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Differential expression of a hydroxyproline-rich cell-wall protein gene in embryonic tissues of *Zea mays* L.

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Abstract. A hydroxyproline-rich glycoprotein (HRGP) component of the maize cell wall was shown to be present in different organs of the plant by extraction of cell wall proteins and detection by Western blotting and immunocytochemistry. Antibodies raised against the protein or against synthetic peptides designed from the protein sequence immunoprecipitated a proline-rich polypeptide which was synthesized in-vitro from poly(A)$^+$RNA extracted from different tissues of the plant and from the complete in-vitro-transcribed mRNA. A very low amount of the protein was found in immature embryos. In particular, the protein could not be detected in the scutellum either by Western blotting or by immunocytochemistry. In agreement with this finding, HRGP mRNA was barely detected in the scutellum, in contrast to its accumulation in the embryo axis. Our results indicate the existence of a unique cell wall structure in embryonic tissues from maize as well as a tissue-specific component of the control of maize HRGP gene expression, distinct to others already described such as cell division.

Key words: Cell wall proteins – Hydroxyproline-rich glycoprotein – Embryo – *Zea*

Introduction

Cell walls play an active role in plant development. During cell division the definition of the cell plate is an essential morphogenetic step. Both protein and carbohydrate components of the wall may be involved in these processes. Oligosaccharides produced by degradation of the cell wall are active growth regulators (Eberhard et al. 1989) while extracellular glycoproteins supplement mutations in carrot embryogenesis (de Vries et al. 1988). Carbohydrate epitopes from the cell wall mark precise steps in plant embryogenesis (Knox et al. 1989).

Hydroxyproline-rich glycoproteins (HRGP(s)) are one of the main protein components in plant cell walls (Varner and Lin 1989). The best known of these proteins are the extensins. They have been described at protein and gene levels in dicotyledonous plants (Cassab and Varner 1989). They are coded by multigene families that show specific patterns of expression as a function of plant development (Memelink et al. 1987), and are induced by fungal infection and wounding (Showalter et al. 1985). Other putative components of the cell wall such as glycine-rich proteins (Keller et al. 1988) and proline-rich polypeptides (Hong et al. 1987) also show precise patterns of mRNA accumulation during development.

In contrast to the situation in dicotyledonous plants, much less information is available for monocotyledonous species. A threonine-proline rich glycoprotein has been purified from maize (Kieliszewski and Lamport 1987) corresponding to a cDNA clone identified in the same species (Stiefel et al. 1988). It has been shown that a small number of genes (Stiefel et al. 1990) code for this protein, and its mRNA has been found to accumulate in dividing organs of maize and upon wounding of young plants (Ludevid et al. 1990). Its mRNA is also transiently accumulated during the early stages of leaf and root vascular differentiation (Stiefel et al. 1990). In the present article it is shown that the HRGP protein can be extracted from the different maize organs examined at levels unrelated to those of mRNA accumulation. However, in immature embryos, and specially in the scutellum, both the protein and the mRNA are present at only a very low level, indicating the existence of a different cell-wall structure in these tissues.

Material and methods

Plant material. All the plant material used in this study derives from seeds of a *Zea mays* L. cv W64A pure inbred line grown in a greenhouse in Barcelona, Spain. When necessary, seeds were germinated in the dark at 25° C. Immature kernels between 2 and 20 d

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Abbreviations: HRGP(s) = hydroxyproline-richglycoprotein(s); DAP = days after pollination

after pollination (DAP) were stored at −80° C prior to RNA extraction. Fresh material was used for immunocytochemistry.

Tissue and protein preparation. All the tissues (6-d-old plantlets, 2-month-old roots and immature embryos from 12 to 20 DAP) were dissected manually with a razor blade. The sections were frozen immediately in liquid nitrogen, and stored at −80° C. For protein extractions, the tissues were ground in a mortar containing liquid nitrogen. To extract the proteins two methods were used. In the *first method*, the resulting powder was extracted with ethanol-acid (Mazeau et al. 1982) and acetone-precipitated. For HRGP purification, the acetone powder was dissolved in sterile H_2O, precipitated with 10% (v/v) trichloroacetic acid and the supernatant chromatographed (after being dissolved in 0.03 M phosphate buffer, pH 7.05) in a Whatman P11 (Whatman Biosystems, Maidstone, Kent, UK) cellulose-phosphate column. In a continuous NaCl gradient, maize HRGP eluates at a ionic strength of 0.25 M NaCl. In the *second method* the homogenate was extracted with 3 vol (v/w) of a solution containing 10 mM Tris-HCl, pH 7.0, 0.3 M NaCl, 1 mM PMSF (phenylmethylsulfonyl fluoride) and 0.1% Triton X-100 (TT buffer). The solution was sonicated in ice for 1 min at 100 MHz, and centrifuged at 12000 · *g* for 30 min at 4° C. The supernatant, diluted 1:3 (v/v) in sample buffer, was subjected to polyacrylamide gel electrophoresis. Protein concentrations were estimated by Coomassie Blue staining, either in solution (BioRad protein assay; BioRad, Richmond, Cal., USA) or by direct staining of calibrated gels.

