrate proteins, while the base subcomplex contains six ATPases designated RPT1-to-RPT6 (Glickman *et al.* 1998, Zwickl *et al.* 1999). The ATPases are assembled into a ring that forms the interface of the 19S complex with the 20S core particle (Baumeister *et al.* 1998). The ATPase ring promotes assembly of the 26S complex and directs translocation of substrate protein into the proteolytic core (Voges *et al* 1999). The RPT ring ATPases belong to the AAA protein super family (ATPases Associated with various cellular Activities) (Dubiel *et al.* 1992) which direct the binding and utilization of ATP in a wide-range of cellular processes (Patel and Latterich 1998). Rpt2 encoding cDNAs have been identified and characterized from the angiosperm species *Oryza sativa* (Suzuka *et al.* 1994) and *Arabidopsis thaliana* (Fu *et al.* 1999) We employ the desiccation-tolerant moss *Tortula ruralis* as a model system for the study of plant vegetative

Received 1 November 2001, *accepted* 25 April 2002.
Abbreviations: ABA - abscisic acid; EST - expressed sequence tag.
Acknowledgements: This work was supported in part by a grant to AJW (USDA NRI#9735100). The nucleotide sequence data appear in GenBank under the accession number (AF432345).
*Corresponding author; fax (+01) 618 4533441, e-mail: wood@plant.siu.edu

desiccation tolerance and post-transcriptional gene control (Wood and Oliver 1999, Oliver *et al.* 2000, Wood *et al.* 2000a,b,c). We postulate that the ubiquitin-proteasome pathway plays a fundamental role in the ability of resurrection plants to survive complete drying of their vegetative structures. In order to pursue our hypothesis at the molecular level, and to further investigate the relationship between salinity-stress and desiccation-stress in *T. ruralis*, a cDNA *TrRpt2* encoding a predicted polypeptide with significant similarity to the AAA protein RPT2 of the human 26S proteasome was isolated and characterized.

Materials and methods

Plants: Gametophytes of *Tortula ruralis* (Hedw.) Gaerten., Meyer & Scherb. (= *Syntrichia ruralis* F. Weber & D. Mohr) were prepared for experimentation as described previously (Wood *et al.* 1999). Hydrated moss was obtained after a 24 h rehydration period. Desiccated moss was obtained by placing cut hydrated gametophytes on a filter paper disc over activated silica gel (rapid-dried, RD) or a stirred saturated solution of sodium nitrite at 20 °C (RH 66 %) for 24 h (slow-dried, SD). The air-dried dry mass is achieved in 1 h for rapid-dried and 6 h for slow-dried gametophytes. Rehydrated moss was obtained by the addition of de-ionized H_2O to desiccated moss. Abscisic acid (ABA) treated moss was obtained by incubation with 50 to 500 µM ABA solutions (*cis*, *trans*, *Sigma*, St. Louis, USA). Salt-treated moss was obtained by the stepwise addition of NaCl (50 mM 2 h, 50 mM + 50 mM 2 h, and 100 mM + 50 mM 2 h; 6 h total application) or by incubation in 50, 100 or 150 mM NaCl for 6 h.

cDNA isolation: The EST clone RNP129 (AI305024) was previously isolated from a *T. ruralis* cDNA expression library cDNA derived from the polysomal, mRNP fraction of desiccated gametophytes (Wood *et al.* 1999). Polymorphic chain reaction (PCR) amplification from the cDNA library was performed using the T3 primer and an antisense primer designed from RNP 129 (primer A, 5'-GAAGCCAAAAGCCATAGAA-3'). The full-length cDNA clone was obtained by 5' random amplification of cDNA ends (5'-RACE) using the FirstChoice RLM-RACE Kit (*Ambion*, Austin, USA) as described by the manufacturer utilizing the gene specific primers primer B (5'-GAAACAACTCCCGCACCA-3') and primer C (5'-GATCAGCTCACTTCCAACAAC-3'). Reverse transcription was done at 50 °C. First and second rounds of PCR amplification was adjusted to 65 and 67 °C, respectively, utilizing *HotstarTaq* DNA polymerase (*Qiagen*, Valenia, USA).

