ELSEVIER

Gene 240 (1999) 355–359

GENE
AN INTERNATIONAL JOURNAL ON GENES AND GENOMES

www.elsevier.com/locate/gene

Structure, organization and expression of the eukaryotic translation initiation factor 5, *eIF-5*, gene in *Zea mays*

Ignacio López Ribera, Pere Puigdomènech *

Departament de Genètica Molecular, Institut de Biologia Molecular de Barcelona, CID-CSIC, Jordi Girona, 18, 08034 Barcelona, Spain

Received 19 June 1999; received in revised form 14 September 1999; accepted 29 September 1999
Received by W. Martin

Abstract

The maize genomic DNA sequence encoding the eukaryotic translation initiation factor 5 (eIF-5) has been isolated from genomic library of maize seedlings and the exon–intron structure determined (accession number AJ132240). The length of genomic DNA sequenced was about 7 kb and contained two exons with the translation start site in exon 2. The only intron is located in the non-coding 5′ region and it is 1298 bp long with the splice acceptor and donor sites conforming to the AG/GT rules. Repetitive sequence fragments are located in the 5′ and 3′ intergenic region. The accumulation of *eIF-5* mRNA was studied by RNA blot and in situ hybridization. The observed distribution of mRNA may correlate with the function of the protein, as it appears to be highly abundant in tissues where the proportion of cells actively dividing is very high, such as meristematic regions. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: Eukaryotic translation initiation factors; *eIF-5* gene; Maize; Meristem

1. Introduction

The eukaryotic translation initiation factor 5 (eIF-5) has been shown to play an essential role in the initiation of protein synthesis, in conjunction with other initiation factors and GTP (Raychaudhuri et al., 1987). Studies have shown that the eIF-5 protein interacts with the 40S initiation complex (40S · mRNA · Met–tRNA$_f$–eIF–2 · GTP) to promote quantitative hydrolysis of the ribosome-bound GTP (Das et al., 1993). Hydrolysis of GTP is essential for the release of eIF-2 and guanine nucleotide (as an eIF-2 · GDP complex) from the 40S ribosomal subunit, and the subsequent binding of the 60S ribosomal subunit to the 40S complex to form a functional 80S initiation complex (80S · mRNA · Met–tRNA$_f$) that is competent for peptidyl transfer (Chakravarti and Maitra, 1993; Chaudhuri et al., 1994).

The eIF-5 protein has a domain with the features expected for a zinc-finger (Klug and Rhodes, 1987) that is conserved in all reported sequences in species as distant as mammals, plants, yeast and nematodes (López et al., 1997). It has also been shown that the interaction of maize eIF-5 with nucleic acids in vitro is dependent on the presence of Zn^{2+} ions, suggesting a functional role for the zinc-finger structure. Following these results we proposed that the zinc-finger plays a role in the interaction of eIF-5 with 40S rRNA, with other regions of the protein possibly also taking part in the interaction (López et al., 1997).

In the present article we describe the structure, organization and expression of the *eIF-5* gene from maize (*Zea mays L.*). We have identified the DNA clone from a genomic library of whole seedlings (two leaf stage) of maize variety B73. A 6.8 kb fragment was sequenced and five regions have been distinguished in it in relation to the coding sequence. The mRNA accumulation patterns have been studied both by RNA blot and in situ hybridization, showing high abundance of the transcript in regions of the embryo and seedling that are rich in dividing cells.

Abbreviations: DAP, days after pollination; DIG, digoxigenin; EDTA, ethylenedinitrilo tetraacetic acid; eIF-2, eukaryotic translation initiation factor 2; eIF-5, eukaryotic translation initiation factor 5.

* Corresponding author. Tel.: +34-9-3400-6129;
fax: +34-9-3204-5904.
E-mail address: pprgmp@cid.csic.es (P. Puigdomènech)

0378-1119/99/$ - see front matter © 1999 Elsevier Science B.V. All rights reserved.
PII: S0378-1119(99)00438-2

