Available online at www.sciencedirect.com
SCIENCE DIRECT®
Biochimie 86 (2004) 439–449
BIOCHIMIE
www.elsevier.com/locate/biochi

In maize, two distinct ribulose 1,5-bisphosphate carboxylase/ oxygenase activase transcripts have different day/night patterns of expression

A. Ayala-Ochoa [a], M. Vargas-Suárez [a], H. Loza-Tavera [a], P. León [b], L.F. Jiménez-García [c], E. Sánchez-de-Jiménez [a,*]

[a] *Departamento de Bioquímica y Biología Molecular de Plantas. Facultad de Química, Universidad Nacional Autónoma de México, Ciudad Universitaria, México, DF 04510, México*
[b] *Departamento de Biología Molecular de Plantas. Instituto de Biotecnología, Universidad Nacional Autónoma de México, Cuernavaca, Morelos, 62271, México*
[c] *Departamento de Biología Celular. Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad Universitaria, México, DF 04510, México*

Received 28 November 2003; accepted 18 June 2004

Available online 08 July 2004

Abstract

Several cDNAs encoding ribulose-1,5-bisphosphate carboxylase/oxygenase activase (Rubisco activase, RCA) were isolated from a maize (*Zea mays* L.) leaf cDNA library. Although all the cDNAs encoded the same polypeptide, the RCA β isoform, they showed two different downstream-like elements (DST-like) at their 3′ untranslated regions (UTRs). The *Zmrca*1 cDNAs had the subdomain I, and II and the *Zmrca*2 cDNAs, besides these subdomains, showed two repeats of the subdomain III. The presence of at least two different *rca* genes in the maize genome was demonstrated by Southern, and by PCR analysis using primers specific for the two cDNAs. Northern analysis with probes specific for each gene showed that the *Zmrca*2 was expressed as a 1.8 kb transcript, the *Zmrca*1 corresponded to a 1.4 kb transcript, and a 1 kb band was a stable degradation product of one or both transcripts. Although both mRNAs showed cyclic variations during a day/night period, with their highest levels before dawn, the *Zmrca*2 transcript showed stronger changes than the *Zmrca*1 transcript, presenting a twofold larger highest to lowest RNA accumulation ratio than the *Zmrca*1 transcript, implying that they have different turnover rates. Our results suggest that post-transcriptional mechanisms, mediated by the DST-like element might be involved in the circadian expression of the maize *rca* transcripts.

Keywords: Rubisco activase; Circadian expression; DST-element; Post-transcriptional regulation

1. Introduction

Ribulose 1,5-bisphosphate carboxylase/oxygenase activase (Rubisco activase, RCA), a soluble chloroplast stromal protein, was initially characterized as a Rubisco activator that promotes dissociation of ribulose 1,5-bisphosphate or other inhibitory sugar-phosphates, from an inactive Rubisco–sugar complex [1]. Subsequent analysis of RCA characteristics suggested that this protein functions like a molecular chaperone [2]. Recently, a temperature-dependant dual function has been proposed for RCA: at optimal temperatures it works in releasing inhibitory sugar phosphates from the Rubisco active site, but during heat stress, RCA might function as a chaperone, protecting the protein synthesis machinery against heat inactivation [3]. Based on sequence similarity, RCA has been classified as a member of the AAA^+ (ATPases associated with a variety of cellular activities) protein family, which includes a variety of proteins with chaperone-like functions and some conserved motifs [4]. As RCA disrupts Rubisco-inhibitor complexes, switching Rubisco conforma-

Abbreviations: BSA, bovine serum albumin; DST, downstream element; EDTA, ethylenediaminetetraacetic acid; FUE, far-upstream element; NUE, near-upstream element; PCR, polymerase chain reaction; RCA, Rubisco activase; Cincadian expression; DST-element; Post-transcriptional regulation; Rubisco, Ribulose-1,5-bisphosphate carboxylase/oxygenase; SAUR, small auxin-up RNA; ORF, open reading frame; SDS, sodium dodecyl sulfate; SSC, sodium saline citrate buffer; TBST, Tris–borate saline tween buffer; 3′UTR, 3′ untranslated region.

* Corresponding author. Tel.: +52-5622-5278; fax: +52-5622-5329.
E-mail address: estelas@servidor.unam.mx (E. Sánchez-de-Jiménez).

0300-9084/$ - see front matter © 2004 Elsevier SAS. All rights reserved.
doi:10.1016/j.biochi.2004.06.007

tion from inactive to active forms, it has been assumed that RCA action is a limiting factor in Rubisco catalytic activity. Based on these criteria, RCA has been called the Rubisco's catalytic chaperone [5].

RCA is nuclear encoded, by one gene in spinach, *Arabidopsis*, and wheat [6,7], by two genes in barley, and cotton [8,9] and, by a multigene family of at least three members in *Nicotiana tabacum* [10]. In rice, different groups have reported one and two genes encoding this protein [11,12]. In spinach, *Arabidopsis*, rice and, the *rca*A gene of barley, alternative splicing near the 3′ end coding region gives rise to two transcripts with 50–85 nt difference. These mRNAs encode two polypeptides: a longer isoform α and a shorter isoform β, with different carboxy termini [8,11–13]. Alternative splicing is not the only mechanism to produce two different RCA isoforms, since in cotton they are encoded by different genes [9]. The physiological reason for the existence of two isoforms seems to be related to differential regulation since the α isoform is redox regulated [14].

By cDNA microarray analysis, changes in the levels of some transcripts associated with circadian rhythms have been observed in *Arabidopsis* [15,16]. Specifically, circadian oscillations in the *rca* mRNA levels have been detected in tomato, apple, *Arabidopsis* and rice [11,17–19]. Changes in mRNA levels may be the result either from transcriptional or post-transcriptional regulation, or both. Transcriptional mechanisms controlling *rca* expression have been observed in *Arabidopsis*, in which mRNA synthesis has been correlated with mRNA accumulation [18]. However, although post-transcriptional control might also play a significant role in *rca* mRNA abundance, its participation has not been addressed.

Regulation of *rca* expression has been mainly studied in C3 plants, which have physiological and biochemical differences with C4 plants. In these plants, although CO_2 fixation is performed in mesophyll cells (MC), Rubisco activity and the pentose phosphate pathway are only present in bundle sheath cells (BSC), showing tissue specific expression of the genes encoding these enzymes. In maize seedlings grown in the dark, the mRNAs for the large and small Rubisco subunits (LS and SS) were both detected in BSC and in MC. However, after greening, both mRNAs disappeared in MC but low levels of the LS and SS peptides were still detected. Hence, post-transcriptional and/or translational mechanisms seem to be involved in the control of tissue specific expression of Rubisco genes [20].

In this work, we addressed several aspects related to *rca* gene expression in maize, a C4 plant. We determined that two *rca* genes are present in the maize genome and that their expression patterns follow specific cyclic day/night changes. The presence of distinctive 3′UTRs sequences suggest that structural elements might be involved in the different expressions of these two genes.

2. Materials and methods

2.1. Plant material and growth conditions

Maize (*Zea mays* L. var. Chalqueño) seedlings were grown in a greenhouse, under a 12 h light/12 h dark photoperiod, at 25 °C (±2 °C). When specified, seedlings were grown under a 14 h light/10 h dark photoperiod, at the same temperature. For the construction of the cDNA library, the second and third leaves at the third stage of seedling development (the first leaf with the exposed ligulae and the second leaf with the ligulae at the level of the first one, but not exposed; approximately 8 days after sowing) [21] were collected at 4:00 a.m., when high *rca* mRNA levels were detected, and used for RNA extraction.

