A maize insulin-like growth factor signals to a transduction pathway that regulates protein synthesis in maize

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Insulin and insulin-like growth factors (IGFs) are well-characterized regulators in higher eukaryotic cells that control biological processes such as cell growth and survival, and selective translation of mRNAs. This research presents the purification of a 20 kDa protein, isolated from maize tissue, with IGF activity. The protein was purified from 48 h-germinated maize embryonic axes by G-50 Sephadex fractionation followed by affinity chromatography through a bovine insulin antibody–Sephadex column. This protein proved to significantly speed up maize germination and seedling growth. At the molecular level, *Zea mays* IGF (ZmIGF) enhanced phosphorylation of S6 ribosomal protein (rp) on the 40 S ribosomal subunit, in a similar way as observed when bovine insulin is applied to maize axes during

germination. Rapamycin, a specific inhibitor of the insulin-stimulated signal transduction pathway, prevented S6 rp phosphorylation in maize axes. Moreover, ZmIGF stimulated [³⁵S]methionine incorporation into rps, above the level of overall cytoplasmic proteins. Either incubation with anti-insulin antibody, heat treatment (60 °C) or trypsin digestion abolished this ZmIGF effect. It is proposed that ZmIGF is an endogenous maize growth factor that regulates the synthesis of specific proteins through a pathway similar to that of insulin or IGFs in animal tissues.

Key words: maize growth factor of peptide nature, protein synthesis regulation, signal transduction pathway.

INTRODUCTION

The relevance of gene expression regulation at translational level in eukaryotes has been widely recognized and, at present, a large variety of translational control mechanisms have been described [1–4].

A well documented mechanism of translational control in various higher eukaryotes is the insulin/growth factor-induced protein synthesis regulation [5–7]. Indeed, insulin and insulin-like growth factors (IGFs), widely distributed among eukaryotic organisms [8–10], stimulate a signal transduction pathway that targets the protein synthesis apparatus by phosphorylating the S6 ribosomal protein (rp) on the 40 S ribosomal subunit [11,12]. Further, the increase in phosphorylated ribosomes tightly correlates with the selective translation of mRNAs containing a specific tract of oligopyrimidines (5'-TOP) cluster at their 5'-untranslated region, mostly transcripts coding for rps and translation factors [12–14].

During seed germination, quiescent maize embryonic axes gradually reinitiate protein synthesis, primarily based on translation of stored mRNAs [15,16]. Within this period translation has shown to be tightly regulated [17]; however, scarce information is available about the mechanisms that control this process.

In plants, several types of evidence have suggested the presence of insulin-like peptides, mainly based on immuno-recognition with anti-bovine or human insulin antibodies [18,19]. And more recently, a protein fraction was isolated from *Canavalia ensiformis* (jack bean) seed coat that contains a polypeptide showing amino

acid sequence identity with the β -chain of bovine insulin [20]. Some of these purified insulin-like peptides have shown stimulation of glucose uptake in target animal tissues [18,20]. However, no endogenous regulatory or physiological role has been investigated for these peptides during plant development.

Previous work in our lab has demonstrated that insulin is capable of inducing activation of a signal transduction pathway in maize cells [21], similar to the one reported to be stimulated by this peptide in animals [7,22], which selectively regulates translation. Indeed, germinating maize embryonic axes fed with [³⁵S]methionine showed fast growth and a selective increase of rp and initiation translation factor, iso4E, synthesis after insulin stimulation [21,23]. These effects are blocked either by rapamycin or wortmannin, specific inhibitors of the insulin-signal-transduction pathway [5]. Based on this previous work, the present report shows the purification of an insulin-like protein, *Zea mays* IGF (ZmIGF), from germinating maize axes and tests its biological action at physiological and biochemical levels in maize tissues. Our analysis showed that the signal transduction pathway revealed in maize axes by this plant protein is similar to that triggered by bovine insulin in non-plant eukaryotic cells.

EXPERIMENTAL

Biological system

Seeds and embryonic axes from *Z. mays* L. cv Chalqueño were used in all experiments. Axes were manually dissected from seeds

Abbreviations used: Ab-I, antibody against insulin; $\Delta 60$ °C, insulin heated for 20 min at 60 °C; DTT, dithiothreitol; $Gt_{1/2}$, half-time of germination; IGF, insulin-like growth factor; MS, Murashige and Skoog; rp, ribosomal protein; TOP, tract of oligopyrimidines; ZmIGF, *Zea mays* IGF; Ab-I, mixture of ZmIGF with antibody against insulin.