2. Materials and methods

2.1. Genomic library screening and sequencing

The *eIF-5* cDNA ZF3 clone (López et al., 1997) was used to screen a genomic library prepared from whole seedlings (two leaf stage) in EMBL-3 (Clontech) from the B73 maize variety using standard methods (Ausubel et al., 1994). To determine the region of the clone corresponding to the cDNA the λ DNA was isolated using the Qiagen kit. The genomic DNA was isolated from the phage arms by digesting with *Sal*I, *Sal*I–*Sph*I and *Bam*HI. The product of the digestion was separated by size in an 0.8% agarose gel and blotted onto a nylon membrane as recommended by the manufacturer (Hybond N, Amersham). Hybridization was carried out at 65°C in 200 mM sodium phosphate (pH 7.2), 1 mM EDTA, 7% SDS, 1% bovine serum albumin, and 0.5 mg/ml sonicated salmon sperm DNA as described by Maniatis et al. (1982). The ZF3 cDNA clone was labelled with ^{32}P to a specific activity of 10^9 cpm/μg by random priming (Boehringer Mannheim). Final washes were carried out at 65°C in 20 mM sodium phosphate (pH 7.2), 1% SDS, and 1 mM EDTA. The membrane was then exposed to Kodak XAR5 film with intensifying screens (Du Pont Lighting Plus) at −70°C. The fragments hybridizing to the cDNA probe were isolated and subcloned into pBluescript SK+ vector. Different fragments were subcloned using the partial restriction map of the gene. Series of fragments of various sizes were also obtained from deletion clones obtained using an ExoIII exonuclease deletion kit (Pharmacia). Nucleotide sequences were determined using the dideoxynucleotide chain terminator method (Sanger et al., 1977) on an automated laser fluorescence (ALF) sequencing apparatus (Pharmacia).

2.2. RNA blot analysis

Tissues were collected from different embryo stages, adult root and leaf of *Z. mays* (W64). All tissues were frozen in liquid nitrogen and kept at −70°C until they were used. Total RNAs were extracted as described by Maniatis et al. (1982). Poly (A)+ RNA used for blotting was isolated using the Poly A Tract mRNA Isolation System (Promega), following the manufacturer's protocol. The *eIF-5* cDNA ZF3 full-length clone (López et al., 1997) was used as probe and labelled by the random priming method (Random Primed DNA labeling kit, Boehringer Mannheim, Germany), following the manufacturer's protocols. The northern blots were fixed, hybridized and washed as described by Church and Gilbert (1984).

2.3. In situ hybridization

Fixation and pre-treatment of the tissues were carried out according to Jackson (1991) and Langdale (1994). A 300 bp *Eco*RV fragment of the 5′ cDNA ZF3 clone was used as probe. Preparation of the riboprobes, hybridization, washes, blocking, antibody incubation and detection were performed according to the supplier's protocols (DIG High Prime Labeling and Detection Starter Kit I and Nonradioactive In Situ Hybridization Manual of Boehringer Mannheim, Germany). Digoxygenin-labelled hybrids were viewed using bright-field microscopy, and photographs were taken using Ektachrome 160 films.

3. Results

3.1. Cloning and sequence of the eIF-5 gene

A *Z. mays* genomic library was screened with an *eIF-5* cDNA probe (López et al., 1997), and the phage showing the strongest hybridization intensity was selected. In order to separate the gene insert from the phage arms, the DNA phage was digested with restriction enzymes in the phage multicloning region (*Sal*I, *Bam*HI and *Sph*I) and hybridized to the cDNA. Three fragments of 4.5 kb, 4.3 kb and 6.5 kb were subcloned in pBluescript. The first two inserts correspond respectively to the 5′ and 3′ regions upstream and downstream to the *Sal*I site of the cDNA, whereas in the 6.5 kb fragment the whole sequence corresponding to the cDNA is included.

The sequence of the whole coding region and the 5′ and 3′ adjacent regions was a total of 6831 bp of genomic region of the *eIF-5* gene. It contained a coding sequence identical to the previously described *eIF-5* cDNA (López et al., 1997), confirming that the genomic sequence was the *eIF-5* gene. The sequence has been submitted to the EMBL database with the accession number AJ132240. At both nucleotide and protein levels the coding region of the gene has 100% homology to *eIF-5* cDNA (accession number X99517).

3.2. Genomic structure and organization of the eIF-5 gene in maize

A schematic drawing of the structural organization of the *eIF-5* gene from maize is shown in Fig. 1. Also shown in Fig. 1 is the partial restriction map where the enzymes used for allowed subcloning the different fragments of the gene are indicated. The 6.8 kb sequence is organized in five well-defined regions that correspond to the percentage distribution of GC:AT of the 6831 bp of the genomic sequence. In general, all the regions are richer in AT nucleotides with the exception of the coding region, which is always richer in GC than the average. The five regions that can be distinguished are as follows.