2.2. Construction and screening of a maize leaf cDNA library

Total RNA was isolated from maize leaves using the guanidinium thiocyanate method [22]. Poly A^+ was purified using a magnetic bead kit (Promega Biotec) following the protocol provided by the manufacturer. Ten micrograms of RNA poly A^+ was used to synthesize double stranded cDNA (Superscript choice system for cDNA synthesis, Life Technologies). cDNA products were cloned into the *Eco*RI/*Not*I sites of λgt11 and packaged using an in vitro packaging kit (Amersham). The most conserved region of the *rca* gene was amplified by PCR, using the pAtRA46 *Arabidopsis* cDNA clone as template (kindly provided by Dr. Ogren) [6] and the oligonucleotides: 5′-TAGCTCCCGCTTCATTGGAC-3′ as forward and 5′-CCTCTTCAAGATGACCGTG-3′ as reverse primers, respectively. The PCR product was radioactively labeled with [^{32}P]-labeled dCTP (Random Primer Extension kit, Du Pont, NEN) and used as probe to screen 7.5×10^5 phage plaques from a maize cDNA library. After plaque lifting, the filters (Hybond-N Plus, Amersham) were prehybridized and hybridized in phosphate buffer (0.5 M Na_2HPO_4/NaH_2PO_4 (pH 7.0), 1 mM EDTA, 7% SDS, 1% BSA (w/v)) at 65 °C. Washes were performed with 2× SSC (1× = 0.3 M NaCl, 30 mM trisodium citrate), 20 min at 65 °C; 1× SSC, 30 min at 65 °C, and 0.5× SSC, 30 min at 65 °C. Filters were exposed to films (X-Omat S, Kodak) at –80 °C. The positive plaques after four rounds of screening were subcloned into pBluescript SK (Stratagene, La Jolla, CA). Eight vector independent clones were isolated and sequenced in both strands by automatic sequencing (Applied Biosystems). The longest clone, *Zmrca*1 was used for comparison analysis. Database searches were performed with the BLAST algorithm [23]. Computer sequence analyses by the PILEUP tool from the GCG program (Genetics Computer Group, Madison, WI) were performed.

2.3. PCR analysis

Specific oligonucleotides for the two 3′UTRs sequences were designed as reverse primers: 5′-AAACTATCCAAGTAATAT-3′ for the *Zmrca*1 (Fig. 2a, R1 primer) and 5′-TTATGGC-AGGAGTGAAAC-3′ for the *Zmrca*2 (Fig. 2a, R2 primer). The forward primer was designed from a conserved sequence in the coding region present in both cDNAs: 5′-AGGAAGCTCGT-CAACTCC-3′ (Fig. 2a, F primer). These primers were used in PCR reactions using maize genomic DNA as template and an annealing temperature of 57 °C.

2.4. Southern blot analysis

Genomic DNA from the maize FR37 and Chalqueño varieties was isolated following the hexadecyltrimethyl-ammonium bromide

procedure [24]. Twenty micrograms of DNA were digested with *Bam*HI, *Hind*III, *Sac*I, *Eco*RI, *Hind*II and *Xba*I endonucleases, fractionated by electrophoresis in a 1.0% agarose gel and blotted onto Genescreen Plus membrane (Dupont, NEN). Prehybridization and hybridization were carried out at 65 °C in phosphate buffer. The probe used in this analysis was the coding region of the *Zmrca*1 gene. After overnight hybridization, the filters were washed with 2× SSC, 30 min at room temperature; 2× SSC, 30 min at 50 °C; 1× SSC, 30 min at 65 °C and exposed to film (X-Omat S Kodak) at –80 °C.

2.5. In situ localization of rca mRNA

Middle leaf sections from the second maize leaf, at the third stage of seedling development [21], were fixed in 4% paraformaldehyde and embedded in paraffin. For in situ hybridizations, 5 μm sections were deparaffinized in xylene and rehydrated to 50% ethanol. Sections were incubated overnight in the hybridization mixture containing the digoxigenin-labelled *rca* probe, at 45 °C. After hybridization, sections were rinsed with 4× SSC/50% formamide at 37 °C and 2× SSC for 30 min each. Sections were then rinsed with TBST (100 mM Tris–Cl (pH 7.5), 0.9% NaCl, 0.1% Tween 20) and incubated with a polyclonal sheep anti-digoxigenin antibody coupled to alkaline phosphatase (Boehringer Manheim, Co. Indianapolis, IN) for 1 h at room temperature. The color reaction was performed by incubating Fast Red chromogen substrate (Biomeda Codo, Foster City, CA) including 0.1 mM of levamisole. Sections were mounted with glycerol and water-soluble mounting medium to prevent fading (DAKO, Carpinteria, CA). Micrographs were taken using bright field microscopy equipped with planapochromatic optics.

2.6. Northern blot analysis

To determine the levels of *rca* transcripts during a day/night cycle, either total or polysomal RNA samples isolated from different times (4:00, 5:30, 7:00, 12:00, 17:00, and 22:00 h) were analyzed. For northern hybridizations polysomal RNA was isolated as reported previously [25]. Thirty micrograms of the RNA samples were resolved in denaturing formaldehyde gels and blotted onto Hybond-N (Amersham). The complete *Zmrca*1 clone was used as probe. To test the integrity of the blotted RNA, hybridization with the rice eIFiso4E probe (clone 54954 from MAFF Bank at Japan) [26] was performed on the same membrane, and exposed the same time as the membrane hybridized with the *rca* probe was. Densitometric analysis of the hybridization signals was performed, and the data were normalized by ribosomal RNA. To determine the nature of the different messages, total and poly A^+ RNA was isolated from leaves collected before dawn, from seedlings growing under 14 h light/10 h dark. Three probes were used to identify the messages. For the 5′ region (5′R), 22 nt of the 5′UTR plus 211 nt encoding the amino terminal sequence from the *Zmrca*1; for the coding region (CR), 510 nt of the central part of the most conserved region from the *Zmrca*1; and for the 3′ region (Z2/3′R), 284 nt containing the two repeats of the DST type III subdomain and the polyadenylation signals from the *Zmrca*2 (Fig. 6a). The synthesis and labeling of the probes and the prehybridization and hybridization procedures were performed as described. Membrane washes were done with 2× SSC, 25 min at 65 °C and 1× SSC, 20 min at 65 °C. The membranes were exposed to film (X-Omat S, Kodak) at –80 °C.

3. Results

3.1. The maize RCA peptide is similar to the short, β isoform

Screening of a maize leaf cDNA library, using the most conserved region of the *Arabidopsis rca* gene as probe, allowed the isolation of eight positive clones. All of these clones encoded the same polypeptide. The largest clone, of 1424 nt, comprised a full length cDNA (*Zmrca*1) (GenBank accession number AF084478) [27] containing a 5′UTR of 22 bp, an open reading frame (ORF) of 1299 nt, and a 3′UTR of 83 bp up to the poly A^+ tail.

Amino acid sequence comparison of the maize RCA peptide with other RCAs of monocotyledonous species is presented in Fig. 1. The first amino acid of the maize RCA protein showed high conservation among monocot sequences, in which the first 17 residues are identical and it includes the translation initiation consensus sequence reported for monocots [28]. The deduced amino acid sequence corresponds to a 433 amino acid polypeptide with a predicted M_r of 48,500. By comparison with other RCA sequences, it can be suggested that the first 52 residues correspond to a chloroplast targeting transit peptide sequence and the mature protein is predicted to start with the AKEVDE sequence, just after MA, a highly conserved amino acid pair (Fig. 1, arrow head). The mature protein is predicted to have 381 amino acids with a M_r of 42,700, and a calculated isoelectric point of 5.37 (ProtParam tool, ExPASy). Based on the size and sequence of this protein it appears to correspond to the reported β isoform that does not contain a long carboxy termini region [9].

Two ATP binding domains, conserved in all RCA proteins, were identified at the positions 161–168 and 218–225 in the maize peptide sequence (Fig. 1) [29]. The segment 161–168 (GGKGQGKS) is identical to the phosphate-binding loop, in which the K169 (K167 in maize) has been correlated with Rubisco activation and ATPase activities in spinach [30]. The sequence LFIND, between 218 and 225, is similar to the Walker B motif, in which the D174 in the tobacco mature protein (D173 in maize) is required for the precise coordination of the γ-phosphate and hence for subunit aggregation [31]. Other relevant amino acids present in the maize mature protein sequence are the W15 (W16 in tobacco), correlated with Rubisco activation [32], the D230 (D231 in tobacco) involved in the coordination of the ATP γ-phosphate and subunit aggregation [31], and the K246 (K247 in tobacco) implicated in ATP hydrolysis [33].