Electrophoresis of the proteins was as described by Laemmli (1970), the acrylamide, bis-acrylamide ratio being 15:0.4, at 15% (Fig. 1) or 10% (Fig. 5) final acrylamide concentration. The gels were transferred onto nitrocellulose filters (BA85, Schleicher and Schüell, Dassel, FRG or Hybond-C, Amersham, UK), using a semi-dry blotter (JKA Biotech, Vaerlose, Denmark) following the instructions of the manufacturers. Silver staining was performed as described by Morrisey (1981). The HRGPs were detected as described by Ludevid et al. (1990) using antiserum raised against the purified protein (working dilution 1:250–1:500) and goat anti-rabbit immunoglobulin G (IgG)-peroxidase conjugates (Dakopatts, Glostrup, Denmark).

Immnunocytochemistry. Six day-old root tips and immature embryos (14 DAP) were fixed and infiltrated with Lowicryl K4M (Agar Aids, Stansted, UK) as described by Ludevid et al. (1990). The blocks were cut with glass knives in an Ultracut (Reichert, Vienna, Austria) microtome. Sections 1 μm thick were picked up with gelatin coated slides, air dried and washed briefly in 0.01 M phosphate buffer (pH 7.4) containing NaCl at a final concentration of 150 mM (PBS). A solution of PBS containing 0.5% (w/v) egg albumin (Sigma, München, FRG) and 0.1% Tween-20 (Sigma; buffer A) was used for saturation and for incubation with specific or pre-immune sera (working dilution, 1:250). After overnight incubation at 4° C, the slides were rinsed once in PBS containing 0.1% Tween-20 and three more times in PBS. Protein A-gold complexes (20 nm; Sigma) diluted 1:20 in buffer A, were used for antibody detection. For examination by optical microscopy the gold complexes were made visible by silver enhancement using an IntenseM kit (Janssen, Olen, Belgium). The signal was allowed to develop for 5 min, and the reaction was then stopped in H_2O. The preparations were air dried and mounted for examination in a Zeiss (Oberkochen, FRG) Axioplan microscope. Photographs were taken with a Zeiss automated camera.

In-vitro transcription, translation and immunoprecipitation. The complete maize HRGP gene was cloned in pBluescript (a kind gift of M. Torrent, this laboratory) and in-vitro transcribed using T7 RNA polymerase (Stratagene, La Jolla, Cal., USA) as described by the manufacturers. The HRGP RNA (0.05 μg · μl^{-1}) or poly(A)$^+$RNA (0.01–0.05 μg · μl^{-1}) extracted from adult (2-month-old) roots or young coleoptiles was in-vitro-translated into protein using wheat germ lysates (Amersham) following the manufacturers instructions. [^{3}H]Proline (1 MBq · ml^{-1}, Amersham) was used as radioactive precursor because methionine and cysteine were absent from protein sequence. After the reaction, the translation mixture was diluted ten fold with immunoprecipitation buffer (50 mM Tris-HCl pH 8.0, 150 mM NaCl, 1.0% (v/v) Nonidet P–40, 1 mM EDTA, 0.05% (w/v) N_3Na and 1 mM PMSF) with antisera at 1:25 final dilution. After overnight incubation at 4° C, 20 μl of Protein A-sepharose (Sigma) pre-equilibrated in immunoprecipitation buffer was added. The samples were washed and analyzed in 12.5% electrophoresis gels. Fluorographs were done using En^{3}Hancer (Du Pont, Boston, Mass., USA), heat-vacuum-dried and exposed to Kodak X-ray preflashed films at −80° C.