(a) 5′ intergenic region. This region is between the nucleotide −1901 and +1 (transcriptional start point).

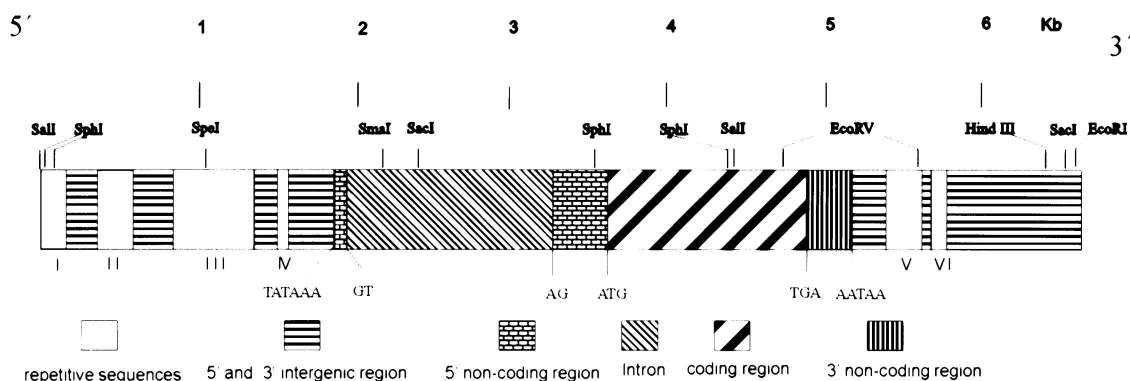

Fig. 1. Schematic drawing of the structural organization of the *eIF-5* gene from maize. Arabic numerals indicate the size in kilobases. The enzymes indicate the partial restriction map. The boxes denote the different regions of the gene. The roman numerals denote the repetitive sequences. The different consensus sequences at the start and end of the different regions of the gene are shown below.

In this region several repetitive sequences are found that have high homology with sequences of other maize genes, in particular with the *Adh* gene (75.4%), the transposon of gene *P* (70.9%), the *waxy* gene (81.7%), the transposable element *DS* (77.3%), the gene of *histoneH 2B* (75.6%), the zein gene *Zc2* (74.3%), the *PRIO* resistance gene of pea (72.0%) and the *Vspe* gene of soybean (75.5%). These similarities indicate the presence of a large number of mobile elements in the intergenic region. Only one region appears unique to this gene and it is proposed that this includes the gene promoter. The only region with no similarity to other genes is the segment of nucleotides −345 and +1, where several consensus TATA boxes are found. We propose that this region contains the promoter of the *eIF-5* gene. The percentage of AT nucleotides in the 5′ intergenic region is 63.1%.

(b) 5′ non-coding region. This region is defined by its identity to the 5′ untranslated region of the *eIF-5* cDNA (López et al., 1997). This begins at nucleotide +1 and ends at position 1642. The only intron present in this gene is found. The intron is located between nucleotides 48 and 1345 (1298 bp long) and it is flanked by the consensus sequence for plant splicing sites, GT and AG (Brown, 1986; Rogers, 1990). This region includes two short open-reading frames. The percentage of AT in this 5′ non-coding region is 58.2%.

(c) Coding region. This region includes nucleotides from position 1643 to 2995 and has an open-reading frame of 1352 nucleotides encoding a protein of 451 amino acids. The percentage of AT in the coding region is 49.8%.

(d) 3′ non-coding region. This region is defined by the length of the 3′ untranslated region of *eIF-5* cDNA. It begins at nucleotide 2999 and ends at position 3231, where the polyA tail begins in the cDNA sequence. A potential polyadenylation signal sequence is present in this region. The percentage of AT in the 3′ non-coding region is 59.6%.

(e) 3′ intergenic region. This region includes nucleotides from position 3283 to the end of the sequence. As found in the 5′ intergenic region, analysis of this region for similarities in databases indicates the presence of repetitive sequences that have high homology with regions of other genes of maize, in particular with the gene *A2* (60%), the transposon found in gene *P* (85%) and with the *Tnr* transposon of rice (60%). The percentage of AT in the 3′ intergenic region is 59%.

