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Rapid changes induced in developmental programmes of the maize embryo detected by analysis of the expression of genes encoding proline-rich proteins

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Abstract The pattern of expression of two genes coding for proline-rich proteins, *zmHyPRP* and *zmHRGP*, in *Zea mays* is modified when the embryogenesis programme is altered by placing the embryos in conditions which promote either precocious germination or callogenesis. *zmHyPRP* gene expression is rapidly arrested when the embryogenesis programme is altered. *zmHRGP* mRNA is highly induced in scutellum within a few hours of callogenesis or precocious germination.

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Key words: Maize; Embryogenesis; Proline-rich protein

1. Introduction

The expression of two maize genes coding for proline-rich proteins has been studied in immature maize embryos. *zmHyPRP* belongs to the group of proline-rich proteins named hybrid proline-rich proteins [1]. The *zmHyPRP* gene is expressed mainly during the morphogenetic stages of maize embryogenesis, in parenchyma cells surrounding the developing vascular tissue of the axis, and is a marker of scutellum differentiation [2,3]. *zmHyPRP* expression is arrested when the maturation stage is reached, after ABA induction [4]. *zmHRGP* codes for a cell wall protein accumulated in tissues rich in dividing cells [5]. During embryo development *zmHRGP* expression is restricted to the morphogenetic stage and is absent from the scutellum [6,7] being a marker of suspensor and vascular tissue development [2,3]. In the present paper *zmHyPRP* and *zmHRGP* have been used as probes to study the changes occurring when development is altered by placing the immature embryos in different media [8,9].

2. Materials and methods

2.1. Biological materials

Unless otherwise stated, plant material was derived from seeds of *Zea mays* cv W64A pure inbred line. Immature embryos of 18 DAP (days after pollination) were stored at −80°C immediately or after being subjected to germination conditions (water) or callogenesis induction (MS2). Where indicated, the embryo axis and scutellum were manually dissected using a razor blade.

2.2. RNA preparation and gel blot analysis

RNA was extracted, blotted and hybridized as previously reported [2]. Fragments from *zmHyPRP* [2] and *zmHRGP* [10] coding regions and *H4* [11] coding and 3′ non-translated regions were used as probes.