Extraction, separation and blot hybridization of RNA. Ribonucleic acid was extracted by the guanidinium-hydrochloride procedure (Logemann et al. 1988). All the RNAs were checked by nondenaturing electrophoresis in 1.5% agarose gels and ethidium-bromide staining, and the concentrations adjusted by reading the absorbance at 260 nm. For blotting, total RNAs were separated in 1.5% agarose-formaldehyde containing gels (Lehrach et al. 1977). The gels were blotted onto nylon membranes (Hybond-N; Amersham), treated as described by the manufacturers and hybridized at 42° C for at least 12 h in 50% formamide-containing solution with specific probes labelled by random priming to a specific activity of 10^8 cpm · μg^{-1}. Hybridized filters were washed to 0.02 M NaCl stringency, and exposed to Kodak X-ray films at −80° C with intensifying screen.

Results

Presence of HRGP in different organs of maize. Using a cDNA clone it was possible to measure the level of

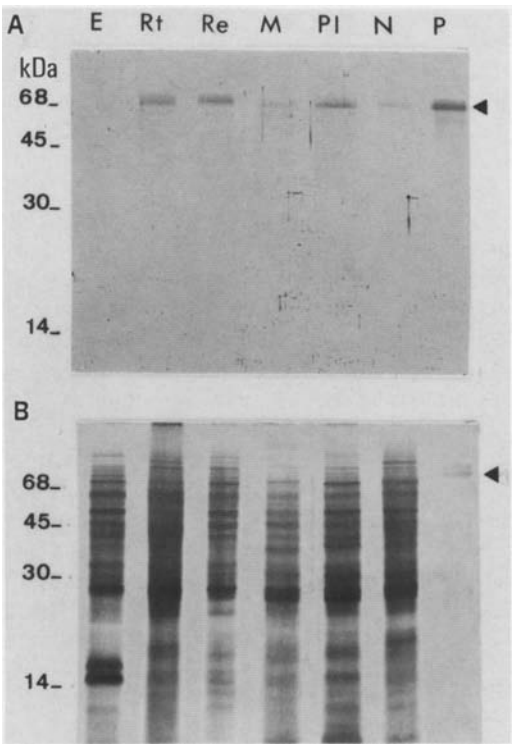

Fig. 1A, B. Presence of HRGP in different maize tissues. Western blot (**A**) and silver-stained replicas (**B**) of gel electrophoresis of total ethanol-acid soluble proteins from 18–DAP embryos (*E*), root tip (*Rt*), elongating root (*Re*), mesocotyl (*M*), plumule (*Pl*), coleoptile node (*N*), and purified maize HRGP (*P*). Ten μg of total proteins, or 0.1 μg of maize HRGP, were loaded in each lane. Detection of anti HRGP antibody was made using goat anti-rabbit IgG-peroxidase conjugates

