

Short communication

cDNA encoding a wheat (*Triticum aestivum* cv. Chinese Spring) glycine-rich RNA-binding protein

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Abstract

A wheat cDNA encoding a glycine-rich RNA-binding protein, whGRP-1, was isolated. WhGRP-1 contains two conserved domains, the RNA-binding motif (RNP motif) combined with a series of glycine-rich imperfect repeats, characteristic of a conserved family of plant RNA-binding proteins. Northern analysis revealed that whGRP-1 mRNA accumulates to high levels in roots and to lower levels in leaves of wheat seedlings. whGRP-1 mRNA accumulation is not enhanced by exogenous abscisic acid in seedlings and accumulates to very high levels during wheat embryo development, showing a pattern different from that of the ABA-inducible wheat Em gene.

RNA-binding proteins have been implicated in the control of gene expression at the levels of mRNA processing, transport, polyadenylation and RNA stability [10, 14]. As increasing numbers of RNA-binding proteins have been identified, several conserved RNA-binding motifs have been defined [5, 19]. The largest and best characterized group contains the RNP motif (sometimes called ribonucleoprotein consensus sequence (RNP-CS) or consensus sequence RNA-binding domain (CS-RBD)). The RNP motif consists of about 90 amino acids present in one or more copies per polypeptide. Two short highly conserved sequences, RNP1 (octamer) and

RNP2 (hexamer) characterize this domain. In plants, RNP motif-containing RNA-binding proteins have been identified in spinach [28], tobacco [15–17, 20, 32], maize [7, 9, 11], *Arabidopsis thaliana* [2, 6, 23, 30], sorghum [8], carrot [29], mustard [13], and *Brassica napus* [4]. Within this group, a subfamily of ca. 16 kDa plant proteins consisting of a single RNP motif in combination with a glycine-rich imperfect repeat domain can be distinguished, represented by cDNAs isolated from maize, *Arabidopsis*, tobacco, sorghum, carrot, mustard, and *B. napus*. The plant glycine-rich RNA-binding proteins (GRPs, following the nomenclature first used [8]) have been

The nucleotide sequence data reported will appear in the GenBank, EMBL and DDBJ Nucleotide Sequence Databases under the accession number U32310 (whGRP-1).

implicated in many physiological processes, including ABA induction [11], water and chemical stress [9, 11], wounding [11, 29], and light and temperature-entrained circadian rhythm [6, 13]. Characterization of genes encoding RNA-binding proteins has revealed examples of developmental and tissue-specific regulation as well as tissue-specific alternative splicing of their products [6, 13, 15]. However to date, knowledge of the specific *in vivo* role of any of these proteins in plant development remains elusive.

We have isolated a full-length cDNA encoding a glycine rich nucleic acid-binding protein from wheat. Northern analysis revealed that the expression pattern of *whGRP-1* is significantly different from that of the ABA-inducible *Em* gene [25], suggesting that unlike the maize RNA-binding protein gene *MA16* [11], *whGRP-1* expression is independent of ABA.

Isolation of a cDNA encoding whGRP-1

In the course of screening a wheat ABA-treated, stage III embryo cDNA library for clones encoding the DNA binding protein *EmBP-1* [24], a phage was isolated that harbored a cDNA insert which upon detailed analysis, appeared to consist of two unrelated cDNAs artifactually fused head to head. At the 5' end of this insert, upstream of an apparently full-length coding region for *EmBP-1*, a short stretch of DNA containing a stretch of T residues begins the sequence, suggesting the reversed fusion of a second cDNA (designated here as *whGRP-1.1*). When the 360 bp 3' end fragment (nt 578–937 in Fig. 1A) was used as a hybridization probe to rescreen the library, 150 clones which hybridized strongly to the probe were identified from ca. 200 000 phage on 5 plates, indicating that these clones are represented in the library at a much higher frequency (1:1333) than *EmBP-1* cDNAs isolated from the same library which occurred at a frequency of 1:80 000 [12, 22]. Preliminary northern blot data using this probe also agreed with this observation, resulting in very strong hybridization signals at a size distinct from that of *EmBP-1* (not shown).

