Available online at www.sciencedirect.com
ScienceDirect
Gene 407 (2008) 86–97
GENE
www.elsevier.com/locate/gene

Chromosomal high mobility group (HMG) proteins of the HMGB-type occurring in the moss *Physcomitrella patens*

Bruno Kiilerich [a], Christian Stemmer [b], Thomas Merkle [c], Dorte Launholt [a], Gilbert Gorr [b], Klaus D. Grasser [a,*]

[a] *Department of Life Sciences, Aalborg University, Sohngaardsholmsvej 49, DK-9000 Aalborg, Denmark*
[b] *Greenovation Biotech GmbH, Heilbronn, Ndl. Freiburg: Boetzinger Str. 29b, D-79111 Freiburg, Germany*
[c] *Genome Research, Faculty of Biology, University of Bielefeld, Universitätsstr. 25, D-33594 Bielefeld, Germany*

Received 21 August 2007; received in revised form 28 September 2007; accepted 29 September 2007
Available online 13 October 2007
Received by G. Theissen

Abstract

High mobility group (HMG) proteins of the HMGB family are chromatin-associated proteins that act as architectural factors in nucleoprotein structures, which regulate DNA-dependent processes including transcription. Members of the HMGB family have been characterised from various mono-and dicot plants, but not from lower plant species. Here, we have identified three candidate HMGB proteins encoded in the genome of the moss *Physcomitrella patens*. The structurally similar HMGB2 and HMGB3 proteins display the typical overall structure of higher plant HMGB proteins consisting of a central HMG-box DNA-binding domain that is flanked by a basic N-terminal and an acidic C-terminal domain. The HMGB1 protein differs from higher plant HMGB proteins by having a very extensive N-terminal domain and by lacking the acidic C-terminal domain. Like higher plant HMGB proteins, HMGB3 localises to the cell nucleus, but HMGB1 is targeted to plastids. Analysis of the HMG-box domains of HMGB1 and HMGB3 by CD revealed that HMGB1box and the HMGB3box have an α-helical structure. While the HMGB3box interacts with DNA comparable to typical higher plant counterparts, the HMGB1box has only a low affinity for DNA. Cotransformation assays in *Physcomitrella* protoplasts demonstrated that expression of HMGB3 resulted in repression of reporter gene expression. In summary, our data show that functional HMGB-type proteins occur in *Physcomitrella* and most likely in other lower plant species.

Keywords: HMG-box domain; Structure-specific DNA interaction; DNA ligation assay; Subcellular localization; Cotransformation assay

1. Introduction

High mobility group (HMG) proteins represent a heterogeneous group of small and relatively abundant non-histone proteins associated with the chromatin of eukaryotic organisms (Bianchi and Agresti, 2005; Bustin and Reeves, 1996; Bustin, 1999). Proteins belonging to the subclass of HMGB proteins (Bustin, 2001) act as architectural factors, facilitating the assembly of nucleoprotein complexes, which are involved in the regulation of transcription and other DNA-dependent processes (Bianchi and Agresti, 2005; Bustin, 1999; Thomas and Travers, 2001; Agresti and Bianchi, 2003). HMGB proteins contain one or two copies of a distinctive ~75-amino acid residue DNA-binding domain, termed the HMG-box domain. The three-dimensional fold of this domain, consisting essentially of three α-helices which are arranged in an L-shape, is well conserved (Thomas and Travers, 2001; Travers, 2000). In addition to chromosomal HMGB proteins, HMG-box domain(s) are found in other DNA-binding proteins including transcription factors (Stros et al., in press). The HMG-box domain of the HMGB proteins mediates non-sequence-specific binding to linear DNA, and high affinity interactions with certain distorted DNA structures (Bustin, 1999; Zlatanova and van Holde, 1998; Thomas and Travers, 2001; Travers, 2000; Agresti and Bianchi, 2003). In complexes with B-DNA, the concave surface of the HMG-box domain binds predominantly the minor groove of

Abbreviations: HMG, High mobility group; EMSA, electrophoretic mobility shift assay; CD, circular dichroism; GFP, green fluorescent protein; NLS, nuclear localisation sequence.

* Corresponding author. Tel.: +45 9635 9126; fax: +45 9814 1808.
E-mail address: kdg@bio.aau.dk (K.D. Grasser).

0378-1119/$ - see front matter © 2007 Elsevier B.V. All rights reserved.
doi:10.1016/j.gene.2007.09.025

the DNA, and the partial intercalation of amino acid residues between base pairs contributes to the bending of the DNA by over 90° (Thomas and Travers, 2001; Travers, 2000).