3.2. The rca cDNAs have different DST-like motifs at their 3′UTRs

All the eight *rca* clones isolated from the screening had identical nucleotide sequences in the coding region. However, at their 3′ UTRs, they showed different motifs similar to the downstream element (DST), which has been related to

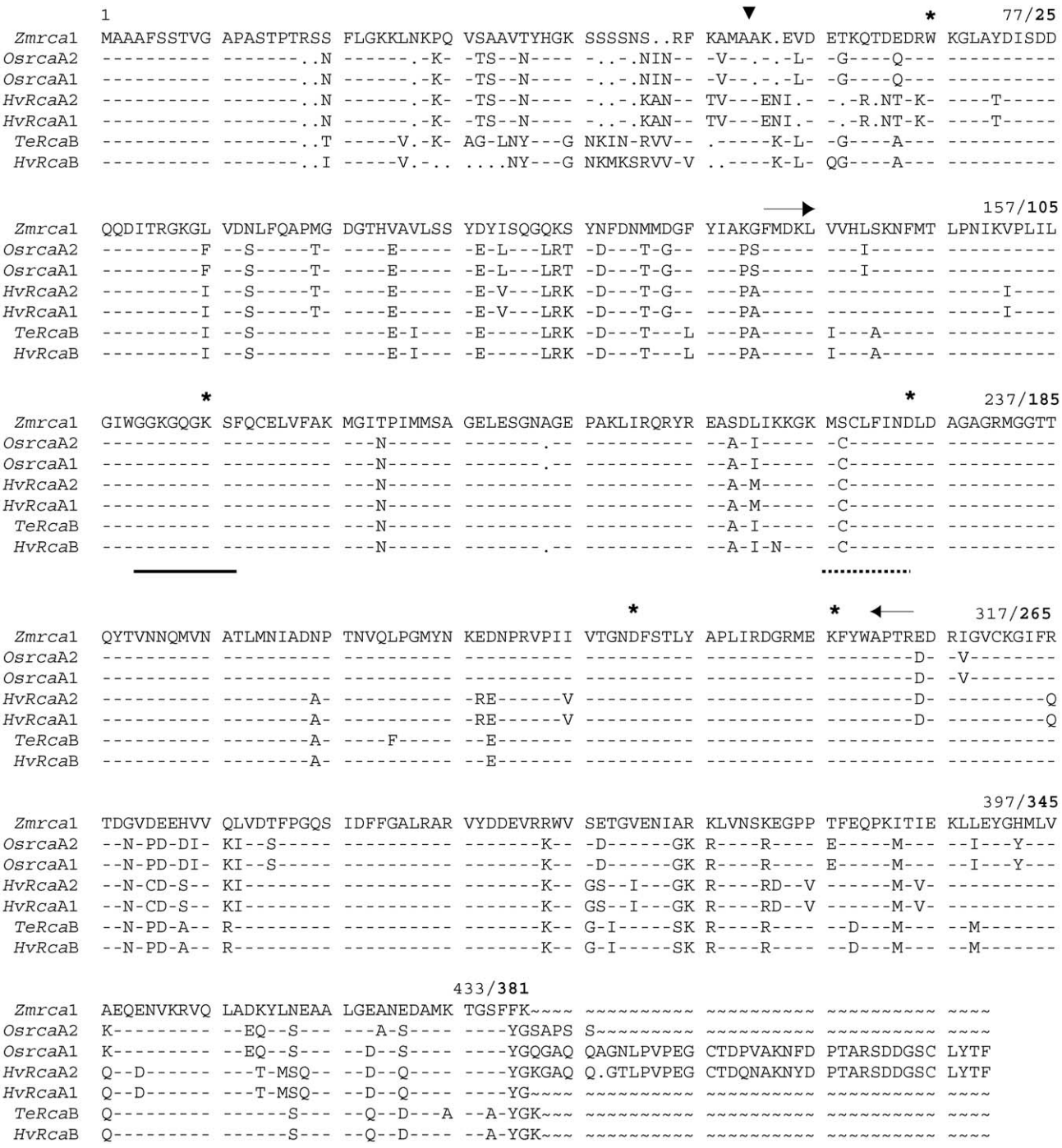

Fig. 1. Monocotyledonous RCA amino acid sequence comparison. The deduced amino acid sequence of maize RCA was aligned with the sequences of monocotyledoneous RCAs using the PILEUP program from GCG (Genetic Computer Group, Madison WI). Numbers show the amino acid position for the maize sequence: regular numbers for the complete protein sequence/bold numbers for the mature protein, without the transit peptide. The arrowhead shows the putative cleavage site for the transit peptide. The conserved ATP-binding domain is underlined and the Walker B domain is underlined in dotted line. Asterisks (*) indicate relevant amino acids involved in Rubisco activation (W15), coordination of ATP γ-phosphate and subunit cooperativity (K115, D173, D230 and K246). Showed between arrows is the amplified sequence used as probe for the conserved region. Gaps introduced into the sequences to optimize the alignment are represented by dots. Dashes represent identical amino acids. GeneBank accession numbers: *Zmrca*1, *Zea mays*, AF305876; *Osrca*A2, *Oryza sativa*, AB034748; *Osrca*A1, *Oryza sativa*, AB034698; *HvRca*A2, *Hordeum vulgare*, M55447; *HvRca*A1, *Hordeum vulgare*, M55446; *TeRca*B, *Triticum aestivum*, AF251264; *HvRca*B, *Hordeum vulgare*, M55448.

mRNA instability [34]. The DST element was identified in the 3′UTR of the rapidly degraded small auxin-up RNAs (SAUR) messages. This DST element was initially described as having three consensus sequences, the subdomains I–III [35], with the subdomains II (ATAGAT subdomain) and III (GTA box) being critical for the DST function [36]. Lately, by microarray analysis of an *Arabidopsis dst*1 mutant several genes with altered expression levels, that showed DST-like

Table 1
DST and DST-like sequences reported for different genes [a, b]

	Subdomains[c]																							
Gene			I				II								III									
*Zmrca*2	31	-	CGA	-	6	-	ATAGGAT	-	108	-	CATGTA	-	85	-	TACGTA									
Classic DST elements[a,d]																								
Soybean 15A	19	-	GGA	-	5	-	ATAGAT	-	12	-	TTTGTA													
Soybean X15	19	-	GGA	-	5	-	ATAGAT	-	12	-	TTGGTA													
Soybean 6B	19	-	GGA	-	4	-	ATAGAT	-	12	-	TTTGTA													
Soybean X10A	19	-	GGA	-	5	-	ATAGAT	-	13	-	TTTGTA													
Soybean 10A5	19	-	GGA	-	5	-	ATAGAT	-	13	-	TTTGTA													
Mungbean Arg7	14	-	GGT	-	2	-	ATAGAT	-	13	-	TTTGTA													
A. thaliana SAUR-AC1	83	-	GGA	-	9	-	ATAGAT	-	13	-	TGCGTA													
TobGNT1/CNT110	64	-	CGA	-	3	-	ATAGAT	-	10	-	TACGTA													
TobGNT35/CNT111	63	-	CGA	-	3	-	GTAGAT	-	8	-	CATGTA													
DST-like elements [b,e]																								
RAP2.4							ATGGAT	-	10	-	TTCGTA	-	8	-	TTTGTA	-	6	-	TTGTA					
CAF1-like							ATTGAT	-	0	-	CTTGTA													
Similar to APR2							ATAGAA	-	116	-	TTTGTA	-	2	-	TTTGTA	-	13	-						
						-	ATAGAC	-	15	-	TATGTA													
Putative patatin protein		-	GGA	-	5	-	AGAGAT	-	12	-	CTTGTA	-	79	-	CTTGTA									
Senescence-associated protein										-	CCTGTA	-	16	-										
						-	GTAGAT	-	43	-	ATAGTT													
Xylosidase						-	ATAGA-GAGA-TAGGT	-	38	-	TTTGTA	-	67	-	ATTGTA									
Similar to CCR protein										-	TTTGTA	-	12	-	TTTGTA	-	44	-						
						-	ATTGTA	-	3	-	ATGGAT													