One of the clones (pXN14) appeared to be near full-length clone (nt 125–965 in Fig. 1A). A second screen was performed using a probe containing the 5' end cDNA sequence from 125 (5' end of pXN14) to 315 (a *SacI* site in pXN14), and 150 additional positive clones were identified from another 200 000 phage. From these two screens, ca. 40 phage isolates were purified and restriction mapped, and of these, 10 were selected for further analysis. Large-scale preparation of purified phage by CsCl equilibrium centrifugation and subcloning of DNA inserts from λ gt11 phage DNA into pUC19 plasmid were performed by standard methods [27]. DNA sequencing of two near full-length clones (pXN28 1–901 and pXN14 125–965 in Fig. 1A), which were identical over their 778 bp overlap, and of 10 partial clones which were also identical in their overlaps resulted in the full-length sequence of *whGRP-1* (Fig. 1A). The *whGRP-1* sequence is 965 bp long with a poly(A) tail of 28 bp. Based on the location of the longest open reading frame, 5'- and 3'-untranslated regions of 100 and 330 bp, respectively, can be identified.

The longest open reading frame within this cDNA (101–604) encodes a protein (designated as *whGRP-1*) which shares strong sequence similarity to a family of RNA binding proteins, the RNPs [5]. The predicted *whGRP-1* protein contains a RNP motif, characteristic of this family of RNA-binding proteins found in organisms ranging from bacteria to humans [5] (Fig. 1B). Within the RNP motif, the two conserved sub-domains RNP-1 (octamer) and RNP-2 (hexamer) were also found in the wheat cDNA. The molecular mass of *whGRP-1* is predicted to be 16 kDa with a pI of 4.9. *whGRP-1* also encodes a domain very rich in glycine residues (Fig. 1), with stretches of glycines interspersed with basic amino acids such as arginine, a characteristic shared with a large group of plant glycine-rich RNA-binding proteins (GRPs). Sequence alignment of 14 plant GRPs indicates that RNA-binding domains (residues 1–86) among these proteins isolated from monocots and dicots are highly conserved, their amino acid sequence similarities ranging from 69% to 94% (Fig. 1B). They all have glycine-rich domains

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CTCCTCCCTTCCGGTTCGGGTTCTGGTTCTGGTTCCGGTCCGAGAGTAGCTAGCTAGTC 60
TAGGGTTTCCGTAGAGAGGAAGGAGAGGAGATCCATCCATGGCCGAGACGGAGTACCG 120
                                     M A E T E Y R
CTGCTTCGTGGCGGCCTCGCTGGGCCACCGACGACAACAACCTCCAGCAGGCCCTTCAG 180
C F V G G L A W A T D D N N L Q Q A F S
                                     RNP2
CCAGTACGGCGAGATCCTCGACGCCAAGATCATCAACGACCCGAGACGGGGAGTCCCG 240
Q Y G E I L D A K I I N D R E T G R S R
CGGGTTCGGCTTCCTCAGCTTCGGCAGCGAGGAGTCGATGCGCCAGGCCATCGAGGAGAT 300
G F G F V T F G S E E S M R Q A I E E M
                                     RNP1
GAACGGCAAGGAGCTCGACGGGGCAACATCACCGTCAACGAGGCCAGTCCCGCCGCTC 360
N G K E L D G R N I T V N E A Q S R R S
GGCGGAGGGGGCGGCGCGCGGCTACGGCGCCAGCGCGCGGTGGCGGAGGTA 420
G G G G G G G G G G Y G G Q R G G G G G Y
CGCGGCGCGCGGCTACGGAGCGCGCGCGGCTACGGCGCCAGGGCGGTGGCGG 480
G G G G G Y G G G G G Y G G Q G G G G
CTACGGCGCCAGCGGTTGGCGCGCGGCTACGGCGCGCGCGGCTACGGCGGTTGG 540
Y G G Q R G G G G G G Y G G G G G Y G G
TGCGGGCTACGGCGCCAGCGCGCGGTTGGCGCGCGGCTACGGCGCCAGTGSAGGAA 600
G G Y G G Q R G G G G G D S G G Q W R N
CTGAGCCTCGAATCCTCCAGTATCGATCTATCACCCTGCTGCTGCTCTTCGTT 660
•
AGCAAGTTATCTTCGGTGTCTGCTGTTCTCTGTGTCAGTCTGAGTCGTGTTGTGTT 720
GTTTTGATCGCGAGCTCGAGTGTCTCTGTCGGGGTAAATAGAGCTGTTACCGGA 780
GGAAGAAACAACATGTGTACTATCTTTCAGTCGGTGGTGGTGGTGGTGGTGGTGG 840
CAGCAATCCCTTCTGTCATGGATTGGGATGTGATGTTGGAATATCTATGTTTGAACAA 900
GTTTAAATGGATGAAGAAAATGCAAGTCATAGCTGCGAAAAAATAAAAAAAAAAAAAA 960
AAAAA 965
    