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and either frozen immediately in liquid nitrogen until used or incubated in Murashige and Skoog (MS) medium [24] for the stated period, as described previously [25].

Germination curves and seedling development

Maize seeds, in sets of 100 seeds each, were imbibed between two rolled filter papers, either in water (control), water plus insulin (a final concentration 200 μ unit/ml bovine pancreas insulin; Sigma, St. Louis, MO, U.S.A.), or water plus ZmIGF (a final concentration of 200 μ unit/ml ZmIGF) during 72 h at $24 \pm 2^\circ\text{C}$ in darkness. At 18 h of imbibition, seeds were evaluated every 2 h for radicle protrusion in all sets. Germination curves were plotted as the average values of five different sets. Results were statistically analysed to calculate $Gt_{1/2}$ values (half-time of germination, i.e. when 50% of seeds have germinated) and total germination percentages, as well as standard deviations for each group. In addition, seedling size (root and coleoptyle) was measured for each group at 72 h of development.

Isolation and purification of ZmIGF protein

Maize seeds were germinated in water-imbibed cotton at $24 \pm 2^\circ\text{C}$ in darkness. After 48 h embryonic axes were dissected. The axes (10 g) were frozen in liquid N_2 , ground in a mortar and then homogenized in grinding extraction buffer [100 mM KCl/20 mM Hepes (pH 7.6)/7 mM 2-mercaptoethanol/0.2 mM EDTA/10% glycerol/0.1% Triton X-100/10 mM benzamidine/2 mM PMSF] in a 1:5 (w/v) ratio. The homogenate was centrifuged at 27000 g for 30 min at 4°C and then the supernatant at 280000 g for 3.5 h. The supernatant was concentrated on poly(ethylene glycol) 6000. The concentrated supernatant was fractionated through a Sephadex G-50 column 1.5 cm $\times$ 24 cm (approx. 15 ml of the column volume), and eluted in 1:10 ratio of elutant to grinding buffer. Absorbance was recorded at 280 nm and analysed for positive reaction with bovine insulin antibody by dot blot (see below). The selected fractions were resolved through affinity chromatography using a column packed with sepharose covalently linked to bovine insulin antibody, prepared according to the manufacturer's instructions (Immunopure IgG; Pierce). The column was washed once with binding buffer (10 mM Tris, pH 7.5) and eluted with buffer (0.1 M glycine, pH 2.8). The A_{280} of the fractions (1 ml) was recorded and analysed by dot blot as indicated above. Fractions containing ZmIGF were treated again with Protein A-sepharose to eliminate remaining insulin antibody. The purified fractions were analysed by Western blotting.

ZmIGF immunodetection

Dot blotting

Each column fraction (50 μ l) was blotted onto PVDF membrane (Immobilon-P; Millipore) using a Dot Micro Filtration Apparatus (Filtration Manifold System, Rockville, MD, U.S.A.).

Western blotting

The samples containing ZmIGF were resolved by SDS/PAGE (using 15% gel), and blotted onto PVDF membrane with a LKB semi-dry blotter (Amersham Pharmacia Biotech, Piscataway, NJ, U.S.A.).

In both cases, guinea-pig polyclonal antibody raised against bovine insulin (Sigma) was used as the primary antibody diluted 1:2000 in 5% non-fat dry milk for ZmIGF immunodetection. After several washes, IgG guinea-pig antibody coupled to per-

oxidase (Sigma), diluted 1:2000, was used for signal detection (ECL[®] system; Amersham Pharmacia Biotech).

Insulin standard curve and ZmIGF units definition

For routine estimation of the relative concentration of ZmIGF, an insulin standard curve was set (50–500 μ unit/ml) and revealed by the dot blotting of 10 μ l aliquots. The ZmIGF concentration was calculated from the insulin standard curve.

In vivo labelling of rps

Maize embryonic axes from seeds imbibed in water during 22 h at 25°C in darkness, were dissected and incubated in nutrient MS medium for 2 h more with either 300 μ Ci [^{35}S]methionine or 330 μ Ci [^{32}P]orthophosphate (Amersham Pharmacia Biotech), in presence or absence of 200 μ unit/ml of either ZmIGF or insulin. In some experiments, rapamycin (0.1 μ M) (Sigma–Aldrich) was applied 30 min before ZmIGF or insulin addition.