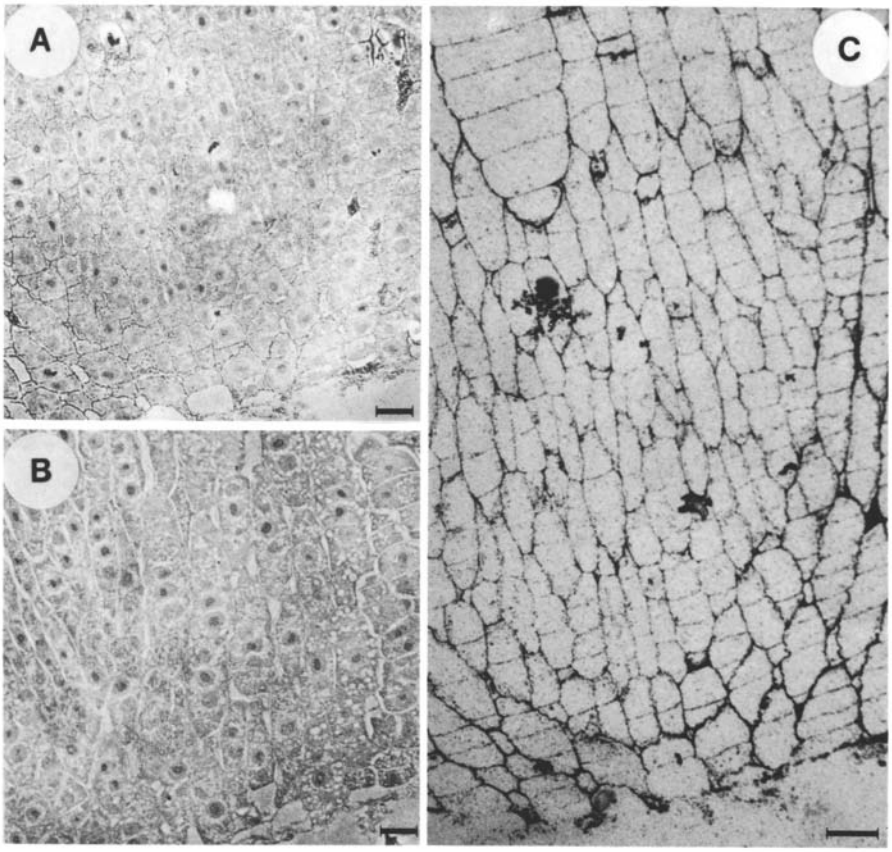

Fig. 2A–C. Immunocytochemical detection of maize HRGP in cell walls of root tip sections. Thin sections (1 μm) of root tips from 6-d-old maize seedlings, embedded in Lowicryl K4M, were incubated with maize HRGP antibodies (1:250 dilution). Silver enhanced, protein A-gold conjugates were used to detect maize HRGP antibodies. Phase contrast microscopy of anti-HRGP treated (*A*) and pre-immune-treated (*B*) sections, as well as bright field microscopy of anti-HRGP treated sections (*C*), indicate specific silver enhancement of protein A-gold particles associated with the wall skeleton of all cells of the root meristem. Bar = 25 μm

mRNA accumulation corresponding to HRGP in RNA extracted from different parts of maize (Stiefel et al. 1988). An association of the HRGP mRNA level with the presence of a high proportion of dividing cells in the tissues observed was found and, in particular, a correlation with the level of histone H4 mRNA (Ludevid et al. 1990). As the HRGP appears to be one of the main protein components of the maize cell wall, it was of interest to analyze the presence of extractable protein in different maize organs. To this end, proteins extracted with ethanol-acid were analyzed by polyacrylamide gel electrophoresis and HRGP polypeptides detected by Western blotting using antibodies raised against the purified protein (Ludevid et al. 1990). The result is shown in Fig. 1.

The pattern of total extracted proteins is shown (Fig. 1B) as well as the blot developed with peroxidase-linked goat anti-rabbit IgG (Fig. 1A). The protein was detected in the two regions of the radicle analyzed (root tip and the elongating root) and in the three segments of coleoptile (mesocotyl, plumules and coleoptile node) although at different levels. No direct correlation with the level of mRNA can be found since the organs where maximum mRNA accumulation occurs, root tip and coleoptile node, are not the places where the highest amount of protein can be detected. The lowest level of protein was observed in the immature embryo. This effect will be discussed below. In order to determine the types of cells in which the protein may be detected, immunocytological studies were carried out on sections of maize root tip (Fig. 2). Bright field microscopy (Fig. 2C) showed that the antibody against maize HRGP is located in the periphery of all the cells, in agreement with previous results using electron microscopy (Ludevid et al. 1990). In fact the wall skeleton of the root tip cells can be observed in this way. The intensity of the signal in the walls around files of cells appeared to be stronger than that in the new, transverse walls dividing the cells of the file. This effect could be the result of a higher HRGP accumulation in older cell walls. It is possible to observe cell morphology better by phase-contrast microscopy, but the silver particles are then barely detectable (see Fig. 2A). No signal at all was observed using pre-immune serum (see Fig. 2B).

In-vitro translation and immunoprecipitation. The relationship between the cDNA and the HRGP protein detected with the antibody can be stated because the protein sequence deduced from the nucleotide sequence was identical to that obtained by protein sequencing (Kieliszewski et al. 1990) and amino-acid analysis. However, this relation was further confirmed by immunoprecipitating both the in-vitro translation products of poly(A) $^+$RNA from adult maize (2-month-old) roots or 6-d-old coleoptiles and the RNA obtained by in-vitro transcription of the complete HRGP mRNA (cloned from the genomic sequence in a pBluescript vector) using T7 polymerase. Fig. 3 shows that in all cases, using antibodies raised either against the purified protein or against a synthetic peptide designed from the cDNA