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RNA Binding Domain

	RNP2	RNP1		RNP2	RNP1	
whGRP-1	MA--ETEYR	CFVGG	AWATDDNNLQQA FYSQYGEILLDAKIINDRETGRS	RGFGFVTF	GSEESMRQAI EE MNGKELDGRNITVNEAQSR 86	
MA16	MAAADVEYR	CFVGG	AWATS SNESLENAFASYGEILLDSKVIIDRETGRS	RGFGFVTF	SSENSMLDA IE MNGKELDGRNITVNEAQSR- 88	
CHEM2	MARSDVEYR	CFVGG	AWATD DHSLHNAFSTYGEVLESKIIIDRETGRS	RGFGFVTF	S TE ERMNR IE MNGKELDGRNITVNEAQSR- 88	
SvS2	MAAADVEYR	CFVGG	AWAT NNETLEQAFANFGQVIDSKVIIDRETGRS	RGFGFVTF	SSEQSMLDA IE MNGKELDGRNITVNEAQSR- 88	
DcGRP	MA--EVEYR	CFVGG	AWAT NDESLEQAFSDFGDIIDSKIIIDRETGRS	RGFGFVTF	KDEKSMRDA IE MNGQELDGRNITVNEAQSRG 86	
AtGRP7	MASGDVEYR	CFVGG	AWATD DRAL E TAFAYGDIVDSKIIIDRETGRS	RGFGFVTF	KDEKAMKDA IE MNGQDLDRSITVNEAQSRG 88	
AtGRP8	MS--EVEYR	CFVGG	AWAT NDEDLQRTFSQFGVIDSKIIIDRESGRS	RGFGFVTF	KDEKAMRDA IE MNGKELDGRVITVNEAQSRG 86	
Ccr1	MS--EVEYR	CFVGG	AWAT NDEDLQRTFSQFGVIDSKIIIDRESGRS	RGFGFVTF	KDEKAMRDA IE MNGKELDGRVITVNEAQSRG 86	
RGP-1a	MA--EVEYR	CFVGG	AWAT TDQTLGEAFSDFGEILLDSKIIIDRETGRS	RGFGFVTF	KDEKAMRDA IE MNGQDLDRNITVNEAQSRG 86	
RGP-1b	MA--EVEYS	CFVGG	AWAT TDRTLADAFQTYGEVLDSKIIIDRETGRS	RGFGFVTF	KDEKCMRDA IE MNGQELDGRSITVNEAQARG 86	
RGP-1c	MA--EVEYR	CFVGG	AWAT TDRTLGEAFSDFGEVLESKIIIDRETGRS	RGFGFVTF	GDEKSMRDA IE MNGQDLDRNITVNEAQSRG 86	
SaGRP1a	MASPDVEYR	CFVGG	AWAT DDRAL E TAFSDFGEVLDKIIIDRETGRS	RGFGFVTF	KDEKSMKDA IE MNGQDLDRSITVNEAQSRG 88	
SaGRP2a	MASPDVEYR	CFVGG	AWAT DRSL E TAFSDFGEVLDKIIIDRETGRS	RGFGFVTF	KDEKSMKDA IE MNGQDLDRSITVNEAQSRG 88	
BnGRP	MS--EVEYR	CFVGG	AWAT GDAL E LRTFSDFGEVLDKIIIDRETGRS	RGFGFVTF	KDEKSMKDA IE MNGKELDGRITVNEAQSRG 86	