The axes were homogenized in liquid N_2 and the frozen powder mixed with extraction buffer [20 mM Tris/HCl (pH 7.8)/5 mM MgCl_2 /20 mM KCl/1 mM PMSF/5 mM NaF/0.5% 2-mercaptoethanol/1% Triton X-100/0.25 M sucrose]. The slurry was clarified at 27000 g at 4°C for 30 min, and to purify the ribosomes from non-structural rps, the supernatant was layered on a sucrose-buffered cushion (0.5 M sucrose and 0.8 M KCl in extraction buffer) and centrifuged at 280000 g for 3.5 h. The ribosome pellet was resuspended in resuspension buffer (20 mM Hepes/20 mM KOH/5 mM magnesium acetate/125 mM potassium acetate/5% 2-mercaptoethanol) and the rps were extracted with acetic acid, precipitated with acetone as described previously [26] and resuspended in resuspension buffer. The cytoplasmic proteins were precipitated with 10% trichloroacetic acid, resuspended in water and neutralized with KOH. [^{35}S]Methionine uptake was measured in this fraction using a liquid scintillation counter (Packard, Downers Grove, IL, U.S.A.).

The labelled [^{32}P]orthophosphate rps were analysed by SDS/PAGE [12% (w/v) gel] [27]. The gels were stained with 0.2% Coomassie Brilliant Blue R-250, destained in 10% ethanol and 15% glycerol during 30 min, and dried or used for Western-blot analysis. Autoradiographs of the dried gels were obtained at -70°C using X-Omat film (Eastman Kodak, Rochester, NY, U.S.A.).

S6 rp immunodetection

For the S6 rp immunodetection, the rps from the gels were electro-blotted on PVDF membrane. Membranes were blocked in TBS [50 mM Tris (pH 7.4)/150 mM NaCl/0.1% (w/v) Tween 20] with 5% non-fat dry milk, incubated with S6 rp rabbit antibody raised against a 17 amino acid peptide from the most conserved region of the S6 rp (Genemed Synthesis, San Francisco, CA, U.S.A.), diluted 1:2000, washed, and developed with 1:2000 diluted rabbit antibody IgG conjugated to horseradish peroxidase (Bio-Rad). Immunoreactive proteins were revealed by ECL[®].

Protein measurements were performed according to Bradford [28] using BSA (Sigma) as a standard.

RESULTS

In order to select the best tissue for ZmIGF purification, different maize tissue extracts were screened by dot blot analysis with insulin antibody. Results indicated the presence of an insulin-like peptide mainly in fast-growing tissues such as emerging seedlings

Figure 1 Immunological detection of ZmIGF in different maize tissues

(A) Dot blot of extracts from non-germinated maize axes (0), 24-h germinated maize axes (24), callus tissue (C), young leaf (L), bovine insulin (stock 1 µg/ml) (I) and bovine serum albumin (A) (stock 1 µg/ml). (B) Dot blot of axes and scutellum extracts from germinated maize seed (0, 24 and 48 h). In both cases, 50 µg of proteins from the extracts were blotted onto a PDVF membrane using a Dot Micro Filtration Apparatus. Insulin antibody (1:2000 dilution) was used as primary antibody and the reaction was detected by ECL[®] system using guinea-pig IgG conjugated with peroxidase.

or calli (Figure 1A). Extracts of 48-h germinating axes were chosen as source of ZmIGF protein (Figure 1B). Maize axes extract containing 8 mg of total protein from 48 h germinating seeds were filtered through a G-50 Sephadex column. The eluted fractions (1 ml) were monitored at A_{280} (Figure 2A) and 10-fold dilutions of these fractions were tested by dot blot with insulin antibody. A peak of positive cross reaction was found in fractions 17–23 (Figure 2B). The highest positive fraction (number 20, indicated by the arrow) was then passed through an affinity chromatography column packed with anti-insulin-sepharose (Figure 3A). Extensive washing of the column was performed before eluting the retained proteins. The eluted fractions were monitored at 280 nm and tested by dot blot analysis. Positive reaction against insulin antibody was recorded from the first eluted fractions (ZmIGF) (Figure 3B). As control, bovine insulin was subjected to the same procedure through the affinity chromatography column and analysed by dot blot (Figures 3C and 3D).

Gel electrophoresis and Western-blot analysis of the eluted fraction was performed (Figure 4). The crude axes extract showed two bands of 20 kDa and 7 kDa by Coomassie-Blue stained gel (Figure 4A) and Western blot (Figure 4B). However, the eluted fraction from the affinity column showed only the band of 20 kDa (ZmIGF) (Figure 4B). All the following experiments were performed with the purified 20 kDa peptide.