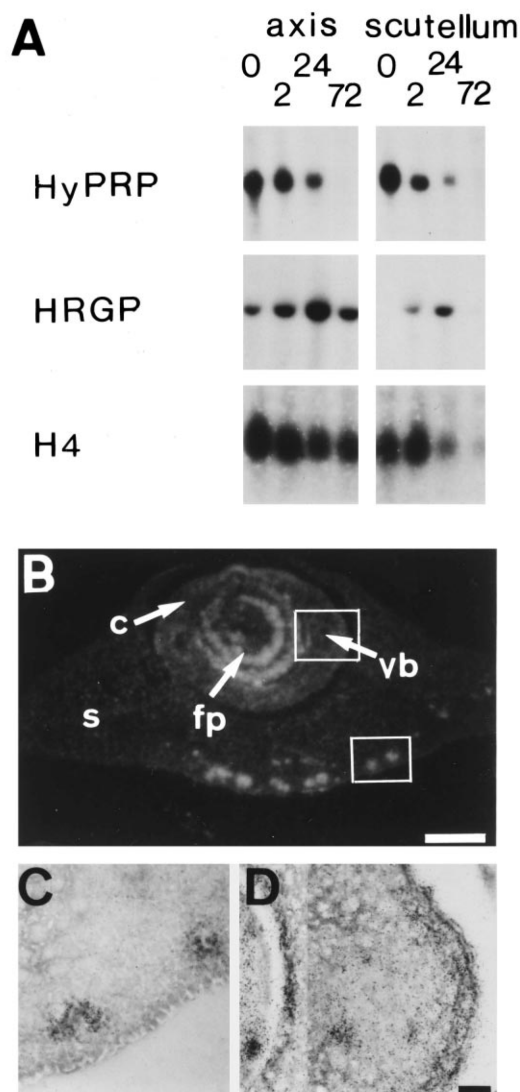

Fig. 1. Precocious germination of maize immature embryos in water. A: RNA gel blot of 18 DAP precociously germinated maize embryos. Total RNAs (10 μg) were hybridized with *zmHyPRP*, *zmHRGP* and *H4* probes. Numbers indicate hours of treatment. Filters were exposed for 2 days with the *zmHRGP* probe or for 3 days with the *zmHyPRP* and *H4* probes. B–D: Localization of *zmHRGP* mRNA by in situ hybridization in a transversal coleoptile section from 18 DAP precociously germinated maize embryo. The upper section is analyzed under dark field microscopy (B). C, D: Details of the same section examined under bright field microscopy. C: Scutellar abaxial side. D: Vascular bundles of the coleoptile. Bar = 250 μm for B and 25 μm for C, D. c, coleoptile; fp, foliar primordia; s, scutellum; vb, vascular bundle.

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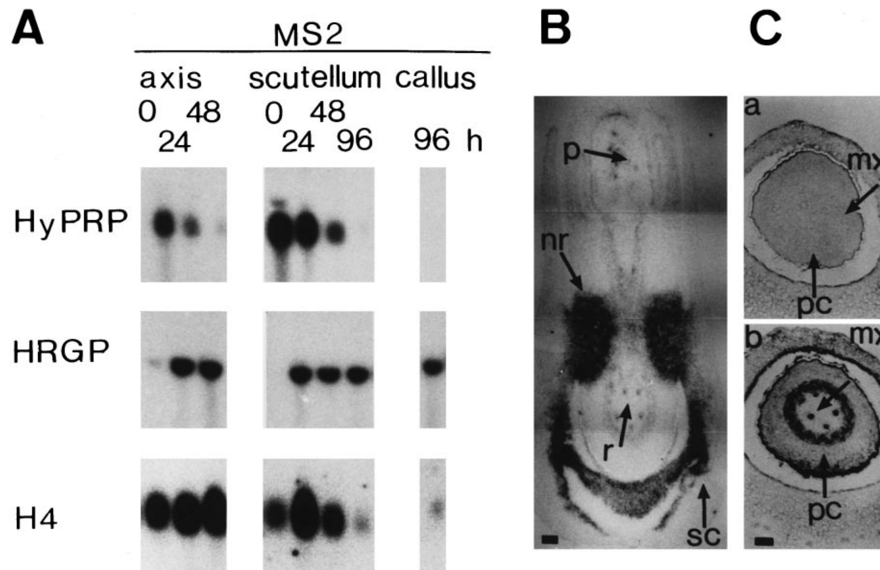

Fig. 2. Induction of callogenesis in 18 DAP maize embryos. A: RNA blot of RNAs (10 μg) hybridized with *zmHyPRP*, *zmHRGP* and *H4* probes. Numbers indicate hours of treatment. Filters were exposed for 1 day with the *zmHRGP* probe and for 4 days for *zmHyPRP* and *H4*. B: Localization of *zmHRGP* mRNA by in situ hybridization to a longitudinal section of a 18 DAP embryo after 24 h in MS2 solid medium hybridized with the antisense probe. Bar = 100 μm. nr, nodular scutellum; p, foliar primordium; r, root; sc, scutellum. C: Localization of *zmHRGP* mRNA by in situ hybridization to a transversal section of a 18 DAP embryo at the root level hybridized with the sense (a) or the antisense HRGP probe (b). Bar = 100 μm. mx, metaxylem precursor cells; pc, procambium.

2.3. In situ hybridization

The in situ hybridization was performed essentially as previously reported [2]. A 670-bp *Dde*I and a 660-bp *Fock*I-*Sna*bI fragment of the 5′ coding region of *zmHyPRP* and *zmHRGP* genes, respectively, were used as templates for the synthesis of sense and antisense riboprobes.