DNA sequence analysis: DNA sequence was determined by the Plant Biotechnology and Genome Center (Southern Illinois University) using an automated sequencer (*ABI model 377*; *Applied Biosystems*, Foster City, USA). Vector NTI suite (*InforMax*, North Bethesda, USA) was used for sequence assembly, analysis and homology searches. Similarity of the *T. ruralis* sequences to nucleotide sequences in GenBank, EMBL, DDBJ, and PDB databases were determined using the FASTA and BLASTN server as described by Wood *et al.* (1999). Molecular mass and pI prediction, and alignment of deduced amino acid sequences were performed using software available on the ExPASy molecular biology server (www.expasy.ch/). Multiple alignments of the deduced amino acid sequence for TrRPT2 and related RTP2 were created using *Clustal-X* and phylogenetic trees were constructed using the Neighbor-Joining algorithm. The amount of support for each node of the resultant trees was examined with 1000 bootstrap replicates. The data set consisted of the following predicted amino acid sequences: *Arabidopsis thaliana* (CAB43918), *Homo sapiens* (Q03527), *Saccharomyces cerevisiae* (546613), *Drosophila melanogaster* (AAF56205), *Neurospora crassa* (CAB88559), *Oryza sativa* (P46466), and *Brassica napus* (CAC 14432).

RNA isolation and RNA blot hybridizations: Total RNA was isolated using the RNeasy kit (*Qiagen*) as described by the manufacturer. RNA (approximately 10 µg) was separated by electrophoresis in a formaldehyde-agarose gel and transferred to nitrocellulose under standard conditions (Duff *et al.* 1999). The DNA probe (*i.e.* the 897 bp *EcoRI* fragment derived from the 5'-RACE product) was labeled with [α^{32}P]-dCTP (*PerkinElmer Life Sciences Inc.*, Boston, USA) using the random prime labeling kit (*Decaprime II* Kit, *Ambion*, Austin, USA). Membranes were prehybridized and hybridized at 42 °C using *ULTRAhybTM* (*Ambion*) as described by the manufacturer. Membranes were washed at 42 °C in 2× standard saline citrate (SSC), 0.1 % sodium dodecyl sulphate (SDS) for 2 × 5 min, followed by washing in 0.1×SSC, 0.1 % SDS for 2 × 15 min. Blots were stripped and re-probed with rRNA-DNA to demonstrate equal loading.

Results and discussion

Characterization of TrRPT2: We have employed EST analysis to discover a number of genes associated with desiccation stress including *Aldh21A1* (Chen *et al.* 2002a), *Aldh7B6* (Chen *et al.* 2002b), *Vac1* (Chen *et al.* 2002c), *Elipa* and *Elipb* (Zeng *et al.* 2002), and the ribosomal proteins *Rpl15*, *Rps14*, *Rps16* and *Rpl23* (Wood *et al.* 2000a, Zeng and Wood 2000). In this paper, we describe the isolation and characterization of an EST-derived *T. ruralis* cDNA encoding a predicted polypeptide with significant similarity to the 26S proteasome regulatory subunit IV. The full-length cDNA was obtained using 5'-RACE and designated *TrRpt2* in accordance with the proposed unified nomenclature for the proteasome (Finley *et al.* 1998). The composite *TrRpt2* cDNA was 1845 bp in length and contained a single, continuous open reading frame from nucleotide 187 to 1524 flanked by a 186 bp 5'UTR, and a 321 bp 3'UTR (data not shown). The initiation and termination sequences conformed to the known consensus sequences in plants (Lutcke *et al.* 1987), and the cDNA contained putative polyadenylation signal sequences (Wood *et al.* 2000b) (data not shown). The ORF encodes a polypeptide of 446 amino acids with a predicted molecular mars of 49.6 kDa and predicted pI of 8.15. The deduced *T. ruralis* polypeptide TrRPT2 is 84 % identical to 26S proteasome RPT2 homologues from *Oryza sativa* and *Arabidopsis thaliana* (data not shown). TrRPT2 contains the AAA cassette (residues 227 - 414, Walker *et al.* 1982) that incorporates the P-loop ATP/GTP-binding site (GEPGTGKT, residues 232 - 239) and the AAA-protein family signature sequence (VKVILATNRIESLDPALLR, residues 331 - 349) (Swaffield and Purugganan 1997).