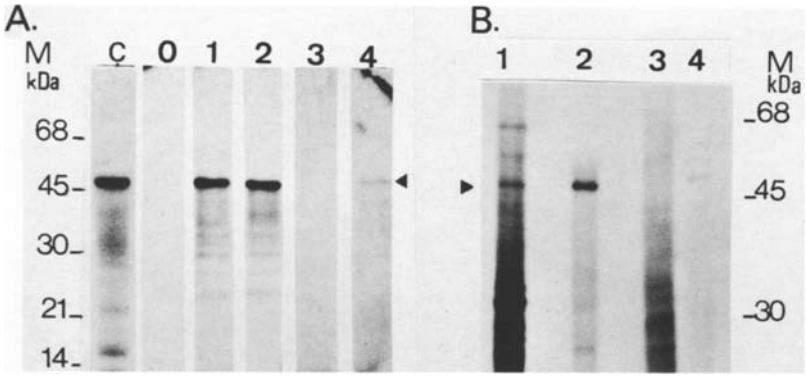

Fig. 3A, B. Immunoprecipitation of in-vitro translation products with HRGP antibodies. **A** Maize HRGP gene transcription/translation product (**C**) immunoprecipitated with antibodies raised against a synthetic peptide deduced from the cDNA sequence (*1*) and antiserum against purified maize HRGP (*2*). Immunoprecipitation of in vitro translated coleoptile poly(A)$^+$RNA (0.5 µg, *lane 4*) gives rise to a single polypeptide with the same Mr as the control HRGP-gene transcription-translation products. *Lanes 0* and *3:* controls consisting of in-vitro-translated maize HRGP gene transcript and coleoptile poly(A)$^+$RNA incubated with preimmune antiserum. **B** In-vitro translated poly(A)$^+$RNA from young coleoptiles (*1*) and adult roots (*3*), immunoprecipitated with antibodies against synthetic peptide (*lanes 2* and *4*). *M*, molecular weight markers. The *arrowheads* show the mobility of HRGP

sequence, the same polypeptide is precipitated. The apparent molecular weight (Mr) of the band is identical to the one obtained by in-vitro transcription-translation of the HRGP gene. It should be noted that the Mr (46–50 kDa) of the immunoprecipitated polypeptide does not correspond to that one observed by protein purification (50–70 kDa) or to that deduced from the complete sequence (35 kDa). This discrepancy can be attributed to the anomalous mobility of basic proline-rich protein in sodium-dodecyl-sulfate gels and to post-translational modifications of the proteins. In fact, a comparison of Fig. 1 and 5, for example, shows that when the electrophoresis conditions are changed, the Mr of the protein varies.

Accumulation of HRGP mRNA in embryonic tissues of maize. The results of Western blots of proteins extracted from different tissues of maize indicated a low abundance of the HRGP protein in immature embryos, 20 DAP (Fig. 1, lane E). Therefore the accumulation of the corresponding mRNA was measured in embryos at different stages of maturation, on the assumption that the expression of the HRGP gene would decrease with maturation in agreement with its association with cell division (Fig. 4). The RNA was extracted either from whole maize kernels from 2 to 12 DAP or from hand-dissected embryos from 12 to 30 DAP. The Northern blot was hybridized with probes corresponding to HRGP, histone H4 (Phillips et al. 1986), α-tubulin (Montoliu et al. 1989) and glutelin-2 (Prat et al. 1985), a specific endosperm protein. Figure 4 shows that the level of HRGP mRNA is low in the embryo throughout maturation; this contrasts with the intensity of the levels of histone H4 and α-tubulin mRNAs that follow the expected pattern of accumulation. In somatic tissues a correlation between the accumulation of the HRGP mRNA and that of the histone genes has been observed (Ludevid et al. 1990) and a difference in mRNA accumulation between these two genes only appeared upon wounding.

Presence of HRGP in specific embryonic organs. To study further the accumulation of HRGP in maize embryos it was of interest to assay the protein in specific parts of the embryo. To this end immature embryos, 20 DAP were hand-dissected into two parts, embryonic axis and scutellum. Protein extracts from the two organs were probed with the antibody against HRGP. The result is shown in Fig. 5, where total protein extracts from 20 DAP embryos are compared with two dilutions of proteins ex-