[a] Newman et al. (1993).
[b] Pérez-Amador et al. (2001).
[c] Numbers indicate the distance in base pairs.
[d] Sequences identified in SAUR transcripts.
[e] Sequences identified in the 3′UTR of genes whose expression levels were altered in the *dst*1 mutant. The ATAGAT and GTA subdomains were present in different number and without a classic DST organization.

motifs, were identified [37]. These DST-like motifs did not have the classic organization; instead they showed different motif number and position (Table 1). In this work, five clones (*Zmrca*1) showed the subdomain I and the subdomain II, with an extra-G (ATAG**G**AT), located 31 bp downstream the translational stop codon (Fig. 2a). Beyond these motifs, a near-upstream element (NUE), a component of the polyadenylation signal [38], was found. The 3′UTR lengths of these five clones were between 83 and 177 nt, without considering the poly A^+ tail. In three other clones, the DST subdomains I and II were also found at identical positions, but in addition, two GTA boxes (subdomain III) were found 104 nt downstream (Fig. 2a). In these clones a far-upstream element (FUE), another component of the polyadenylation signal [38], was followed by at least four NUE motifs. The poly A^+ tails of these clones also started at variable distances from the last NUE sequence. The 3′UTR lengths from these three clones varied from 403 to 432 nt. Due to the differences among the two identified clones the largest sequence that belongs to this group was reported as *Zmrca*2 cDNA (GeneBank accession number AF305876). Comparison of the DST-like elements found in the *Zmrca* genes with different classic and DST-like elements from other plants is presented in Table 1.

3.3. At least two rca genes are present in the maize genome

In spinach, *Arabidopsis*, barley and rice, have been reported that an alternative splicing generates two different types of *rca* messages [8,11,13]. This alternative splicing site has been mapped at the 3′ end of the *rca* coding region, producing two RCA proteins with different carboxy termini. In our case, the two types of isolated cDNAs code for the exact same polypeptides within the coding region and the differences between them start at 56 nt downstream the stop codon, at the 3′UTR. Although an alternative splicing signal sequence was not detected in maize, these two transcript types could correspond to either an alternative splicing in the 3′UTR of one single gene or from two independent genes. To discern between these two possibilities, PCR experiments using genomic DNA and 3′ end-specific primers for either the *Zmrca*1 (R1) or the *Zmrca*2 (R2) transcripts were performed. When the R1 specific primer was used, a band of approximately 290 nt was synthesized. In contrast, when the reaction was set using the R2 primer, a band of around 630 bp was observed (Fig. 2b). The identity of these PCR products was corroborated by sequence, showing the expected sequences for either *Zmrca*1 or *Zmrca*2 transcripts. These re-

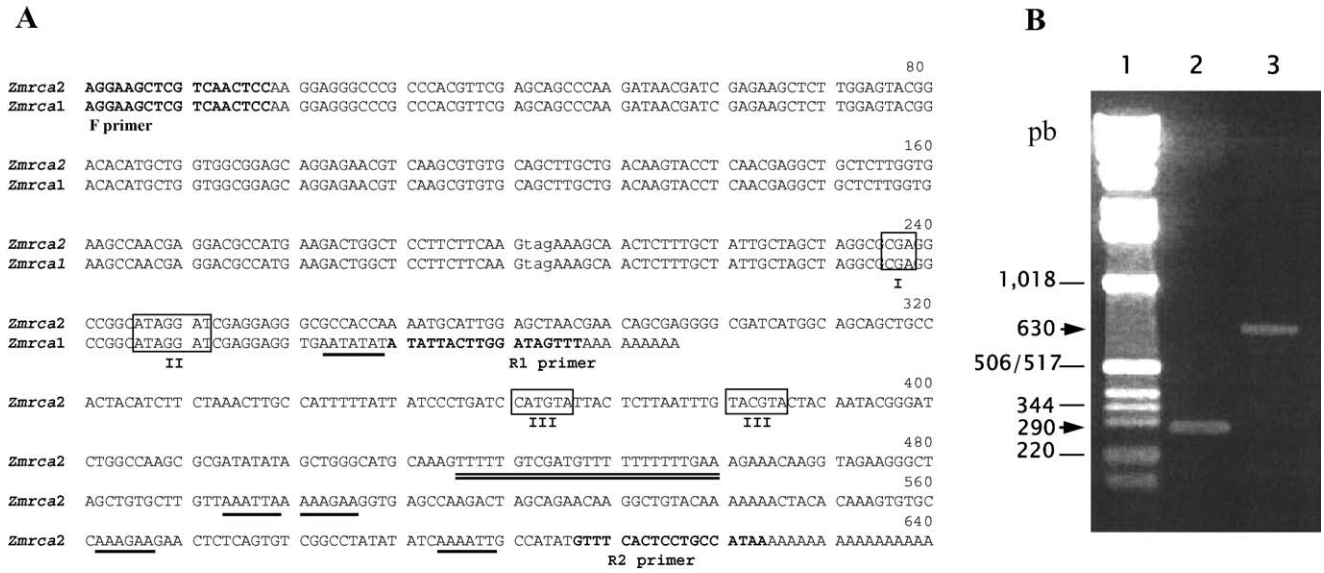

Fig. 2. Different 3′UTRs of the *rca* cDNA clones, and PCR analysis on genomic DNA. Representative 3′UTRs from *Zmrca*1 and *Zmrca*2 cDNA clones. The stop codon is shown in small letters. The subdomains for the DST-like element are in boxes numbered accordingly: subdomain I, **I**; subdomain II, **II**; subdomain III, **III**. Polyadenylation signal components are underlined: near-upstream element (NUE), one line; far-upstream element (FUE), double line. Primers used for PCR genomic analysis are in bold: forward (F); reverse for *Zmrca*1 (R1); reverse for *Zmrca*2 (R2). (**B**) PCR analysis was performed using the primers shown in **A** and genomic DNA as template. The PCR products were resolved in 1% agarose gels, and stained with ethidium bromide. Molecular size standards (lane 1); PCR product from the reaction with F and R1 primers (lane 2); PCR product from the reaction with F and R2 primers (lane 3). Lines at the left show sizes of the molecular weight markers, arrowheads indicate the estimated sizes of PCR products.

sults indicate that the two types of isolated transcripts are synthesized from two independent *rca* genes and not as a result of an alternative splicing from one gene.

To estimate the number of *rca* genes present in the maize genome, a Southern blot analysis with genomic maize DNA using the complete *Zmrca*1 sequence as probe was performed. DNA digestions were performed with *Hind*III, *Bam*HI, *Sac*I and *Sst*I endonucleases. A *Sac*I site is present in the position 144 of the *Zmrca* sequence, but no *Hind*III or *Bam*HI sites exist in this clone. Four bands were observed when the DNA was cut with *Sac*I (Fig. 3, lane 2), two bands when the DNA was cut with *Hind*III (lane 3), and three bands when it was cut with *Bam*HI (lane 4). When *Sst*I, a methylation susceptible endonuclease *Sac*I isoschizomer, was used to digest the DNA, only two bands were observed, suggesting that the *rca* genes might be methylated at this position (Fig. 3, lane 1). Analysis using *Eco*RI, *Hind*II and *Xba*I digested DNA (data not shown) showed comparable results. These results, together with the PCR analysis, indicate that RCA is encoded by at least two genes in the maize genome.

3.4. The expression of rca correlates with the Kranz anatomy

Rubisco is differentially expressed in the chloroplasts of BSC in maize leaves [20]. To test whether the expression of RCA was associated with a specific cell type, in situ hybridizations were performed in leaves using the full *Zmrca*1 as probe. Results obtained show a positive signal only in BSC (Fig. 4), indicating that *rca* gene expression is restricted to the same cell type where Rubisco is expressed. Cell specific and coordinated expression of Rubisco (*rbc*S and *rbc*L) and *rca* genes has been observed during barley leaf development [39] and in C3 and C4 *Atriplex* species [40]. The same tissue specific *rca* expression was observed by differential screening in sorghum, another C4 plant [41].