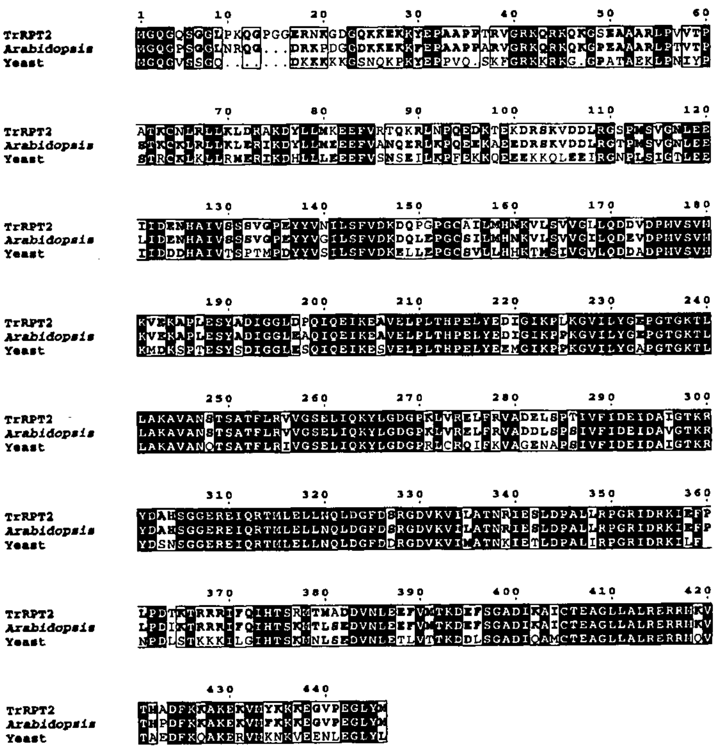

Fig. 1. Alignment of the deduced polypeptide sequence of TrRPT2 from *Tortula ruralis* (AF432345), *Arabidopsis* (CAB43918) and yeast (S46613). The sequences are numbered from the presumed translation initiation methionine (M) and are aligned using *ClustalV* to give maximal alignment.

Phylogenetic analysis of TrRPT2: To examine the structural relationship between the predicted polypeptide TrRPT2 and similar RPT2 subunits, the deduced amino acid sequences were analyzed by the Neighbor-Joining method. The gene tree was assembled from the pairwise alignment of these deduced polypeptide sequences (Fig. 2). To our knowledge, TrRPT2 is the first full-length RPT2 homologue to be characterized in bryophytes and this analysis demonstrates its evolutionary relationship to other RPT2 sequences. The RPT2 sequences could be reproducibly grouped into 2 major clades, one for plants and animals, and one for fungi. TrRPT2 is distinct from animals and tracheophytes, and forms a discrete evolutionary group.

Expression of TrRpt2 in *T. ruralis* gametophytes: The steady-state mRNA accumulation of *T. ruralis TrRpt2* transcripts were analyzed by RNA blot hybridization in response to a desiccation-rehydration cycle, ABA-treatment or NaCl treatment (Fig. 3). Total RNA was isolated from hydrated, slow-dried, slow-dried rehydrated, rapid-dried, rapid-dried rehydrated, ABA-treated or salinized gametophytic tissues as described (see Materials and methods). To enable normalization of the hybridization signals to account for loading anomalies, the membrane was re-probed after the initial analysis using a plant 18S nuclear rRNA probe. *T. ruralis TrRpt2* hybridized to a single mRNA species of approximately 1850 bp (Fig. 3). *TrRpt2* steady-state mRNA transcript levels are unchanged in response to desiccation or rehydration cycle (Fig. 3*A*), ABA-treatment (Fig. 3*B*), or stepwise application of NaCl and NaCl shock (Fig. 3*C*).