Glycine Rich Domain

whGRP-1	SSGGGGGGGGYGGQR--GGGGGY-GGG--GGYGGGGGY--GGGGGGYGGQGGGG--GYGGGG-YGG---GGG-YGG-QR-GGGGGDGGQWRN 167
MA16	--GGGGGGGGYGGRR--GGGGY-GGGRRDGGYGGGGYGGRRGGGGGY--GG-YGG--RREGG--GGYGG--GGGWR-D 157
CHEM2	--GRRGG--GGGGY-GGGRRGGGGYGGGG--RRDGGGGYGG--GG--GYGGGG-YGG---GGGGYGGNR-GGGYGNDSGNWRN 155
SvS2	--GGGGGGGGYGGC--GGGY-GG-REGGGYGGGGGGYGGRRGGGGY--GG--GYGGGGGGYGG--REGGGYGGGGYGGNRGDSGGNWRN 168
DcGRP	SSGGGG--RREGGGGGYGGC--G-YGRR--EGGGGGYGG-RREGG-GG-GYGGGGGGYGGRR-EGGDGGY-----GGGGGSRW 157
AtGRP7	SSGGGGHRGGGGGGYRSGGGGGYSGGG--GSYGGGG--RREGGGGGYSGG--GG-GYSSRGGGGGGYGGRR-REGGGYGGGGG-GGYGGSGGGGGW 176
AtGRP8	SSGGGGRRGGGGGGYRSGGGGGYSGGG--GGYSGG--GGGY--ERRSGGYSGG-GGGGRGYGGG-RREGGGYGGGG-G-SYGGGGGGW 169
Ccr1	SSGGGGRRGGGGGGYRSGGGGGYSGGG--GGYSGG--GGGYE--RRSGGYSGG-GGGGRYGGG-RREGGGYGGG--GGYGGGGGG--W 169
RGP-1a	SSGGGG--GGYRGGSGGGYGGGRREGGYG--GGGGYGGRRREGG-YG-G--GGGGYGGGR-R-EGGY-----GGGSEGNWRS 156
RGP-1b	SSGGGG--YGGRRREGGGGGYGGC--G-YGGR--REGGGGGYGGRRREGG-GC-GYGGG--YGGG-RY 148
RGP-1c	SSGGGG--GGFRGGRR--GGGGYGG--G-YGGR--REGGGGGYGGGGYGG--RDRGYGGDRGYGG--DGGSRYSG--GGSDGNWRN 165
SaGRP1a	SSGGGGRRGGGG--YRSGGGGGYGGC--GGYGG--GREGG--YSGG--GG-GYSSRGGGGGGYGGG--RRDGG--EG-GGYGGSGGGGGW 166
SaGRP2a	SSAGGGRRGGGG--YR--GGGGYGGC--GGYGG--RREGG-YSGG--GG-GYSSRGGGGGGYGGG--RRDGGYGGGG-GGYGG-GGGGGW 169
BnGRP	--GGGGGG--GGYGGRRGGGGY-GGG--GGYGGRR--GGGGY--GSGGGRRGGGGYGGGGRRDGGYGGGG-G-YGGSGGGGGW 169