3.3. mRNA accumulation corresponding to the maize eIF-5 *gene*

The accumulation of the gene transcript corresponding to the insert of the *eIF-5* gene was studied by RNA blot hybridization of polyA+ RNA isolated from various maize organs, and in different stages of development. RNA blot analysis with total RNA was not possible, probably due to the low abundance of mRNA in the maize organs studied. The results for embryos of 12, 14, 16, 18 and 20 days after pollination (DAP), adult roots and leaves are shown in Fig. 2. The analysis revealed

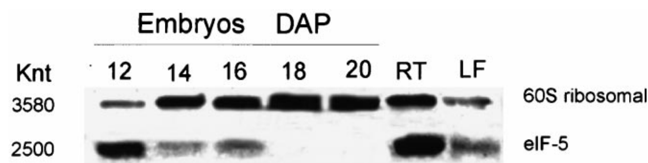

Fig. 2. RNA blot analysis of the *eIF-5* gene. RNA polyA+ was isolated from different embryos and different parts of the plant using *eIF-5* cDNA as a probe. Embryos are of 12, 14, 16, 18 and 20 DAP; RT, root tip and LF, leaves. The gene transcript corresponding to the ribosomal *60S* was used as control.

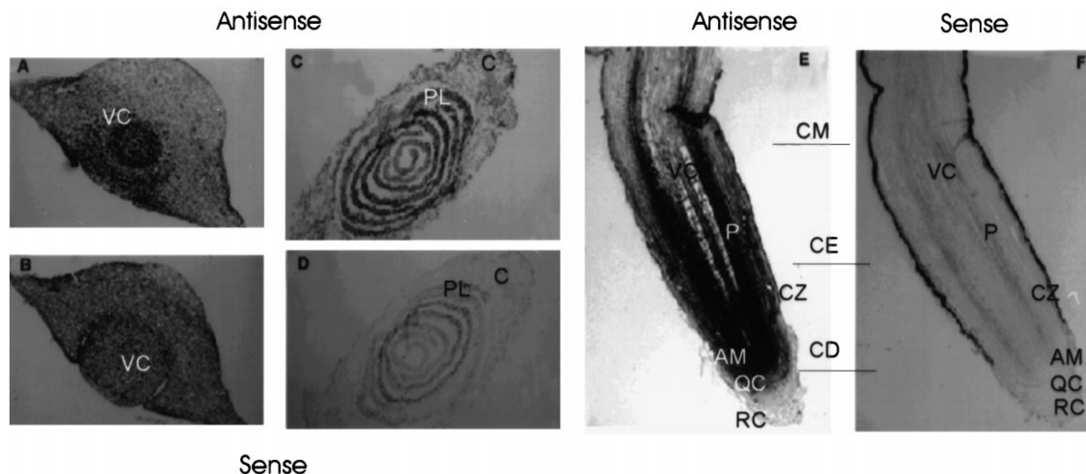

Fig. 3. In situ hybridization of *eIF-5* mRNA. Hybridization of: antisense (**A**) and sense (**B**) probes with transverse sections from embryos of 14 DAP; antisense (**C**) and sense (**D**) transverse sections of leaves; antisense (**E**) and sense (**F**) longitudinal sections of root tip. VC, vascular cylinder; C, coleoptile; PL, plumule. The different regions of the root tip are CD (cell division), CE (cell elongation), and CM (cell maturation). The situation of the root cap (RC), quiescent center (QC), apical meristem (AM), cortical zone (CZ), and pericycle (P) are shown.

that the *eIF-5* mRNA runs at approximately 2.5 knt. This value indicated a lower electrophoretic mobility than that predicted from the cDNA sequence (1.9 knt). The highest levels of accumulation are found in embryos of 12 DAP seeds and root tips (RT) with less accumulation of mRNAs in embryos of 14 and 16 DAP and in young leaves. mRNA was not accumulated in embryos of 18 and 20 DAP, indicating that the mRNA of the *eIF-5* gene starts to accumulate at the beginning of embryo development, and later decreases until it is not detectable at 18 DAP. Fig. 2 also presents the control of RNA abundance and hybridization with a probe corresponding to the *60S* ribosomal gene.