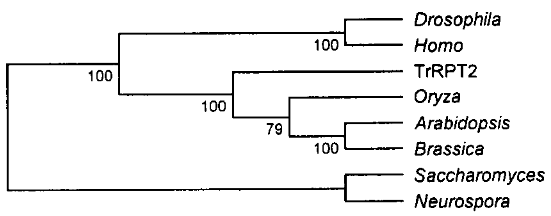

Fig. 2. Gene tree derived from a data set of deduced polypeptides for the *Tortula ruralis* TrRPT2 and related deduced polypeptides. The unrooted Neighbor-Joining tree was constructed using *ClustalV*. See Materials and methods for species name and accession numbers; the *scale bar* indicates the number of changes per unit length. Numbers below the lines represent bootstrap percentages (based on 1000 replicates).

The proposed mechanisms of desiccation tolerance differ between bryophytes and tracheophytes. *T. ruralis* employs a constitutive protection system and an active rehydration induced recovery mechanism (Oliver *et al.* 2000). This is in contrast to the strategy proposed for the angiosperm *Craterostigma plantagineum* that utilizes a drying induced elevation of abscisic acid to trigger the accumulation of gene products that mediate the establishment of a cellular protection system prior to desiccation (Ingram and Bartels 1996, Bartels and Salamini 2001). Unlike many plant stress responses, the alteration in gene expression within *T. ruralis* gametophytes elicited by desiccation stress is primarily regulated at the post-transcriptional level as a result of differential selection and/or recruitment of rehydrin mRNAs from a qualitatively constant mRNA pool (Oliver 1991). We have hypothesized that genes essential to recovery and cellular repair are preferentially expressed upon rehydration of desiccated gametophytes, *i.e.* the rehydrins (Oliver and Bewley 1997, Duff *et al.* 1999, Wood and Oliver 1999). We have also postulated that constitutive transcripts that are maintained in the slow dried state consist of both important house keeping genes and those genes that establish the constitutive desiccation tolerance protection system(s). TrRPT2 must play a role in the maintenance of 26S proteasome integrity and/or function during drying, in the desiccated state, and upon rehydration.