Fig. 1. A. Nucleotide and deduced amino acid sequences of the cDNA encoding the RNA binding protein whGRP-1. The conserved RNA binding motifs, octa-amino acids (RNP1) and hexa-amino acids (RNP2), in the RNA binding domain are boxed and indicated on the sequence. B. Amino acid sequence alignment of plant GRPs from maize (MA16 [11]; CHEM2 [9]), sorghum (SvS2 [8]), carrot (DcGRP [29]), *Arabidopsis* (AtGRP7 and AtGRP8 [30]; Ccr1 [6]), tobacco (RGP-1a, RGP-1b and RGP-1c [15]), *Sinapis alba* L. (SaGRP1a and SaGRP2a [13]) and *Brassica napus* (BnGRP [4]). The RNA-binding domain (upper panel) and glycine-rich domain (lower panel) are indicated. The conserved octa-amino acids (RNP1) and hexa-amino acids (RNP2) in the RNA binding domain are shaded and in bold type. Other highly conserved amino acids in RNA binding domain and glycine-rich domain are also in bold type. Sequence alignments were performed using Clustal analysis with the PAM250 lookup table implemented on a Macintosh Centris 650 computer (DNASTAR, Madison, WI).

and approximately uniform size (148 to 176 amino acids). Detailed examination indicates that whGRP-1 differs from all other plant GRPs in several positions; threonine (T) at position 4 and arginine (R) at position 86. whGRP-1 is also unique in containing several glutamine (Q) residues in the glycine-rich domain, a characteristic not found in any other plant GRPs identified to date. Whether these variations result in biochemical and biological differences remains unknown. Of the plant GRPs, whGRP-1 is most similar to maize CHEM2 and MA16 proteins and to sorghum SvS2, the only other monocot GRPs in the database.

Northern blot analysis

Expression of the whGRP-1 and Em genes in wheat leaves, roots, embryos and seedlings ger-

minated with or without ABA as detected by northern blot analysis is shown in Fig. 2. Wheat total RNA was isolated by phenol extraction by a standard method [1] from plants which were grown to maturity in a greenhouse, and from developing wheat embryos excised and staged as previously described [26]. Root and leaf samples were excised from seedlings imbibed on MS salts on filter papers in a glass dish incubated in a growth room with lights for 5 days. For ABA treatment, wheat seeds were incubated in the dark for 3 days on filter papers soaked with MS salts with or without 0.1 mM ABA. As judged by hybridization to a 16S rRNA probe, the RNA amounts loaded in each lane were approximately the same.

The whGRP-1 mRNA steady state levels are relatively low in leaves as compared to the relatively high expression levels in roots and embryos.