3. Results

3.1. Developmental expression of zmHyPRP and zmHRGP genes in precociously germinating Zea mays embryos

At the end of embryo morphogenesis, both *zmHyPRP* and *zmHRGP* gene expression is arrested by the initiation of the maturation and desiccation programmes by ABA induction [3,4]. In vitro it is possible to bypass these programmes by promoting the germination of freshly excised 18 DAP embryos by placing them in a hydrating medium. We analyzed how *zmHyPRP* and *zmHRGP* mRNA levels were modified during such precocious germination. *zmHyPRP* expression decreased in the embryo axis and scutellum after the induction of germination, and it was not detectable at time points more than 24 h after hydration (Fig. 1A). *zmHRGP* expression was not arrested in the embryo axis after hydration but, curiously, *zmHRGP* mRNA was transiently observed in the scutellum, where it is absent during unperturbed embryogenesis, and disappeared after 1 day of treatment (Fig. 1A). As a control, the histone *H4* gene was studied in parallel, as a marker of cell division [11]. Cell proliferation was arrested in the scutellum after 24 h of treatment while it was continuously activated in the axis (Fig. 1A).

The cells transiently responsible for *zmHRGP* expression in the scutellum were identified by in situ mRNA hybridization. Fig. 1B shows a transversal embryo section at the level of the coleoptile after 24 h of hydration. Scutellum morphological analysis showed that some scutellar cells become modified as a result of hydration. The scutellar expression of *zmHRGP* was observed as cell patches located below the epidermis of the embryo abaxial side (Fig. 1B and C for a detailed analysis). In the foliar primordial axis *zmHRGP* cellular expression was observed in vascular bundles, as in immature embryos, but induced expression was also observed in groups of cells. Expression in the coleoptile bundles was restricted to a few patches of cells and induced in parenchyma cells surrounding the coleoptile bundles (Fig. 1B and D for a detailed analysis).

3.2. zmHyPRP and zmHRGP mRNA expression after induction of callogenesis

Embryogenesis can also be precociously arrested after the induction of de-differentiation by promoting non-specific proliferation. The embryo axis begins to germinate but scutellar cells de-differentiate inducing callus formation. Under these conditions *zmHyPRP* mRNA expression is arrested in the embryo as well as in the scutellum (Fig. 2A). *zmHRGP* expression increases in the embryo axis and is induced in the scutellum where no mRNA can be detected during normal embryogenesis [6]. The mRNA levels of histone *H4* increased rapidly in both the axis and the scutellum, but after 48 h they decreased in the scutellum. In situ hybridization studies allowed the identification of the cells responsible for the induction of *zmHRGP* expression which are localized in the scutellar node and in the regions surrounding the root (Fig. 2B and C).

4. Discussion

We have studied how the expression of *zmHyPRP* and *zmHRGP* in maize is modified when the embryogenic programme is altered by induction of precocious germination or callogenesis. The results indicate that *zmHyPRP* is very responsive to the induction of germination or callogenesis as its mRNA rapidly diminished and was not detected after 24 h of placing the embryo in the appropriate conditions. This behavior differs from the *zmHRGP* expression pattern. After germi-

nation and callogenesis induction, *zmHRGP* mRNA is induced in the scutellum, with a different cell expression pattern in each case. After germination the pattern of expression observed in the axis, as well as in the scutellum, is very similar to that described for *H4*, a marker of cell division actively expressed during embryo morphogenesis [6,7]. As *zmHRGP* and *H4* have a similar time expression in hydrating immature maize embryos, we conclude that *zmHRGP* morphological expression pattern is associated with the new cell division centers activated by germination. In contrast, after callogenesis induction, *zmHRGP* is expressed in those cells first differentiating to callus in the embryo nodular region. In this case the correlation of *zmHRGP* and *H4* expression is lost in the scutellum, as has been described after wounding [5]. Our results indicate that the factors controlling the expression of genes coding for proline-rich proteins in the embryo are affected within 24 h of culturing in conditions that alter the developmental programme of the embryo. These results are of importance when interpreting results of gene expression or promoter activity of embryogenic plant cells in culture and in understanding the factors controlling the early stages of embryo development.

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