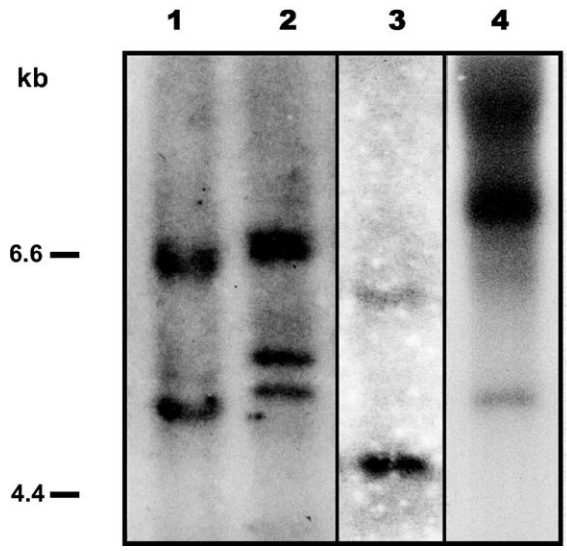

Fig. 3. Southern blot analysis of maize genomic DNA. Hybridization analysis using 5 μg of genomic DNA isolated from maize leaves and digested with *Sst*I (lane 1), *Sac*I (lane 2), *Hind*III (lane 3), and *Bam*HI (lane 4) was performed using the complete sequence *Zmrca*1 as probe. Molecular sizes are shown at the left. A representative blot of three replicates is shown.

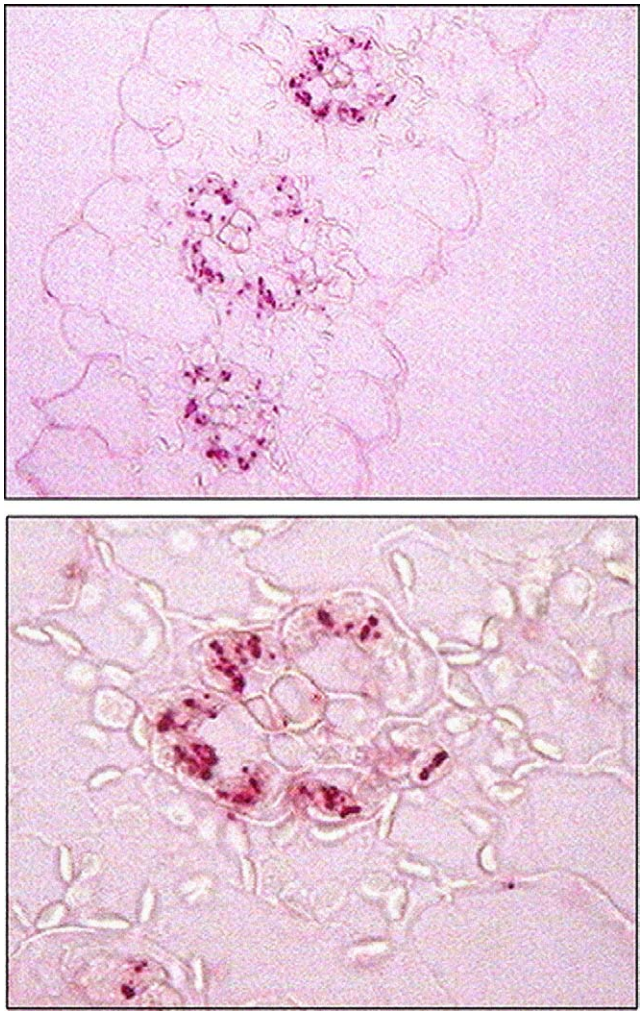

Fig. 4. In situ localization of *rca* mRNA in maize leaves. Leaves of maize seedlings were sectioned, embedded in paraffin and hybridized with digoxigenin-labeled *Zmrca*1 probe. The hybridization signal was revealed by polyclonal sheep anti-digoxigenin antibody coupled to alkaline phosphatase. Upper panel: middle leaf section (X140); bottom panel: amplified middle leaf section (X420).

3.5. The levels of rca transcripts differently change during a day/night cycle

It has been found that in several C3 plants, like tomato, rice, *Malus* and *Arabidopsis, rca* transcript levels change rhythmically during the day, displaying a circadian rhythm regulation [11,17–19]. In this work we analyzed whether the *rca* transcripts from a C4 plant, like maize, also present rhythmic changes. Northern analysis was performed with polysomal RNA extracted at different moments during a 24 h cycle from maize leaves. Changes of *rca* transcripts were found during a day/night cycle using the complete *Zmrca*1 as probe (Fig. 5a,b). Three bands of 1.8, 1.4 and 1.0 kb were observed (Fig. 5a). The abundance of these bands varied widely during the 24 h period. The 1.8 kb message was strongly expressed early in the morning between 4:00 and 7:00 h, peaking at 5:30, and progressively diminished throughout the day, to reach its lowest level at 17:00 h, and it started to increase again, at 22:00 h (Fig. 5a,b). The 1.4 kb

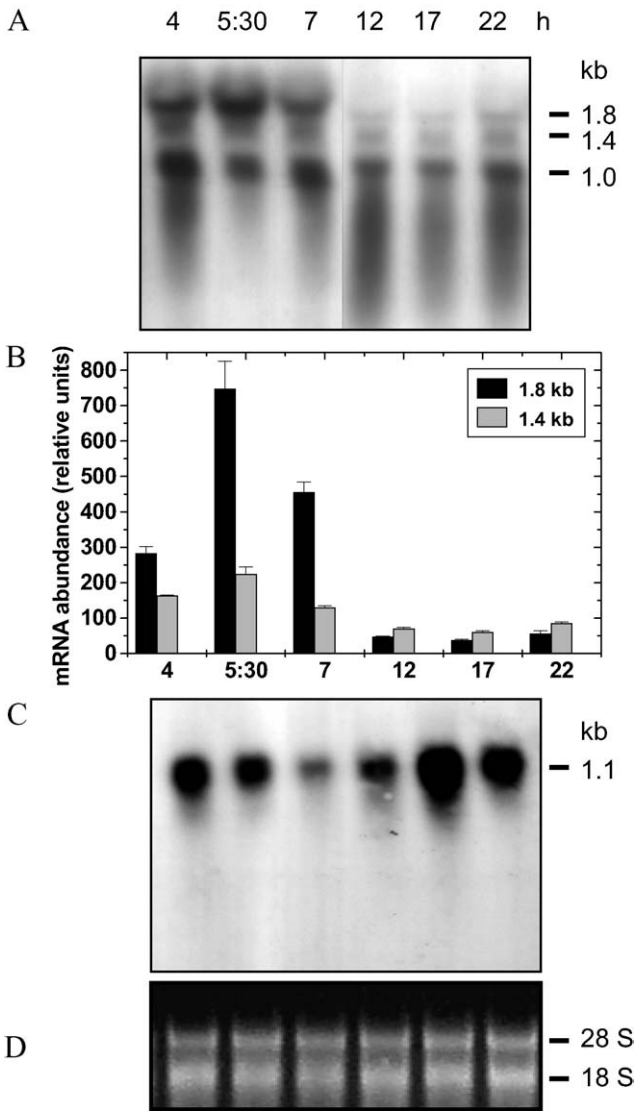

Fig. 5. Expression of maize *rca* during a day/night cycle. (**A**) Polysomal RNA (30 μg) isolated from maize leaves at different times during a day/night cycle were resolved in a denaturing agarose gel and transferred to a nylon membrane. The membrane was hybridized with the *Zmrca*1 cDNA clone and autoradiographed. The sizes in kb of the main hybridization signals are shown at the right. (**B**) Densitometric measurements of the 1.8 kb (black bar) and 1.4 kb (gray bar) bands, normalized by the 18S rRNA signal are showed. (**C**) To test RNA integrity, the same membrane was rehybridized with a control probe (eIFiso4E) and autoradiographed. The molecular size of the hybridization signal is shown at right. (**D**) Ethidium bromide stained gel showing 28S and 18S ribosomal RNAs. A representative blot of three replicates is shown.

transcript expression was less strong and less variable than the 1.8 kb message and also accumulated at its highest level at 5:30 h (Fig. 5a,b). The 1 kb band was very strong before dawn, and decreased during the light period (Fig. 5a). Densitometric analysis of the 1.8 and 1.4 kb bands indicated that, although the two *rca* transcripts showed a diurnal rhythm, they are differentially expressed. At 5:30 h, the amount of *Zmrca*2 message was threefold larger than the *Zmrca*1, and six and a half hours later, the content of both messages was almost the same (Fig. 5a,b). In all the samples, hybridization

with the *rca* probe showed RNA smears below the 1 kb band. However, after the same membrane was re-probed with the rice eIFiso4E cDNA, a clear hybridization signal of the expected size (1.1 kb) without signs of degradation was detected (Fig. 5c). The ethidium bromide stained gels also showed the intactness of the ribosomal RNA (Fig. 5d) indicating that the smears observed in the hybridization with the *rca* probe were not caused by indiscriminate degradation of the mRNA samples, but by specific degradation of the *rca* transcripts. Experiments with total RNA showed similar results (data not shown).