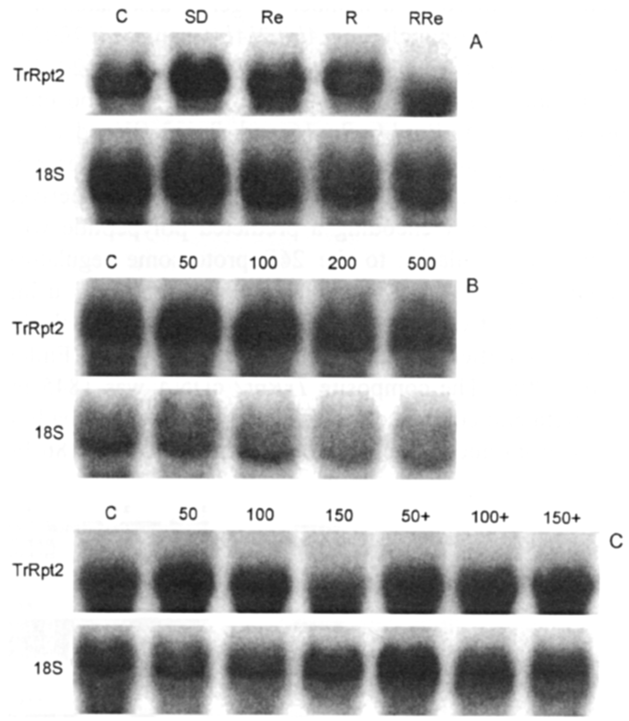

Fig. 3. RNA blot analysis of *TrRpt2* using the total RNA fraction of desiccated (*A*), ABA-treated (*B*) or salt-treated (*C*) *Tortula ruralis* gametophytes. *A*: C - control, SD - slow-dried, Re - rehydrated, R - rapid-dried, and RRe - rapid-dried rehydrated. *B*: gametophytes incubated in solutions of 50, 100, 200 or 500 µM ABA. *C*: C - control, 50 - 50 mM, 100 - 100 mM or 150 - 150 mM NaCl for 6 h or the step-wise addition of NaCl 50+ - 50 mM 2 h, 100+ -50 mM + 50 mM 2 h, and 150+ - 100 mM + 50 mM 2 h. RNA was extracted as described in Materials and methods. RNA (approximately 10 µg) was separated by electrophoresis in a formaldehyde-agarose gel and transferred to nitrocellulose under standard conditions. The resulting RNA blot was hybridized with ^{32}P-labeled cDNA probe for *TrRPT2* and blots were re-probed with rRNA-DNA to demonstrate equal loading.

Efficient targeted gene disruption (*i.e.* homologous recombination) is a well-established tool in the moss *Physcomitrella patens* (Schaefer and Zryd 1997). This capability is at present unique amongst all plants and represents an extremely powerful technique for the functional analysis of plant genes (Reski 1999, Cove 2000, Wood *et al.* 2000b, Schaefer and Zryd 2001). Girod *et al.* (1999) have characterized the 26S proteasome subunit MCB 1 (RPN 10) using homologous recombination in *P. patens*. MCB 1 was found to have high affinity for multiubiquitin chains and was postulated to play a key role in hormone-triggered developmental processes. In addition, the *P. patens* EST Programme

(PEP) (www.moss.leeds.ac.uk) has identified several 26S proteasome associated ESTs including the RPT2 subunit (AW599382). Mosses such as *T. ruralis* and *P. patens* will be key experimental systems for the study of 26S proteasome function *in vivo*, and will provide greater insight to the role which the ubiquitin-proteasome pathway plays in adaptation to elevated NaCl concentration, desiccation tolerance, and to stress-inducible post-transcriptional gene control.

References

Bartels, D., Salamini, F.: Desiccation tolerance in the resurrection plant *Craterostigma plantagineum*. A contribution to the study of drought tolerance at the molecular level. - Plant Physiol. **127**: 1346-1353, 2001.

Baumeister, W., Walz, J., Zuhl, F., Seemuller, E.: The proteasome: paradigm of a self compartmentalizing protease. - Cell **92**: 367-380, 1998.

Becker, F., Buschfeld, E., Schell, J., Bachmair, A.: Altered response to viral infection by tobacco plants perturbed in ubiquitin system. - Plant J. **3**: 875-881, 1993.

Chen, X., Zeng, Q., Wood, A.J.: The stress-responsive gene *ALDH21A1* describes a novel eukaryotic aldehyde dehydrogenase protein family. - J. Plant Physiol. **159**: 677-684, 2002a.

Chen, X., Zeng, Q., Wood, A.J.: *ALDH7B6* encodes a turgor-responsive aldehyde dehydrogenase homologue that is constitutively expressed in *Tortula ruralis* gametophytes. - Bryologist **105**: 177-184, 2002b.

Chen, X., Kanokporn, T., Zeng, Q., Wilkins, T.A., Wood, A.J.: Characterization of the V-type $H^{(+)}$-ATPase in the resurrection plant *Tortula ruralis*: accumulation and polysomal recruitment of the proteolipid C subunit in response to salinity. - J. exp. Bot. **53**: 225-232, 2002c.

Coux, O., Tanaka, K., Goldberg, A.L.: Structure and functions of the 20S and 26S proteasomes. - Annu. Rev. Biochem. **65**: 801-847, 1996.

Cove, D.J.: The moss *Physcomitrella patens*. - J. Plant Growth Regul. **19**: 275-283, 2000.

Duff, R.J., Oliver, M.J., Wood, A.J.: A *Tortula ruralis* cDNA encoding small-subunit ribosomal protein S3a: polysomal retention of transcript in response to desiccation and rehydration. - Bryologist **102**: 418-425, 1999.

Dubiel, W., Ferrell, K., Pratt, G., Rechsteiner, M.: Subunit 4 of the 26S protease is a member of a novel eukaryotic ATPase family. - J. biol. Chem. **267**: 22699-22702, 1992.