To test the physiological activity of ZmIGF, sets of 100 seeds each were germinated in water or in water plus 200 µunit/ml of either insulin or ZmIGF, and the % of germinated seeds was recorded after 18 h at 2 h intervals. At the end of a total 72 h incubation period the size of roots and coleoptyles was measured (Table 1). A representative seedling from each set is shown in Figure 5. Two negative controls were set by germinating the seeds with either ZmIGF previously mixed with insulin antibody (Ab-I) or insulin heated for 20 min at 60 °C ($\Delta 60$ °C) (Table 1).

Figure 2 Sephadex G-50 column chromatography

Crude protein extract from 48-h germinated maize axes were fractionated by applying 3 ml of extract (8 mg of protein) to a 1.5 cm × 24 cm column (approximately 15 ml bed volume). Elution was performed with extraction buffer (1:10 dilution). Fractions (1.5 ml) were collected and assayed by dot-blot with bovine insulin antibody. (A) 280 nm elution profile. (B) Dot blot of the fractions (1:10 dilution). Blots were incubated with the same polyclonal antibody as in Figure 1.

$Gt_{1/2}$ was determined using the average values from each group. Significant acceleration of seed germination in insulin and ZmIGF sets was observed, as compared with the control (Table 1). The Ab-I and $\Delta 60$ °C groups gave similar $Gt_{1/2}$ values as the control. Roots and coleoptyles from the corresponding seedlings after 72 h of growth were measured. Significantly larger values were found for the experimental groups germinated with insulin or ZmIGF as compared with the controls, the Ab-I or $\Delta 60$ °C groups ($P < 0.01$). The largest values were obtained for coleoptyles from the ZmIGF-stimulated axes (Table 1).

Insulin is known to target the translation apparatus by inducing phosphorylation on the S6 rp of the 40 S ribosomal subunit, both in animal [12] and maize [21] tissues. To investigate if ZmIGF has the same effect on maize tissues as insulin, axes were stimulated either with ZmIGF, insulin or not at all (water) and exposed to [32 P]orthophosphate for the last 2 h of the incubation period. One additional group was also set where rapamycin, a powerful inhibitor of the insulin-stimulated signal transduction pathway, was added to the axes 30 min prior to ZmIGF stimulation. At the end of the incubation period, the rps were obtained from the ribosomes of each axis set, and aliquots with equal protein content were resolved by SDS/PAGE and revealed by autoradiography (Figure 6A). A phosphorylated 31 kDa labelled band (S6 rp) was observed in all samples tested. Stronger phosphorylation on S6 rp from axes stimulated with insulin or ZmIGF was observed, compared with the control. On the other

Figure 3 Affinity chromatography column

Purification of ZmIGF was performed through an affinity chromatography column of Sepharose–Protein A matrix covalently attached to insulin antibody. (A) Fraction number 20 from the Sephadex G-50 gel filtration column (arrow in Figure 2) (ZmIGF approximately equivalent to 3.3 units of insulin) was loaded onto the affinity chromatography column. After extensive washing the column was eluted with 0.1 M glycine buffer and the 280 nm elution profile was recorded. (B) Aliquots of each fraction were assayed by dot blot with bovine insulin antibody (1:2000 dilution), as described in the Experimental section. (C) Bovine insulin (0.15 mg, approx. 3.28 units) was applied to the same type of affinity column as the control. (D) Dot-blot analysis of fractions from column (C).

hand, the set of axes containing rapamycin did not exhibit the enhanced S6 rp phosphorylation induced by ZmIGF (Figure 6A). Densitometric analysis of the 31 kDa labelled bands clearly shows these differences (Figure 6C).

Identification of the 31 kDa phosphorylated band as the S6 rp was performed by Western-blot analysis using a specific antibody raised against a 17 amino acid peptide from the most conserved region of the S6 rp (see the Experimental section). Results indicated positive immunorecognition of the 31 kDa band with the specific antibody in all the sets confirming previous identification made by the full S6 rp antibody [26] (Figure 6B). These results also showed that the concentration of S6 rp in each channel was approximately the same, regardless of the experimental treatment applied.