3.6. The 1.8 kb mRNA is the Zmrca*2, the 1.4 kb is the* Zmrca*1, and the 1 kb is a deadenylated mRNA*

As three hybridization bands were detected in the Northern blot analysis with the complete *Zmrca*1 probe, and since it was established the existence of at least two different *rca* genes in maize, it was important to determine whether the different bands corresponded to one or both of these maize genes. With this purpose, Northern blot analysis with total and poly A^+ RNA from maize leaves grown under 14 h light/10 h dark photoperiod, was carried out using specific fragments of the *Zmrca*1 and *Zmrca*2 cDNAs as probes

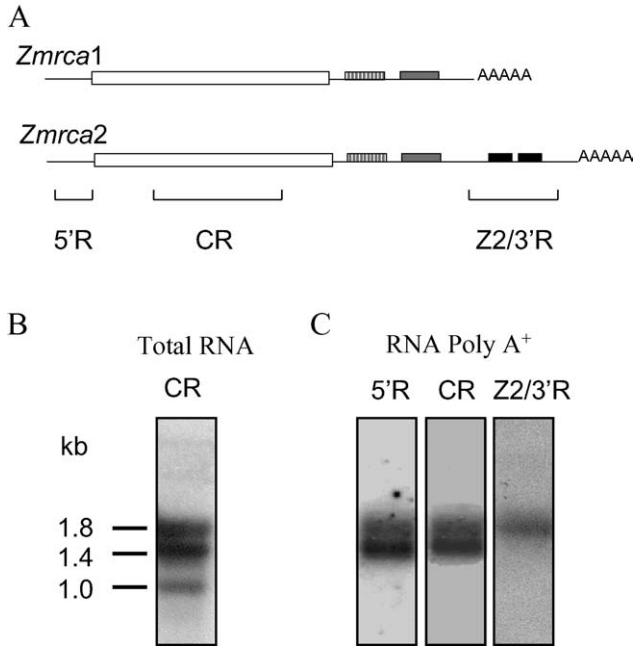

Fig. 6. Characterization of the *rca* transcripts. (**A**) Schematic representation of the *Zmrca*1 and *Zmrca*2 cDNAs. Clear box: coding region; vertical sliced box: type I DST subdomain; shaded box: type II DST subdomain; black box: type III DST subdomain. The specific probes used to identify the RNA messages are shown: 5′R, segment of the 5′ region; CR, coding region; Z2/3′R, 3′UTR fragment containing the type III DST subdomains from *Zmrca*2. (**B**) Total (15 μg), or (**C**) poly A^+ (5 μg) RNA was isolated from the third leaf of maize seedlings grown under 14 h light/10 h dark photoperiod, and collected at 4:00 h. The samples were resolved in a denaturing gel, transferred to nylon membranes and hybridized with the specific probes indicated on the top of the figure. Molecular sizes of the observed hybridization signals are depicted at the left. A representative blot of three replicates is shown.

(Fig. 6a). When total RNA was analyzed using the probe for the conserved *rca* coding region (CR), the three bands of 1.8, 1.4, and 1.0 kb were observed (Fig. 6b). When poly A^+ RNA was hybridized against either a 5′ region (5′R) or the CR, only two hybridizing bands were observed (1.8 and 1.4 kb), suggesting that both bands corresponded to complete messages. However, when the specific probe for *Zmrca*2, which contains the DST type III subdomains (Z2/3′R) was used, only the 1.8 kb band hybridized (Fig. 6c). These data indicate the presence of two complete poly A^+ RCA mRNAs, one of 1.8 kb corresponding to the *Zmrca*2 gene, and the other of 1.4 kb, that stands for the *Zmrca*1 gene. Since the 1.0 kb band is observed only in the hybridization with total RNA and not in the poly A^+ fraction, and its length is not enough to encode for a complete RCA ORF (1.3 kb), it is likely that it corresponds to a stable deadenylated degradation fragment from either one or both RCA transcripts. Stable intermediates have been reported for the degradation of the SRS4 mRNA (encoding Rubisco small subunit) in soybean, which shows different mRNA fragments, probably produced by stochastic endoribonuclease attack [42]. In our work, the presence of this molecule might be the result of an endonuclease activity, although the smear observed in the Northern analysis (Fig. 5a) implies that an exoribonuclease activity might also be involved. Contrarily to what was observed in the previous experiment, the amount of the 1.4 kb band was larger than the 1.8 kb band, at 4:00 h. This difference might be related to the growing conditions since the plants for the analysis of day/night expression were grown under 12 h light/12 h dark photoperiod, whereas the plants used to identified the transcripts were grown under 14 h light/10 h dark photoperiod.

4. Discussion

The number of genes encoding RCA varies in different plants. In several species a gene with alternative splicing produces two RCA isoforms (6–8,11–13). However, other *rca* genes do not present alternative splicing. For example in cotton, two different genes encode the two different RCA isoforms [9] and in barley, the *rca*B gene, which does not show alternative splicing, encodes a β isoform [8]. In this paper we report the isolation of two different maize cDNAs, *Zmrca*1 and *Zmrca*2, encoding Rubisco activase. These transcriptional units contain the same coding region but have different 3′UTRs. Although we do not know whether these transcripts have the same 5′UTR, our results indicate that these cDNAs were retro-transcribed from two mRNAs encoded by two different genes, and they were not the result of alternative splicing from one gene. These conclusions are supported by several findings: (a) eight cDNAs from only two types were obtained in the screening (Fig. 2), (b) the differential PCR experiments showed the presence of the two distinct 3′UTR sequences in the maize genome DNA (Fig. 3), (c) the Southern blot analysis (Fig. 4), and (d) the absence of alternative splicing consensus sequences in the cDNAs.

Most of the plant species studied until now contains two different RCA isoforms, differing only at their carboxy-terminus [8–9,11–13]. Meanwhile the M_r of the α isoform oscillates between 45,000 and 47,000, the M_r for the β isoform changes between 41,000 and 44,000. We previously reported that in maize leaf extracts, two RCA polypeptides of M_r 41,000 and 43,000 were detected [43] and, in this work, two different cDNA sequences, encoding the same polypeptide of M_r 42,700 similar to the β isoform, were found. Since a smaller cDNA sequence encoding the M_r 41,000 RCA form was not found in our screening, either the mRNA encoding this isoform is very low abundant, or the peptide is a proteolytic product derived from the 43,000 RCA isoform. In vitro translation experiments using poly A^+ maize RNA produced only one RCA product of M_r 49,000, the size expected for the M_r 43,000 precursor, supporting the later possibility (Vargas-Suárez M, et al., unpublished results). On the other hand, the presence of the long carboxy terminus on the α isoform has been implicated in the regulation of the enzyme activity by the chloroplast redox-state [14,44]. Thus our finding that in maize only the β isoform is present, is in agreement with published work reporting that, in this plant, Rubisco is not extensively regulated in response to changes in light intensity, as has been observed in other species [45].