Members of the HMGB family of proteins have been characterised from various higher plant species, revealing that they share many properties with their counterparts from other eukaryotic organisms, and that they are different in some respects (Pedersen et al., 1991; Ritt et al., 1998b; Spiker, 1984; Webster et al., 1997; Webster et al., 2000; Wu et al., 2003b; Yamamoto and Minamikawa, 1998; Zhang et al., 2003). Plant genomes encode a variety of HMGB proteins ranging from ~13–27 kDa. Thus, five different HMGB proteins were characterised from the monocot plant maize (Ritt et al., 1998b; Stemmer et al., 1999) and seven HMGB proteins encoded in the genome of the dicot plant *Arabidopsis thaliana* have been analysed (Grasser et al., 2004; Grasser et al., 2006; Stemmer et al., 1997). Typically, plant HMGB proteins have a single HMG-box domain, which is flanked by a basic N-terminal domain and an acidic C-terminal domain. The HMG-box domains of the various plant HMGB proteins are relatively conserved, but the basic and acidic flanking regions vary considerably in length and sequence (Grasser et al., 2007a). Based on Northern/Western blotting and expression analysis of *HMGB* gene promoter-reporter gene fusions, HMGB proteins are widely expressed in the plant (Kwak et al., 2007; Launholt et al., 2007; O'Neill and Zheng, 1998; Stemmer et al., 1999; Wu et al., 2003a; Yamamoto and Minamikawa, 1998). Plant HMGB proteins bind linear DNA non-sequence-specifically with moderate affinity, but recognise specifically certain DNA structures such as minicircles and four-way junctions, and they severely bend linear DNA upon binding (Ritt et al., 1998b; Stemmer et al., 1997; Webster et al., 2001; Wu et al., 2003b). In the cell nucleus, HMGB proteins display a high dynamics, interacting with DNA/chromatin only transiently before moving on to the next binding site, thereby scanning the nuclear space for binding sites (Launholt et al., 2006).

To date, HMGB proteins have been identified and characterised from a variety of mono-and dicot plants, but not from lower plant species. Here, we have searched databases for HMGB proteins encoded by the moss model *Physcomitrella patens*. Our survey yielded three candidate HMGB proteins that share structural features with higher plant HMGB proteins. Analysis of two *Physcomitrella* proteins revealed structural and functional similarities as well as differences compared to HMGB proteins of higher plants.

2. Materials and methods

2.1. Analysis of sequences encoding HMGB-type proteins

EST sequences encoding putative *Physcomitrella* HMGB proteins were identified by searching the translated NCBI nucleotide database (http://www.ncbi.nlm.nih.gov/) using TBLASTN and the amino acid sequence of the HMG-box DNA-binding domain of the maize HMGB1 protein as a query. The obtained sequences were assembled into contigs using the software SeqMan (DNASTAR). Sequences were aligned pairwise (http://www.ebi.ac.uk/emboss/align/index.html) and by multiple sequence alignments (http://www.ebi.ac.uk/Tools/clustalw/index.html).

2.2. Plasmid constructions

All PCR reactions were performed using Deep Vent DNA polymerase (NEB) or Pfu DNA polymerase (MBI Fermentas) and *Physcomitrella* cDNA as a template, unless stated otherwise. All plasmid constructions were checked by restriction enzyme digestions and DNA sequencing. The plasmid constructions performed in this study are summarised in Table 1.

2.3. Localisation assays with GFP and DsRed fusion proteins

Protoplasts were prepared from dark-grown tobacco BY-2 cells, and transiently transformed with plasmids encoding GFP and/or DsRed fusions (and control constructs) by PEG-mediated transformation as described previously (Haasen et al., 1999). Excitation of GFP was performed with a standard UV light source and fluorescein isothiocyanate (FITC) filters, while for the excitation of DsRed tetramethyl-rhodamine isothiocyanate (TRITC) filters were used. For confocal laser scanning microscopy, samples were directly examined under oil with a 63x objective and a DM RE TCS4D microscope (Leica) equipped with an argon-krypton laser (excitation 488 nm, beam splitter 500 nm, detection 500–560 nm for GFP; excitation 488/543 nm, double dichroic 488/543, detection 500–530 nm and 580–700 nm for simultaneous excitation and detection of GFP and DsRed) using Leica Scanware. Analysis of the localisation of the GFP and/or DsRed fusion proteins was performed in three independent experiments, representing approximately 60–80 transformed protoplasts.

2.4. Protein production

Using plasmids pQE9-HMGB1box and pQE9-HMGB3box, recombinant HMG-box domains were expressed as 6xHis-tagged proteins in *E. coli* and purified by three-step FPLC column chromatography (Ni–NTA agarose, S Sepharose Fast Flow, Phenyl–Sepharose) as described previously (Ritt et al., 1998a). Purified proteins were checked by SDS–PAGE and MALDI/TOF mass spectrometry.

2.5. Circular dichroism (CD)

CD measurements using 10 μM HMGB1box and HMGB3box were performed using a Jasco J-810 instrument as described previously (Stemmer et al., 2002).

2.6. Electrophoretic mobility shift assay (EMSA)

DNA binding of HMG-box domains to linear and circularised DNA fragments was examined by EMSAs as previously described (Grasser et al., 2006).

2.7. DNA ligation assays

The ability of the HMG-box domains to promote DNA end joining and circularisation of DNA fragments was tested