Finley, D., Tanaka, H., Mann, C., Feldmann, H., Hochstrasser, M., Vierstra, R., Johnston, S., Hampton, R., Haber, J., Mccusker, J., Silver, P., Frontali, L., Thorsness, P., Varshavsky, A., Byers, B., Madura, K., Reed, S.L, Wolf, D., Jentsch, S., Sommer ,T., Baumeister, W., Goldberg, A., Fried, V., Rubin, D.M., Glickman, M.H.: Unified nomenclature for subunits of the *Saccharomyces cerevisiae* proteosome regulatory particle. - Trends biochem. Sci. **23**: 244-245, 1998.

Fu, H.Y., Doelling, J.H., Rubin, D.M., Vierstra, R.D.: Structural and functional analysis of the six regulatory particle triple-A ATPase subunits from the *Arabidopsis* 26S protease. - Plant J. **18**: 529-539, 1999.

Genschik, P., Jamet, E., Philipps, G., Parmentier, Y., Gigot, C., Fleck, J.: Molecular characterization of a (3-type proteasome subunit from *Arabidopsis thaliana* co-expressed at a high level with an a-type proteasome subunit early in the cell cycle. - Plant J. **6**: 537-546, 1994.

Genschik, P., Criqui, M.C., Parmentier, Y., Derevier, A., Fleck, J.: Cell cycle-dependent proteolysis in plants: Identification of the destructive box pathway and metaphase arrest produced by the proteasome inhibitor MG132. - Plant Cell **10**: 2063-2076, 1998.

Girod, P-A., Fu, H., Zryd, J-P., Vierstra, R.D.: Multiubiquitin chain binding subunit MCB 1 (RPN10) of the 26S proteasome is essential for developmental progression in *Physcomitrella patens*. - Plant Cell **11**: 1457-1472, 1999.

Glickman, M.H., Rubin, D.M., Fried, V.A., Finley, D.: The regulatory particle of the *Saccharomyces cerevisiae* proteasome. - Mol. cell. Biol. **18**: 3149-3162, 1998.

Groll, M., Ditzel, L., Lowe, J., Stock, D., Bochtler, M., Bartunik, H.D., Huber, R.: Structure of 20S proteasome from yeast 26S protease subunit. - Nature **366**: 355-357, 1997.

Homma, S., Horsch, A., Pouch, M.N., Petit, F., Briand, Y., Schmid, H.P.: Proteasomes (prosomes) inhibit the translation of tobacco mosaic virus RNA by preventing the formation of initiation complexes. - Mol. Biol. Rep. **20**: 57-61 1994.

Ingram, J., Bartels, D.: The molecular basis of dehydration tolerance in plants. - Annu. Rev. Plant Physiol. Plant mol. Biol. **47**: 377-403, 1996.

Ito, N., Seo, S., Ohtsubo, N., Nakagawa, H., Ohashi, Y.: Involvement of proteasome-ubiquitin system in wound-signaling in tobacco plants. - Plant Cell Physiol. **40**: 355-360, 1999.

Lutcke, H.A., Chow, K.C., Mickel, F.S., Moss, K.A., Kern, H.F., Scheele, G.A.: Selection of AUG initiation codons differs in plants and animals. - EMBO J. **6**: 43-48, 1987.

Oliver, M.J.: Influence of protoplasmic water loss on the control of protein synthesis in the desiccation-tolerant moss *Tortula ruralis*: Ramifications for a repair-based mechanism of desiccation-tolerance. - Plant Physiol. **97**: 1501-1511, 1991.

Oliver, M.J., Bewley, J.D.: Desiccation-tolerance of plant tissues: a mechanistic overview. - Hort. Rev. **18**: 171-212, 1997.

Oliver, M.J., Velten, J., Wood, A.J.: Bryophytes as experimental models for the study of environmental stress tolerance: desiccation-tolerance in mosses. - Plant Ecol. **151**: 73-84, 2000.

Patel, S., Latterich, M.: The AAA team related ATPases with diverse functions. - Trends cell. Biol. **8**: 65-71, 1998.