The DST element was identified as an instability determinant sequence present in the 3′ UTRs of rapidly degraded SAUR mRNAs [35]. Although several *rca* gene sequences from different plants and algae have been reported [6–12], and some of the messages show rapid turnover because of their circadian oscillations [18,19], the presence of the DST element in their 3′UTRs has not been described. We identified different DST-like elements in the *rca* genes of maize. Although the two genes have the DST subdomains I and II, the later with an extra G than the classic subdomain, only the *Zmrca*2 gene contains the type III subdomain, repeated twice and located farther apart from what has been observed for the classic DST element (Table 1). Variations in the sequence and organization of the classic DST element are not rare; DST-like sequences observed in transcripts of the *Arabidopsis thaliana dst*1 mutant, deficient in the degradation of some mRNAs, have been reported (Table 1) [37]. Experiments where a DST element was introduced downstream of the β-glucuronidase reporter gene in stable transformed tobacco cells generated unstable GUS transcripts [35]. Mutants with two-base substitutions in the subdomain II showed that the first four bases are critical for instability in tobacco cell cultures, whereas mutations with two-base substitution in the subdomain III inactivate DST function in tobacco leaves but not in cell cultures [36]. These results indicated that there is not a unique DST element, that the DST element might be differentially recognized in different cell types, and that the different subdomains may probably function as modules for stability/instability recognition [46].

Circadian expression of the *rca* transcript has been observed in several plants. Particularly in *Arabidopsis*, descending levels of the message are observed during the day and increasing levels during the night [17]. On the other hand, even in the species with several or differently spliced *rca* genes, only one transcript with 1.6–1.9 kb has been identified [6,8,11,13]. In this work two transcripts of 1.8 and 1.4 kb corresponding to the *Zmrca*2 and the *Zmrca*1 genes, respectively, showed distinctive day/night changing patterns of expression. Although the levels of both transcripts reached their maximum at the same time, early in the morning, the intensity of their changes were different. The *Zmrca*2/*Zmrca*1 mRNA ratio was almost twofold at 4:00 h and even larger later on at 7:00 h, but it was less than 1 from 12:00 to 22:00 h, implying different turnover rates for the two transcripts thorough the day (Fig. 5b). Moreover, other environmental conditions, such light, temperature etc., might also be involved in this phenomenon since samples taken at the same moment but grown under different light conditions differently expressed the two *rca* transcripts (compare Fig. 5a, at 4:00 h vs. Fig. 6b).

The contribution of transcription in this chronobiological phenomenon was undoubtedly demonstrated when elements, localized within 317 bp upstream the transcription initiation site, were sufficient to confer clock-regulated transcription to the *Arabidopsis rca* gene [47]. Although the existence of clock-regulated promoters with different transcriptional strength in the maize *rca* genes are not excluded, our results suggest that the presence of the subdomain III in the *Zmrca*2 message might be a factor involved in the stability of this transcript that would contribute to generate different turnover rates. Thus, besides transcriptional control, post-transcriptional mechanisms might also be involved in the regulation of the expression of *rca* genes. Correlation between the DST element and transcripts showing circadian rhythms was recently suggested [37]. Moreover, the *rca* gene was identified between a group of unstable transcripts (At-GUTs), by a microarray analysis in *A. thaliana*, showing a half-life of 57.5 min (data presented as supporting information in [16]). The presence of DST-like elements in the maize *rca* mRNAs may provide the plant, with mechanisms to match *rca* expression with the clock-mediated coordination of physiological pathways, which has been proposed to transcriptionally interconnect the genes responding to specific circadian rhythms [15]. Nevertheless, other levels of regulation might also be involved in controlling this processes, for example, it would be feasible that translational and/or post-translational mechanisms, acting in response to environmental signals demanding rapid changes on protein activity, may superimpose the circadian control, therefore changes in the mRNA levels are not necessarily echoed by changes in protein levels.

In this work, we reported unique features of the maize *rca* gene expression and organization. We found that at least two *rca* genes are present in the maize genome. These genes encode the same β isoform, but they have different DST-like elements. The *Zmrca*2 transcript has the three DST subdomains, and changes abruptly during a day/night cycle, whereas the *Zmrca*1 has only two subdomains, and showed

little changes in its transcript levels. These results suggest that post-transcriptional mechanisms, signaled by different subdomains of the DST-like element, might be involved in controlling daily cyclic changes of *rca* expression in maize. We are presently analyzing the physiological implications of these findings. Whether this is a general or an exclusive mechanism for C4 plants remains to be elucidated.

Acknowledgments

Authors are thankful to Dr. W.L. Ogren for kindly providing the plasmid containing the *Arabidopsis thaliana* cDNA, to the MAFF Bank in Japan for the eIFiso4E rice clone, to Dr. J. Polacco for the revision of the manuscript and to the editorial reviewers for their suggestions to improve it. A.A.-O. and M.V.-S. acknowledge CONACYT for their Ph. D. scholarships. This work was supported by a CONACYT grant to E.S.-J. This work is dedicated to the memory of Alfredo Ayala-Ochoa who worked to generate the data but was unable to see it finished.