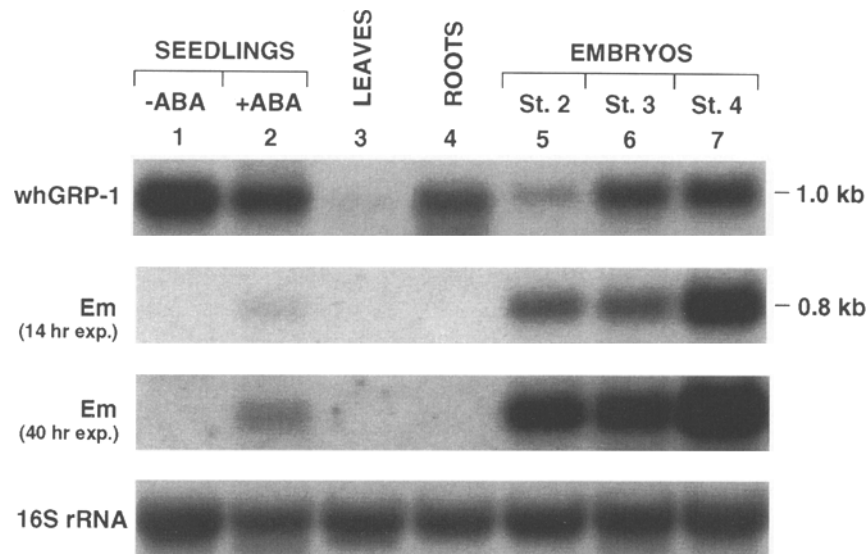


Fig. 2. Northern analysis of whGRP-1 gene transcripts in different tissues or at different development stages as indicated at the top of each lane. Approximately 10 μ g of total RNA from each tissue were separated on a 1.2% agarose-formaldehyde gel and transferred to a nylon membrane [1]. Lanes 1 and 2, seedlings without and with ABA induction; lane 3, leaves; lane 4, roots; lanes 5–7, stage 2, 3, and 4 of embryos. As controls, Em gene transcripts and 16S rRNA on the same northern blot were also analyzed. A longer exposure (40 h exposure) is to show ABA induction of Em gene expression in seedlings. DNA probes were; a 320 bp *SacI*-digested whGRP-1 cDNA fragment, a 680 bp *PstI*-digested Em cDNA fragment [18], and a 400 bp *EcoRI*-digested 16S tomato ribosomal cDNA fragment which were gel-purified and labeled by the random primed method. Hybridization was in 5 \times SSC (1 \times SSC is 0.15 M NaCl, 0.015 M sodium citrate pH 7.0), 5 \times Denhardt's solution, 0.5% (w/v) SDS, 100 μ g/ml denatured salmon sperm DNA with a [α - 32 P]dCTP-labeled probe at 68 $^{\circ}$ C for 16 h. The filters were washed twice in 2 \times SSC, 0.1% (w/v) SDS at room temperature and twice in 0.1 \times SSC, 0.1% (w/v) SDS at 68 $^{\circ}$ C for 30 min each. Between probes, the filter was stripped by immersion in near-boiling 0.1% (w/v) SDS followed by cooling to room temperature and air-drying before rehybridization to a second probe as described above. Autoradiography was performed at -80° C with intensifying screens and Kodak XAR 5 film.

Although we have not quantitatively determined the percentage of mRNA represented by the whGRP-1 transcript, judging from the very high signal strengths (exposure times of 7 h in Fig. 2), whGRP-1 hybridizing mRNA appears to be very abundant in these tissues. Our results for whGRP-1 expression in leaves and roots are consistent with the expression patterns of several other plant GRP genes [11, 15].

Expression of whGRP in seedlings in not ABA-induced

The maize MA16 gene (which is closely related to whGRP) was shown to be ABA inducible in embryos [11]. It has been proposed that MA16 may function in some aspect of ABA regulated gene expression. Because of the high similarity in sequence between MA16 and whGRP-1 (Fig. 1B), we tested if the whGRP-1 gene can be similarly induced by exogenous ABA in young seedlings. As a positive control for ABA induction, the well characterized Em gene [3, 25] was used as a second hybridization probe. The northern blot depicted in Fig. 2 reveals that the whGRP-1 gene is highly expressed in seedlings, however, exogenous ABA had no or a small repressive effect on whGRP-1 mRNA accumulation (compare lane 1 and lane 2 in Fig. 2). As expected, Em mRNA accumulation was induced by exogenous ABA applied to the seedlings [3], although the level of Em message was apparently much lower than that of the whGRP gene.

Expression of whGRP-1 during wheat embryogenesis

The whGRP cDNA was isolated from a cDNA library made from ABA treated wheat embryos, and thus embryo development presented another opportunity to test the potential ABA inducibility of the whGRP-1 gene. Northern blots of RNA extracted from dissected embryos at several stages of development [26] were hybridized to the whGRP-1 and Em probes (Fig. 2, lanes 5 to 7).

Steady-state mRNA levels of both the whGRP-1 and Em transcripts are at relatively low levels early in development and accumulate to very high levels during later stages, but the timing of the increase in expression levels differs between the genes. A sharp increase in whGRP-1 transcripts can be observed between stage 2 and 3 embryos, prior to the known period of rapid ABA accumulation and induction of the maturation pathway [24]. As expected, although detectable in stage 2 embryos, a sharp increase in Em transcripts occurs later, during the transition from stage 3 to 4 of embryo development consistent with previous results [21, 31].

From these data it appears that the whGRP gene is regulated in a manner quite different from the ABA-inducible Em gene both in tissue specificity and during embryo development. The whGRP gene does not appear to be regulated by ABA in seedlings, and reaches maximal expression in developing embryos prior to the large increase in ABA levels during the transition to the maturation pathway.

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