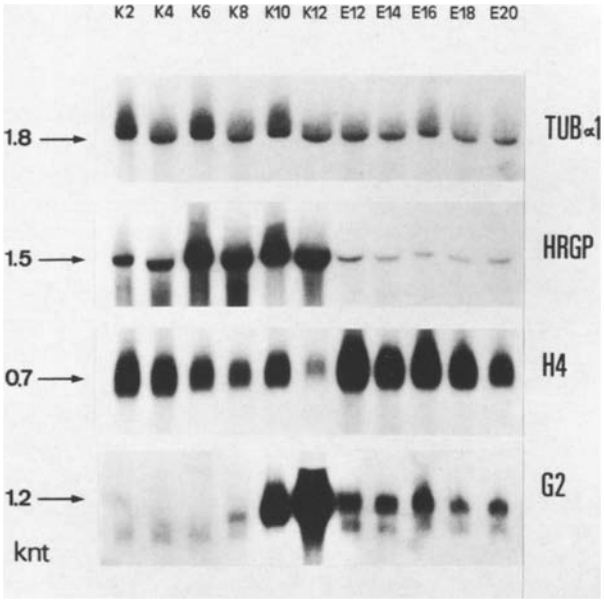

Fig. 4. Accumulation of maize HRGP mRNA during the early stages of seed and embryo development. Northern blotting of total RNAs from maize kernels between 2 and 12 DAP *(lanes K2 to K12)* and embryos between 12 and 20 DAP *(lanes E12 to E20)*. The same filter was hybridized with specific probes for maize α-tubulin gene *(tubα1)*, maize histone H4 (*H4*), maize storage protein glutelin-2 (*G2*) and maize *HRGP*. Each lane was loaded with 50 µg of total RNA

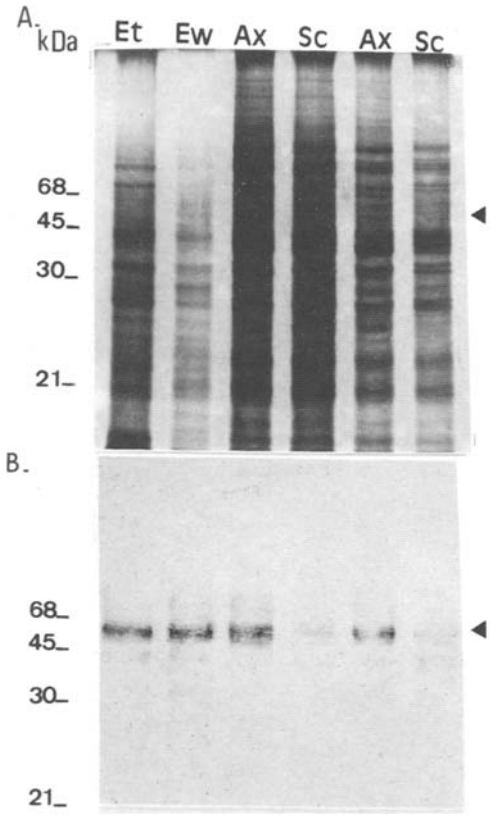

Fig. 5A, B. Detection of maize HRGP in protein extracts of scutellum and embryo axis. Protein extracts from 20 DAP whole embryos (*Et* and *Ew*), scutellum (*Sc*) or axis (*Ax*), were subjected to gel electrophoresis and silver stained (**A**) or blotted onto nitrocellulose filters and hybridized with anti-HRGP antibodies (**B**). The proteins loaded in *Ew* are total ethanol-acid soluble proteins. All the other extracts were made with TT-buffer. Duplicated *Sc* and *Ax* lanes were loaded with 25 or 50 μg of protein. *Arrowheads*, maize HRGP