According to previous reports [13,14], insulin-induced S6 rp phosphorylation of the ribosomes stimulates selective translation

Figure 4 Western blot of immunoreactive fractions from affinity column

(A) Protein (50 µg) from crude extract of 48-h germinated maize axes (I), 0.78 µg of purified ZmIGF (II) and 2 µg of insulin (III) were loaded on SDS/PAGE [12% (w/v) gel] and stained with 0.2% Coomassie Brilliant Blue R-250. MM, molecular mass markers. (B) Proteins were blotted on PVDF membranes and revealed as described in the Experimental section. Arrows indicate the immuno-reacting peptides against insulin antibody.

Table 1 Physiological effect of insulin and ZmIGF on maize germination and seedling growth

$Gt_{1/2}$, total germination and seedling size were recorded for each maize set (100 seeds). The percentage of germinated seeds was recorded every 2 h from 18–72 h of germination. After that, the length of coleoptyles and roots was measured in the maize seedlings from each treatment. The mean values of five independent replicates were used for statistical analysis (*t*-test). * $P < 0.01$ (statistically significant difference with respect to control).

Treatment	$Gt_{1/2}$ (h)	Germination (%)	Seedling size at 72 h	
			Root length (cm)	Coleoptyle length (cm)
Control	37	92	7.4 ± 0.1	13.3 ± 0.2
Insulin	34*	97	$8.9 \pm 0.4^*$	$15.0 \pm 0.6^*$
ZmIGF	32*	96	$9.7 \pm 0.1^*$	$17.1 \pm 0.2^*$
Ab-I	35	90	7.0 ± 0.2	13.1 ± 0.3
$\Delta 60^\circ\text{C}$	37	92	6.8 ± 0.4	12.7 ± 0.2

Figure 5 Effect of purified ZmIGF on maize seed growth

100 maize seeds were surface sterilized for 5 min with 5% hypochlorite, washed thoroughly with sterilized water and germinated at $24 \pm 2^\circ\text{C}$ in darkness in presence of either 200 µunit/ml bovine insulin, ZmIGF equivalent to 200 µunit/ml bovine insulin or in water as a control. Representative seedlings from each group after 72 h of germination are shown.

Figure 6 Effect of ZmIGF on S6 rp phosphorylation

Maize seeds were imbibed for 22 h in water and then the embryonic axes (1.0 g each) were manually dissected and incubated for 2 h in MS medium in the presence of 330 μ Ci [32 P]orthophosphate and final concentration of 200 μ unit/ml insulin (I) or ZmIGF (Z), ZmIGF and rapamycin (0.1 μ M) (Z + R) or none (C). (A) SDS/PAGE and autoradiography of phosphorylated S6 rp. Ribosomes were purified as indicated in the Experimental section and the rps (60 μ g) extracted and subjected to SDS/PAGE and analysed by autoradiography. (B) Identification of S6 rp by Western blotting; rps from (A) were blotted onto PVDF membrane and S6 rp immunodetection was performed by antiserum raised against S6 rp 17 residue peptide, as described in the Experimental section. (C) Densitometric analysis of the labelled 31 kDa band. These experiments were reproduced twice with similar results.

Table 2 Effect of ZmIGF and insulin on rp synthesis

Eight groups of maize seeds were imbibed in water for 22 h, embryonic axes were then manually dissected (1.0 g of each) and incubated for 2 h in MS medium in presence of 400 μ Ci [35 S]methionine and 200 μ unit/ml of insulin, insulin and 2 mM DTT, ZmIGF, ZmIGF and 2 mM DTT, ZmIGF and 1 μ g/ml of trypsin, 500 μ g/ml of protein extract devoid of ZmIGF, 500 μ g/ml lysozyme or no treatment (Control). Radioactivity incorporated in ribosomal and cytoplasmic proteins was determined. The values are presented as the ratio (*R*) of the radioactivity in rp (c.p.m./mg) to that in cytoplasmic protein (c.p.m./mg). Values are the mean of three independent experiments. **P* < 0.01 (statistically significant difference with respect to control).