To elucidate the spatial expression pattern in specific tissues of maize, we used in situ hybridization to detect the *eIF-5* gene mRNA in tissue sections from embryos 14 DAP, seedling leaves and root tips. Analyses of transverse sections of 14 DAP embryos (Fig. 3**A** and **B**) indicate that, at this developmental stage, the *eIF-5* transcripts are present in the meristematic cells of the vascular cylinder (VC). In the transverse sections of seedling leaf tissues (Fig. 3**C** and **D**) the accumulation of mRNA in the plumule (PL) can be observed, but there is no mRNA accumulation in the coleoptile (C). Fig. 3**E** and **F** also shows longitudinal sections of the root and different regions of the root tip: cell division (CD), cell elongation (CE), and cell maturation (CM) regions. Also shown is the position of the root cap (RC), quiescent center (QC), apical meristem (AM), cortical zone (CZ), pericycle (P) and vascular cylinder (VC). It is in the apical meristem where greater accumulation of *eIF-5* mRNA appears. The level of expression decrease with distance from the root tip, but even in more differentiated regions of the root mRNA accumulation persists in the pericycle.

4. Discussion

The genomic sequence corresponding to the maize eukaryotic translation initiation factor-5, eIF-5, has been isolated and 6.8 kb of the insert sequenced. The structure of the gene has the main characteristics observed for maize genes. In particular, the regions flanking the coding sequences, both upstream and downstream of the gene, show similarity to abundant repetitive fragments having the features of mobile elements. This has been found in other maize genes, but in this case the abundance of such elements is particularly remarkable. The *Z. mays* genome consists of 60–80% repetitive DNA (Flavell et al., 1974), the majority of which is dispersed throughout the genome interspersed with unique or other repetitive sequences (Hake and Walbot, 1980). This DNA is mostly made up of retrotransposons or MITE elements (SanMiguel et al., 1996). In the case of the *eIF-5* gene the elements found are probably retrotransposon fragments. Among the sequences coding for *eIF-5* gene in the different organisms described, only one other genomic sequence, that of *Saccharomyces cerevisiae*, is available, but the flanking sequences of this gene do not contain any repetitive sequences, as is the case in maize.

The maize gene has only one intron, located in the 5′ non-coding region, and is very long (1298 bp) for a maize intron. The presence of an intron in this location is not unusual among plant genes, and maize genes in

particular. In at least one case the intron has been shown to be important for the regulation of the expression of the corresponding gene (Luehrsen and Walbot, 1991). Whether this is the case for the *eIF-5* gene is a question that the analysis of the promoter could decide in the future. Within the maize genomes, only a 5′ intergenic region, from nucleotide 1621 to 1921, which includes a TATA box, has no detectable similarity to other genomic sequences and is a good candidate for the promoter of the gene.

When the maize *eIF-5* mRNA is analyzed, a difference in size between the cDNA (1975 bp) and the size of the mRNA (2.5 knt) measured in the polyA+ RNA blot hybridization is observed. A similar situation was described in mammal (rat and human) *eIF-5* RNA blots analyzed (Kausik et al., 1996). Although in this case bands of different sizes were found, the use of alternative polyadenylation signals was proposed. In the case of maize the use of alternative polyadenylation signals or the existence of secondary structures in these mRNAs can also be proposed. The eIF-5 protein is an essential component of the translational machinery and it may be considered a constitutive gene. However, differential mRNA accumulation in different tissues of the plant can be observed. The highest level of expression of the gene is in the embryo 12 DAP and the root tip, with intermediate expression in embryos 14 and 16 DAP and young seedling leaves. Accumulation of mRNA in embryos of 18 and 20 DAP is not observed, due to lower level of expression of *eIF-5*. At this stage of embryo development they begin to prepare the dormancy phase, during which protein synthesis is reduced. It can be concluded that the *eIF-5* gene is expressed, above all, in meristematic regions where there is a high percentage of cells undergoing division. These results are confirmed by the experiments of in situ hybridization in embryos of 14 DAP, leaf and root tip. It seems reasonable to propose that in dividing cells the translational machinery has to be built, so new protein, and consequently the mRNA of genes such as *eIF-5*, essential for this function, is needed. In these types of gene a plus high expression in dividing cells can be expected, and this is the case for *eIF-5*. A similar situation was found with another maize gene coding for an important element of cellular machinery, the clathrin coat assembly protein AP17 (σ2), where a strong decrease of the accumulation of mRNA is observed in embryos of 20 DAP, with respect to younger embryos (Roca et al., 1998). In this sense, in genes coding for essential proteins of the cell function, although the protein may be at a constant level, the mRNA steady state level is observed to be higher in tissues rich in proliferating cell where the cell machinery is being built.