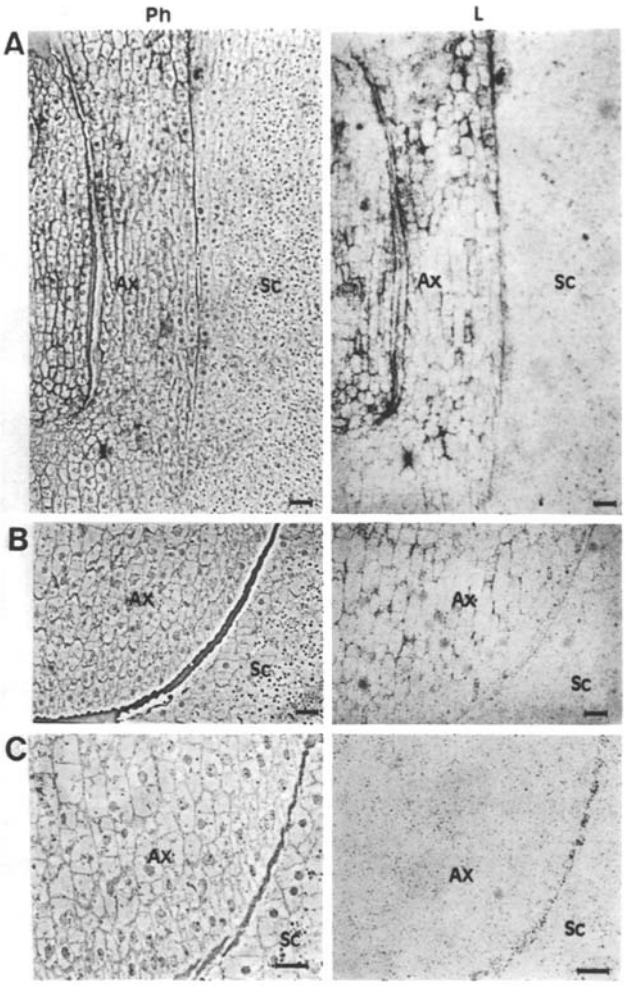

Fig. 6A–C. Detection of maize HRGP in scutellum (*Sc*) and embryo axis (*Ax*). Lowicryl embedded thin sections (1 μm) of 14–DAP embryos were incubated with anti-HRGP antibodies (**A, B**) or preimmune serum (**C**). Silver enhanced, protein A-gold complexes were used for antibody detection. The cell wall skeleton of the axis (*Ax*) bright field image, but not of the scutellum (*Sc*), can be seen. *Ph*, phase contrast. *L*, bright field. Bars = 25 μm

tracted from embryo axis and scutellum. The same protein is detected in all cases. The result indicates that the concentration of polypeptides reacting with these antibodies is substantially lower in the scutellum than in the axis.

The results described in the previous paragraph were confirmed by immunocytological techniques. Sections of immature embryos (14 DAP) were incubated with anti-HRGP antibodies and developed with silver-enhanced protein A-gold conjugate. The result is shown in Fig. 6 where two parts of the embryo, the developing coleoptile (Fig. 6A) and the coleorhiza (Fig. 6B), and the proximal part of scutellum are presented. The phase contrast image (Ph) is compared with the immunocytological image under bright field (L) and with the result using a preimmune serum (Fig. 6C). It can be seen that in the embryo axis the antibody reacts with the cell wall in a manner similar to that observed previously (Fig. 2) in root tip. However, no reaction was observed in the scutellum in accordance with the result from the Western blot.

In order to check whether the lack of detection of the HRGP protein in the scutellum was due to a lower accumulation of mRNA, a Northern blot of RNA extracted from immature embryo, axis and scutellum was probed with the HRGP cDNA (Fig. 7). Two amounts of RNA were compared with coleoptile RNA. The blot clearly shows that HRGP mRNA is substantially (at least 10 times) more abundant in the embryo axis than in the scutellum, thus confirming an uneven distribution of the HRGP protein in different regions of the immature embryo and showing that this differences arise from the corresponding mRNA levels. Similar levels of histone H4 mRNA were detected between embryo axis and scutellum (result not shown) indicating that the observed difference in HRGP mRNA in these two organs is not a consequence of a difference in their mitotic activities. The accumulation of HRGP mRNA in the embryo axis is lower (approx. 4 times less) than that in the coleoptile. The differences observed between coleoptile and total embryo can be then explained as a result of the differential expression of the HRGP gene in these tissues, and specially to a strong diluting effect of total embryo

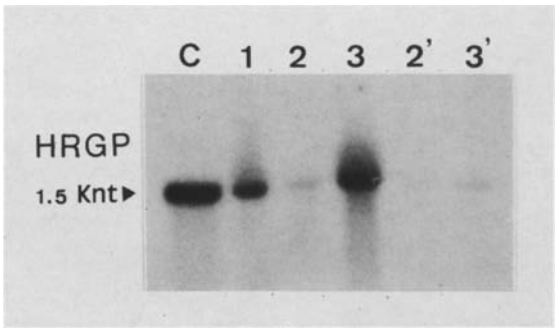

Fig. 7. Accumulation of maize HRGP mRNA in the scutellum and embryo axis. Northern blotting of total RNA extracted from 20–DAP embryos (*lane 1*, 10 μg), 20 DAP embryo scutellum (*lane 2*, 10 μg; *lane 2'*, 1 μg) and 20 DAP embryo axis (*lane 3*, 10 μg; *lane 3'*, 1 μg), hybridized with a maize HRGP specific probe. Total RNA from coleoptiles (*C*, 5 μg) was loaded as a positive control

mRNA as a result of low expression of the HRGP gene in the scutellum.

Discussion

The HRGP studied here is one of the main cell wall proteins extractable from maize following protocols adapted from dicotyledonous systems. The same protein has been isolated from pericarp (Hood et al. 1988), cell suspension cultures (Kieliszewski and Lamport 1987) and coleoptiles (Stiefel et al. 1990). This protein corresponds to a cDNA first described by Stiefel et al. (1988). The complete protein and gene sequence have recently been published (Stiefel et al. 1990).