Treatment	<i>R</i>
Control	0.286 $\pm$ 0.017
Insulin	0.786 $\pm$ 0.020*
Insulin and DTT	0.363 $\pm$ 0.037
ZmIGF	0.690 $\pm$ 0.011*
ZmIGF and DTT	0.636 $\pm$ 0.017*
ZmIGF and trypsin	0.386 $\pm$ 0.032
Extract devoid of ZmIGF	0.346 $\pm$ 0.042
Lysozyme	0.383 $\pm$ 0.023

of 5'-TOP mRNAs, rp mRNAs being among this group. Thus, the effect of ZmIGF on rp synthesis was determined on axes fed with [35 S]methionine during the last 2 h of the incubation period. A positive control with axes stimulated with a similar amount of insulin was also carried out. Cytoplasmic and ribosomal proteins were separately isolated from the axes and the incorporated labelled precursor was measured in these proteins. Relative values of the label incorporated into the rps, with respect to the cytoplasmic proteins, are presented (Table 2). Axes stimulated with either insulin or ZmIGF showed a significantly larger ribosomal to cytoplasmic protein ratio of 35 S incorporation than the control. This increment was not observed either when insulin was pre-treated with dithiothreitol (DTT), or

ZmIGF with trypsin (Table 2). It is interesting to notice that the stimulatory effect of ZmIGF was not affected by DTT, suggesting that ZmIGF biological activity does not depend on disulphide bridge structure. Other proteins than ZmIGF added to the axes during the same part of the incubation period, such as maize axes extract devoid of ZmIGF or lysozyme, did not show effect on rp synthesis (Table 2). On the other hand, addition of bovine insulin antibody to axes, with previous exposure to either insulin or ZmIGF, avoided the induced stimulation of rp synthesis (results not shown).

DISCUSSION

Up to now, only few peptides have been recognized as signal molecules functioning in plants [29]. On the other hand, IGFs are widespread peptides known to be present in lower and higher eukaryotes [10,30–32]. In animals, these highly conserved peptides are known to play important roles in promoting basic biological processes such as cell cycle progression, cell growth, survival and cell differentiation [8,31,33]. Similar peptides have also been found in plants (Figures 1–4) [19,20], suggesting that they have a very early evolutionary origin [18]; however, their biological activity has not been investigated in these organisms.

This study presents the purification of a maize insulin-like peptide, ZmIGF (Figures 1–3), and demonstrates its biological activity in maize seeds at physiological (Figure 5) and biochemical levels (Figure 6 and Table 2). To our knowledge, this is the first study to recognize an endogenous regulatory role of a plant insulin-like peptide. This peptide (ZmIGF) is specifically recognized by bovine insulin antibody and stimulated maize seed germination and seedling growth in a selective manner (Figure 5 and Table 1) similarly as shown to occur with insulin [21].

Growth-mediated protein synthesis regulation has been observed in many organisms after insulin/IGF stimulation [6,34,35]. Regulation of this process is particularly relevant during growth and differentiation in order to ensure the production of stage-specific proteins. A complex network of intracellular signalling pathways mediates the specific mechanisms of translational control exerted by insulin/IGF. This mechanism initiates at the cell membrane by effector–receptor recognition and targets the translational apparatus by phosphorylation of S6 rp on the 40 S ribosomal subunit [12,36]. Stimulation of S6 rp phosphorylation preferentially induces translation of 5'-TOP mRNAs. These transcripts mostly code for proteins of the translational apparatus, such as rps, and initiation and elongation translation factors [14,23,35,37]. In the present case, induction of S6 rp phosphorylation was clearly observed after ZmIGF-stimulation of maize axes. This reaction was reversed by rapamycin addition to the axes a few minutes before they were stimulated with ZmIGF (Figure 6). In animal cells, rapamycin has been demonstrated to block the insulin-induced signal transduction pathway [5].

These results suggest that there might be a ZmIGF-induced signal transduction pathway in maize, similar to the one described for insulin in animal tissues that selectively regulates protein synthesis. Accordingly, in this research ZmIGF was shown to stimulate S6 rp phosphorylation on the 40 S ribosomal subunit (Figure 6) and to enhance further the synthesis of rps without a significant increase in general cytoplasmic protein synthesis (Table 1 and 2). Several reports of insulin/IGF action on sea urchin eggs, as well as on mammalian cells, exhibited similar stimuli on S6 rp phosphorylation and induced selective synthesis of proteins of the translational apparatus including ribosomal proteins [4,12,13]. Recently, insulin-induced mobilization of S6 rp and initiation translation factor iso4E mRNAs (two 5'-TOP-

like mRNAs) into S6 rp phosphorylated polysomes has also been shown in maize axes [23]. All these results predict the existence of a cell membrane receptor in maize tissues for specific recognition of ZmIGF. Cell membrane receptors with predicted protein kinase activity have been reported in plant tissues [20,29], and particularly in maize [38], which strengthens the interpretation of our results regarding ZmIGF as a maize growth factor of a peptide nature that regulates specific protein synthesis, in a similar manner as insulin/IGFs in non-plant eukaryotes.

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