Rechsteiner, M.: The 26S proteasome. - In: Peters, J.M., Harris, J.R., Finley, D. (ed.): Ubiquitin and the Biology of the Cell. Pp. 147-189. Plenum Press, New York 1998.

Reski, R.: Molecular genetics of *Physcomitrella*. - Planta **208**: 301-309, 1999.

Ruegger, M., Dewey, E., Grey, W.M., Hobbie, L., Turner, J., Estelle, M.: The TIR1 protein of *Arabidopsis* functions in auxin response is related to human SKP2 and yeast Grrlp. - Gene Dev. **12**: 198-207, 1998.

Schaefer, D., Zryd, J.P.: Efficient gene targeting in the moss *Physcomitrella patens*. - Plant J. **11**: 1195-1206, 1997.

Schaefer, D., Zryd, J.P.: The moss *Physcomitrella patens*, now and then. - Plant Physiol. **127**: 1430-1438, 2001.

Suzuka, L, Koga-Ben, Y., Minobe, Y., Hashimoto, J.: Identification of cDNA clones for nice homologs of the human immunodeficiency virus-1 Tat binding protein and subunit 4 of human 26S protease (proteasome). - Plant Sci. **103**: 33-40, 1994.

Swaffield, J.C., Purugganan, M.D.: The evolution of the conserved ATPase domain (CAD): reconstructing the history of an ancient protein module. - J. mol. Evol. **45**: 549-563, 1997.

Voges, D., Zwickl, P., Baumeister, W.: The 26S proteasome; A molecular machine designed for controlled proteolysis. - Annu. Rev. Biochem. **68**: 1015-1068, 1999.

Walker, J.E., Saraste, M.J., Runswick, J.J., Crray, N.J.: Distantly related sequences in the α- and β-subunits of ATPase, myosin, kinases, and other ATP-requiring enzymes and a common nucleotide-binding fold. - EMBO J. **1**: 945-951, 1982.

Wood, A.J., Duff, R.J., Oliver, M.J.: Expressed sequence Tags (ESTs) from desiccated *Tortula ruralis* identify a large number of novel plant genes. - Plant Cell Physiol. **40**: 361-368, 1999.

Wood, A.J., Duff, R.J., Oliver, M.J.: The translational apparatus of *Tortula ruralis*: polysomal retention of transcripts encoding the ribosomal proteins RPS 14, RPS 16, and RPL23 in desiccated and rehydrated gametophytes. - J. exp. Bot. **51**: 1655-1662, 2000a.

Wood, A.J., Duff, R.J., Zeng, Q., Oliver, M.J.: Molecular architecture of bryophyte genes: putative polyadenylation signals in cDNA 3'-ends of *Tortula ruralis*. - Bryologist **103**: 44-51, 2000b.

Wood, A.J., Oliver, M.J.: Translational control in plant stress: characterization of messenger ribonucleoprotein particles (mRNPs) in desiccated *Tortula ruralis*. - Plant J. **18**: 359-370, 1999c.

Wood, A.J., Oliver, M.J., Cove, D.J.: Frontiers in bryological & lichenological research. I. bryophytes as model systems. - Bryologist **103**: 128-133, 2000.

Zeng, Q., Wood, A.J.: A cDNA encoding ribosomal protein RPL 15 from the desiccation-tolerant bryophyte *Tortula ruralis*: mRNA transcripts are stably maintained in desiccated and rehydrated gametophytes. - BioSci. Biotech. Biochem. **64**: 2221-2224, 2000.

Zeng, Q, Chen, Y, Wood, A.J.: Two early light-inducible protein (ELIP) cDNAs from the resurrection plant *Tortula ruralis* are differentially expressed in response to desiccation, rehydration, salinity and high-light. - J. exp. Bot. **53**: 1197-1205, 2002.

Zwickl, P., Voges, D., Baumeister, W.: The proteasome: amacromolecular assembly designed for controlled proteolysis. - Phil. Trans. roy. Soc. London **354**: 1501-1511, 1999.