Antibodies raised against a synthetic peptide deduced from the cDNA sequence, and against the purified maize HRGP protein, immunoprecipitate the in-vitro transcription-translation product of the HRGP gene, as well as a predominant proline-rich peptide, from in-vitro translated mRNAs from actively dividing (young coleoptiles) and 2-month-old (adult root) tissues. This protein migrates with an electrophoretic mobility corresponding to the same Mr as the control gene product. This fact gives further support to the idea that both immunological and DNA probes are detecting the protein product and the RNA of the same gene. This is so in spite of the apparent discrepancy between the expected molecular weight for the HRGP immature protein (35 kDa), the observed molecular weight of the in-vitro translated proteins (46–50 kDa) and the polypeptides detected by immunoblotting of extractable cell wall and total proteins for many tissues (ranging from 50 to 70 kDa, depending on the percentage of acrylamide in the electrophoresis gel). These differences can be attributed to the physicochemical properties of basic and proline-rich proteins (Noelken et al. 1981), as well as to posttranslational modifications (hydroxyprolination and glycosylation), that could account for a 10-kDa difference between the mature and immature protein (Kieliszewski et al. 1990).

By using the antibodies raised against the extractable maize HRGP we have demonstrated the presence of the protein in extracts from different tissues of the plant (Fig. 1). The pattern of protein distribution contrasts with the mRNA steady state pattern. The highest RNA level is associated with cell division and with procambial cells during early leaf and root development (Ludevid et al. 1990; Stiefel et al. 1990). The fact that the antibodies recognize the whole wall skeleton in the root tip, as revealed by immunocytochemistry, is in agreement with the idea of a distinct turnover of HRGP mRNA production (associated with cell division), and HRGP deposition in cell walls. The effect can be seen in Fig. 2. The difference in staining of different walls may be interpreted as the result of the increase in thickness in older cells that allows blocks of cells with a common origin to be distinguished. In this interpretation, newly formed walls, associated with plate formation and cell division planes, are poorly marked with what seems to be a single line of deposition of gold particles. We have previously shown that the mRNA accumulation is low during cell elongation or differentiation (Ludevid et al. 1990) yet the immunocytochemistry results suggest that the highest HRGP levels are present in cell walls at late stages of wall formation. Therefore, it is possible that HRGP mRNA accumulation and HRGP protein deposition in cell walls are not synchronized. It is interesting to note that there is no relationship between tissues rich in HRGP mRNA (coleoptile node, root tip) and the amount of extractable protein that can be detected in Western blots (see Fig. 1).

Surprisingly, maize embryos have the lowest amount of HRGP protein. This is valid also for mRNA accumulation. Although cell division activity in the immature embryos is high (see the patterns of H4 histone and α-tubulin mRNA accumulation in Fig. 4), there was a very low and constant level of HRGP mRNA between 12 and 20 DAP. Different expression patterns for proline-rich protein genes have been already described for other embryonic systems (Hong et al. 1989), indicating a certain uniqueness of cell wall composition during seed development and embryogenesis. However, two main parts can be distinguished in the embryo: the axis, committed to the formation of the plant after germination, and the scutellum, a fully differentiated storage organ in maize (Murray 1984). By dissecting the embryo into these two parts, we have shown that expression of the HRGP gene is at least 10 times higher in the axis than in the scutellum. This cannot be attributed to a difference in mitotic activity, since H4 histone mRNA levels are the same in the maturation period studied (20 DAP). We confirmed this result by immunoblotting of total axis and scutellar proteins, and finally by immunocytochemistry of younger (14 DAP) embryos, where the protein is located in the walls of the axis in a similar fashion to root tips, but no signal at all is detected in scutellar cell walls, in agreement with the results obtained after immunoblotting. These results indicate that the HRGP gene is under a tissue-specific control in embryonic tissues. Although no data are presently available on the composition of the scutellum cell wall, our results indicate that it is unique compared with the walls of other maize organs.

Cell wall structure is an obvious constrain for many processes in the plant (Varner and Lin 1989). Differences

in composition and organization may have a functional significance. In maize the expression of the HRGP gene is found to be under the control of a number of distinct factors. Those include divisional activity and stress conditions such as wounding (Ludevid et al. 1990). The transient accumulation of its mRNA in developing vascular tissue has been observed (Stiefel et al. 1990). In the present article we propose the existence of a distinct, tissue-specific control of the HRGP-gene expression in the maize embryo. All these factors make the maize HRGP an attractive system for studying the linkage between cell wall structure, tissue function and gene expression.

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