References

Ausubel, F.M., Brent, R., Kingston, R.E.Moore, D.D., Seidmar, J.G., Smith, J.A., Struhl, K. (Eds.), Current Protocols in Molecular Biology 1994. Wiley, New York.

Brown, J., 1986. A catalogue of splice junction and putative point sequences from plant introns. Nucl. Acids Res. 14, 9549–9559.

Chakravarti, D., Maitra, U., 1993. Eukaryotic translation initiation Factor 5 from *Saccharomyces cerevisiae*. J. Biol. Chem. 268, 10 524–10 533.

Chaudhuri, J., Das, K., Maitra, U., 1994. Purification and characterization of bacterially expressed mammalian translation initiation Factor 5 (eIF-5): demonstration that eIF-5 forms a specific complex with eIF-2. Biochemistry 33, 4794–4799.

Church, G.M., Gilbert, W., 1984. Genomic sequencing. Proc. Natl. Acad. Sci. USA 81, 1991–1995.

Das, K., Chevesich, J., Maitra, U., 1993. Molecular cloning and expression of cDNA mammalian translation initiation factor 5. Proc. Natl. Acad. Sci. USA 90, 3058–3062.

Flavell, R.B., Bennett, M.D., Smith, J.B., Smith, D.B., 1974. Genome size and the proportion of repeated nucleotide sequence DNA in plants. Biochem. Genet. 12, 257–269.

Hake, S., Walbot, V., 1980. The genome of *Zea mays*, its organization and homology to related grasses. Chromosoma 79, 251–270.

Jackson, D.P., 1991. In situ hibridization in plants. In: Bowle, D.J., Gurr, S.J., McPhereson, M. (Eds.), Molecular Plant Pathology: a Practical Approach. Oxford University Press, Oxford, pp. 163–174.

Kausik, Si., Das, K., Maitra, U., 1996. Characterization of multiple *mRNAs* that encode mammalian translation initiation Factor 5 (eIF-5). J. Biol. Chem. 271, 16 934–16 938.

Klug, A., Rhodes, D., 1987. Zinc fingers: a novel protein motif for nucleic acid recognition. Trends Biochem. Sci. 12, 464–469.

Langdale, J.A., 1994. In situ hybridization. In: Freeling, M., Walbot, V. (Eds.), The Maize Handbook. Springer, New York, pp. 165–180.

López, I., Ruíz-Avila, L., Puigdomènech, P., 1997. The eukaryotic translation initiation factor 5, eIF-5, a protein from *Zea mays*, containing a zinc-finger structure, binds nucleic acids in a zinc-dependent manner. Biochem. Biophys. Res. Commun. 236, 510–516.

Luehrsen, K.R., Walbot, V., 1991. Intron enhancement of gene expression and the splicing efficiency of introns in maize cells. Mol. Gen. Genet. 225 (1), 81–93.

Maniatis, T., Fritsch, E.F., Sambrook, J., 1982. Molecular Cloning: a Laboratory Manual. Cold Spring Harbor University Press, Cold Spring Harbor, NY.

Raychaudhuri, P., Chevesich, A., Ghosh, S., Maitra, U., 1987. Characterization of eukaryiotic initiation factor 5 from rabbit reticulocytes. J. Biol. Chem. 264, 5134–5140.

Roca, R., Stiefel, V., Puigdomènech, P., 1998. Characterization of the sequence coding for the clathrin coat assembly protein AP17 (σ2) associated with the plasma membrane from *Zea mays* and constitutive expression of its gene. Gene 208, 67–72.

Rogers, J., 1990. The role of introns in evolution. FEBS Lett. 268, 339–343.

Sanger, F., Nickler, S., Coulsen, A.R., 1977. DNA sequencing with chain-terminating inhibitors. Proc. Natl. Acad. Sci. USA 74, 5463–5467.

SanMiguel, P., Tikhonov, A., Jin, Y.-K., Motchoulskaia, N., Zakharov, D., Melake-Berhan, A., Springer, P.S., Edwards, K.J., Lee, M., Avramova, Z., Bennetzen, J.L., 1996. Nested retrotransposons in the intergenic regions of the maize genome. Science 274, 765–768.