References

[1] A.R. Portis Jr., Regulation of ribulose-1,5-bisphosphate carboxylase/oxygenase activity, Annu. Rev. Plant Physiol. Plant Mol. Biol. 43 (1992) 415–437.
[2] E. Sánchez-de-Jiménez, L. Medrano, E. Martínez Barajas, Rubisco activase, possible new member of the molecular chaperone family, Biochemistry 34 (1995) 2826–2831.
[3] A. Rokka, L. Zhang, E.M. Aro, Rubisco activase: an enzyme with a temperature-dependent dual function? Plant J. 25 (2001) 463–471.
[4] A.F. Neuwald, L. Aravind, J.L. Spouge, E.V. Koonin, AAA+: a class of chaperone-like ATPases associated with the assembly, operation, and disassembly of protein complexes, Genome Res. 9 (1999) 27–43.
[5] A.R. Portis Jr., Rubisco activase—Rubisco's catalytic chaperone, Photosynth. Res. 75 (2003) 11–27.
[6] J.M. Werneke, R.E. Zielinski, W.L. Ogren, Structure and expression of spinach leaf cDNA encoding ribulosebisphosphate carboxylase/oxygenase activase, Proc. Natl. Acad. Sci. USA 85 (1988) 787–791.
[7] R.D. Law, S.J. Crafts-Brandner, High temperature stress increases the expression of wheat leaf ribulose-1,5-bisphosphate carboxylase/oxygenase activase protein, Arch. Biochem. Biophys. 386 (2001) 261–267.
[8] S.J. Rundle, R.E. Zielinski, Organization and expression of two tandemly oriented genes encoding ribulosebisphosphate carboxylase/oxygenase in barley, J. Biol. Chem. 266 (1991) 4677–4685.
[9] M.E. Salvucci, F.J. van de Loo, D. Stecher, Two isoforms of Rubisco activase in cotton, the products of separate genes not alternative splicing, Planta 216 (2003) 736–744.
[10] J. Qian, S.R. Rodermel, Ribulose-1,5-bisphosphate carboxylase/oxygenase activase cDNAs from *Nicotiana tabacum*, Plant Physiol. 102 (1993) 683–684.
[11] K.Y. To, D.F. Suen, S.C.G. Chen, Molecular characterization of ribulose 1,5-bisphosphate carboxylase/oxygenase activase in rice leaves, Planta 209 (1999) 66–76.
[12] Z. Zhang, S. Komatsu, Molecular cloning and characterization of cDNAs encoding two isoforms of ribulose-1,5-bisphosphate carboxylase/oxygenase activase in rice (*Oryza sativa* L.), J. Biochem. 128 (2000) 383–389.
[13] J.M. Werneke, J.M. Chatfield, W.L. Ogren, Alternative mRNA splicing generates the two ribulosebisphosphate carboxylase/oxygenase activase polypeptides in spinach and *Arabidopsis*, Plant Cell 1 (1989) 815–825.
[14] N. Zhang, A.R. Portis Jr., Mechanisms of light regulation of rubisco: a specific role for the larger rubisco activase isoform involving reductive activation by thioredoxin-f, Proc. Natl. Acad. Sci. USA 96 (1999) 9438–9443.
[15] S.L. Harmer, J.B. Hogenesch, M. Straume, H.S. Chang, B. Han, T. Zhu, X. Wang, J.A. Kreps, S.A. Kay, Orchestrated transcription of key pathways in *Arabidopsis* by the circadian clock, Science 290 (2000) 2110–2113.
[16] R.A. Gutiérrez, R.M. Ewing, J.M. Cherry, P.J. Green, Identification of unstable transcripts in *Arabidopsis* by cDNA microarray analysis: rapid decay is associated with a group of touch- and specific clock-controlled genes, Proc. Natl. Acad. Sci. USA 99 (2002) 11513–11518.
[17] S. Martino-Catt, D.R. Ort, Low temperature interrupts circadian regulation of transcriptional activity in chilling sensitive plants, Proc. Natl. Acad. Sci. USA 89 (1992) 3731–3735.
[18] M.L. Pilgrim, C.R. McClung, Differential involvement of the circadian clock in the expression of genes required for ribulose-1,5-bisphosphate carboxylase/oxygenase synthesis, assembly, and activation in *Arabidopsis thaliana*, Plant Physiol. 103 (1993) 553–564.
[19] B. Watillon, R. Kettman, P. Boxus, A. Burny, Developmental and circadian pattern of rubisco activase mRNA accumulation in apple plants, Plant Mol. Biol. 23 (1993) 501–509.
[20] J.-Y. Sheen, L. Bogorad, Differential expression of C4 pathway genes in mesophyll and bundle sheath cells of greening maize leaves, J. Biol. Chem. 262 (1987) 11726–11730.
[21] H. Loza-Tavera, E. Martínez-Barajas, E. Sánchez-de-Jiménez, Regulation of Ribulose-1,5-bisphosphate carboxylase expression in second leaves of maize seedlings from low and high yield populations, Plant Physiol. 93 (1990) 541–548.
[22] P. Chomczynski, N. Sacchi, Single step method of RNA isolation by acid guanidinium thiocyanate–phenol–chloroform extraction, Anal. Biochem 162 (1987) 156–159.
[23] S.F. Altschul, T.L. Madden, A.A. Schaffer, J. Zhang, Z. Zhang, W. Miller, D.J. Lipman, Gapped BLAST and PSIBLAST: a new generation of protein database search programs, Nuc. Acid Res. 25 (1997) 3389–3402.
[24] S.L. Della Porta, J. Wood, J.B. Hicks, A plant DNA minipreparation: version II, Plant Mol. Biol. Rep. 1 (1983) 19–21.
[25] E. Beltrán-Peña, A. Ortiz-López, E. Sánchez-de-Jiménez, Synthesis of ribosomal proteins from stored mRNAs early in seed germination, Plant Mol. Biol. 28 (1995) 327–336.
[26] T.D. Dinkova, R. Aguilar, E. Sánchez-de-Jiménez, Expression of maize eukaryotic initiation factor (eIF) iso-4E is regulated at the translation level, Biochem. J. 351 (2000) 825–831.
[27] A. Ayala-Ochoa, H. Loza-Tavera, E. Sánchez-de-Jiménez, A cDNA from maize (*Zea mays* L) encoding Ribulose-1,5-bisphosphate carboxylase/oxygenase activase (Accession No. AF084478), (PGR98-207), Plant Physiol. 118 (1998) 1535.
[28] C.P. Joshi, H. Zhou, X. Huang, V.L. Chiang, Context sequences of translation initiation codon in plants, Plant Mol. Biol. 35 (1997) 993–1001.
[29] M.E. Salvucci, W.L. Ogren, The mechanism of Rubisco activase: insights from studies of the properties and structure of the enzyme, Photosynth. Res. 47 (1996) 1–11.
[30] J.B. Shen, E.M. Orozco, W.L. Ogren, Expression of the two isoforms of spinach ribulose 1,5 bisphosphate carboxylase activase and essentiality of the conserved lysine in the consensus nucleotide-binding domain, J. Biol. Chem. 266 (1991) 8963–8968.
[31] F.J. van de Loo, M.E. Salvucci, Involvement of two aspartate residues of rubisco activase in coordination of the ATP γ-phosphate and subunit cooperativity, Biochemistry 37 (1998) 4621–4625.
[32] F.J. van de Loo, M.E. Salvucci, Activation of ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) involves rubisco activase Trp16, Biochemistry 35 (1996) 8143–8148.

[33] M.E. Salvucci, R.R. Klein, Site-directed mutagenesis of a reactive lysyl residue (Lys-247) of Rubisco activase, Arch. Biochem. Biophys. 314 (1994) 178–185.
[34] B.A. McClure, G. Hagen, C.S. Brown, M.A. Gee, T.J. Guilfoyle, Transcription, organization, and sequence of an auxin-regulated gene cluster in soybean, Plant Cell 1 (1989) 229–239.
[35] T.C. Newman, M. Ohme-Takagi, C.B. Taylor, P.J. Green, DST sequences, highly conserved among plant SAUR genes, target reporter transcripts for rapid decay in tobacco, Plant Cell 5 (1993) 701–714.
[36] M.L. Sullivan, P. Green, Mutational analysis of the DST element in tobacco cells and transgenic plants: identification of residues critical for mRNA instability, RNA 2 (1996) 308–315.
[37] M.A. Perez-Amador, P. Lidder, M.A. Johnson, J. Landgraf, E. Wisman, P.J. Green, New molecular phenotypes in the *dst* mutants of *Arabidopsis* revealed by DNA microarray analysis, Plant Cell 13 (2001) 2703–2717.
[38] H.M. Rothnie, Plant mRNA 3′-end formation, Plant Mol. Biol. 32 (1996) 43–61.
[39] R.E. Zielinski, J.W. Werneke, M.E. Jenkins, Coordinate expression of rubisco activase and rubisco during barley leaf cell development, Plant Physiol. 90 (1989) 516–521.
[40] G.S. Hudson, R.E. Dengler, P.W. Hattersley, N.G. Dengler, Cell-specific expression of Rubisco small subunit and rubisco activase genes in C3 and C4 species of Atriplex, Aust. J. Plant Phys. 19 (1992) 89–96.
[41] R. Wyrich, U. Dressen, S. Brockmann, M. Streubel, C. Chang, D. Qiang, A.H. Paterson, P. Westhoff, The molecular basis of C4 photosynthesis in sorghum: isolation, characterization and RFLP mapping of mesophyll- and bundle-sheath-specific cDNAs obtained by differential screening, Plant Mol. Biol. 37 (1998) 319–335.
[42] M.M. Tanzer, R.B. Meagher, Faithful degradation of soybean *rbc*S mRNA in vitro, Mol. Cell. Biol. 14 (1995) 2640–2650.
[43] E. Martínez-Barajas, J. Molina-Galán, E. Sánchez-de-Jiménez, Regulation of rubisco activity during grain fill in maize: possible role of rubisco activase, J. Agric. Sci. 128 (1997) 155–161.
[44] N. Zhang, R.P. Kallis, R.G. Ewy, A.R. Portis Jr., Light modulation of Rubisco in *Arabidopsis* requires a capacity for redox regulation of the larger Rubisco activase isoform, Proc. Natl. Acad. Sci. USA 99 (2002) 3330–3334.
[45] R.F. Sage, J.R. Seeman, Regulation of ribulose-1,5-bisphosphate carboxylase/oxygenase activity in response to reduced light intensity in C4 plants, Plant Physiol. 102 (1993) 21–28.
[46] R.A. Gutiérrez, G.C. MacInstosh, P.J. Green, Current perspectives on mRNA stability in plants: multiple levels and mechanisms of control, TIPS 4 (1999) 429–438.
[47] Z. Liu, C. Ching Taub, R. McClung, Identification of an *Arabidopsis thaliana* ribulose-1,5-bisphosphate carboxylase/oxygenase activase (RCA) minimal promoter regulated by light and the circadian clock, Plant Phys. 112 (1996) 43–51.