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The *Tubα3* gene from *Zea mays*: structure and expression in dividing plant tissues

(α-Tubulin; recombinant DNA; maize; corn; genomic cloning; transcript accumulation; cell division)

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SUMMARY

A gene (*Tubα3*) coding for an α-Tub, expressed in dividing tissues, has been cloned from *Zea mays*. The deduced amino acid (aa) sequence, 450 aa long, is very similar to the other plant α-Tub (85–89% homology) so far reported, and in particular to the other two aa sequences (α1-Tub and α2-Tub) already published from the same species (93% homology). The genomic structure is also very similar, having three introns located at the same positions as in the *Tubα1* and *Tubα2* genes, one of them placed at the same position in the homologous genes from *Arabidopsis thaliana*. Nevertheless, the noncoding sequences are very different from the two other maize genomic sequences. In particular, no homology has been found either in the 5′ upstream or in the 3′-untranslated sequences. Using specific 3′ probes, it has been possible to detect the mRNA coded by this gene in many of the plant organs measured, but its highest abundance is observed in the organs rich in dividing cells, a pattern correlated with that of the histone H4-encoding gene. A cDNA clone has been identified in maize coleoptiles and sequenced, confirming the expression of the *Tubα3* in this organ. No preferential accumulation in any organ of the plant was found, in contrast with what was observed in the *Tubα1* and *Tubα2* genes already described. The *Tubα* gene family seems to consist in maize by at least two groups of homologous sequences, each one including a maximum of two or three coding units.

INTRODUCTION

Microtubules are basic elements of cytoskeleton and they take part in essential cellular functions, such as cell division, motility and transport. Consequently, their protein components are abundant and present in all eukaryotic cell types. Tubulin (Tub), a heterodimer formed by two different subunits, α and β, is the major constituent of microtubules. Tubs are coded by a number of genes that vary from species to species. In mammals, up to 20 genes coding for Tubs are detected, most of them being pseudogenes (Cleveland and Sullivan, 1985). In plants, *Tub* genes appear to be formed by much simpler families, at least in the two species already analyzed, *A. thaliana* (Silflow et al., 1987) and *Z. mays* (Montoliu et al., 1989). Both in animals and in plants, genes coding for tubulins having specific expression in defined parts of the organism or in particular developmental stages have been detected and they offer interesting systems to study gene regulation (Silflow et al., 1987; Sullivan, 1988).

A tandem repeat of *Tubα* genes has been identified in *Z. mays* (Montoliu et al., 1989). The two genes seem to be expressed and both show a preferential accumulation of their mRNA in the root system of the plant. Using a maize cDNA probe (MR19) corresponding to a segment of α-Tub sequence conserved in proteins from different species, more

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Abbreviations: *A.*, *Arabidopsis*; aa, amino acid(s); α-Tub, α-tubulin; bp, base pair(s); kb, kilobase(s) or 1000 bp; nt, nucleotide(s); SSC, 0.15 M NaCl/0.015 M Na_3·citrate pH 7; SSPE, 0.18 M NaCl/0.001 M Na_2EDTA/0.01 M Na·phosphate pH 7.7; *Tubα1*, *Tubα2*, *Tubα3*, genes encoding α1-Tub, α2-Tub and α3-Tub proteins, respectively; Z., *Zea*.

than two genes were detected by Southern blots. It was therefore of interest to try to characterize other maize *Tubα* genes and to analyze their sequence and expression. Here we present the identification in maize of a third *Tubα* gene, and a corresponding cDNA clone, which is accumulated in dividing tissues of the plant, but lacking the preferential accumulation in radicular tissues observed in the already published genes.

RESULTS AND DISCUSSION

(a) Structure and expression of the *Tubα3* gene

Two genes (*Tubα1* and *Tubα2*) coding for α-Tub and placed in tandem, within 9 kb, have been identified in the maize genome (Montoliu et al., 1989). These two genes are expressed at different levels in organs rich in dividing tissues and they show a preferential accumulation in the radicular system of the plant. When a cDNA probe (MR19a; Montoliu et al., 1989), that corresponds to the central and most conserved part of the protein (and is 95% homologous between the two genes), was used in Southern blots, the bands corresponding to the two cloned genes were observed, but a limited number of other genomic bands were also found. It was of interest to try to find other *Tubα* genes in the maize genome that might show different patterns of expression. To this end, a λCh35 genomic library of *Z. mays* L. (inbred line W64A) (Gallardo et al., 1988) was further screened searching for other genes that code for similar sequences.

A number of clones hybridizing with a maize cDNA probe, MR19a, were detected. One of those (MG19/15 clone) had a restriction map different from the other genomic clones already analyzed (clones MG19/14 and MG19/6; Montoliu et al., 1989). The gene has been termed *Tubα3*. In parallel, an incomplete cDNA (MC19/1 clone) was found in a pBR322 library constructed from maize coleoptiles (Stiefel et al., 1988). This cDNA sequence was found to be identical to the *Tubα3* nt sequence, excluding introns (Fig. 1). No evidence for the presence of another gene in the vicinity of *Tubα3* has been found at similar distances to those existing between *Tubα1* and *Tubα2*. These genes are separated by 1452 bp between the stop

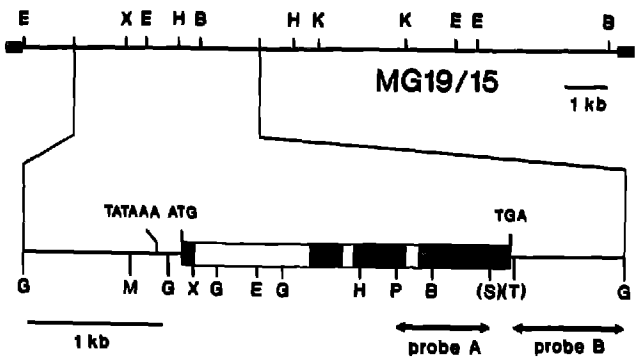

Fig. 1. Restriction map of maize genomic clone MG19/15 showing the coding unit for the *Tubα3* gene. Open boxes represent introns and black ones the translated segments of the gene. The positions of putative TATA box, start and stop codons are indicated. The *Bgl*II genomic fragment shown below corresponds to the sequence presented in Fig. 3. The 3'-untranslated probe for the *Tubα3* (probe B), as well as the one having the most conserved part of the α3-Tub (probe A), are indicated by two-headed arrows. B, *Bam*HI; E, *Eco*RI; G, *Bgl*II; H, *Hin*dIII; K, *Kpn*I; M, *Sma*I; P, *Pst*I; S, *Bss*HII; T, *Taq*I and X, *Xho*I. The sites in parentheses, used for subcloning the probes, are not unique in the genomic clone.

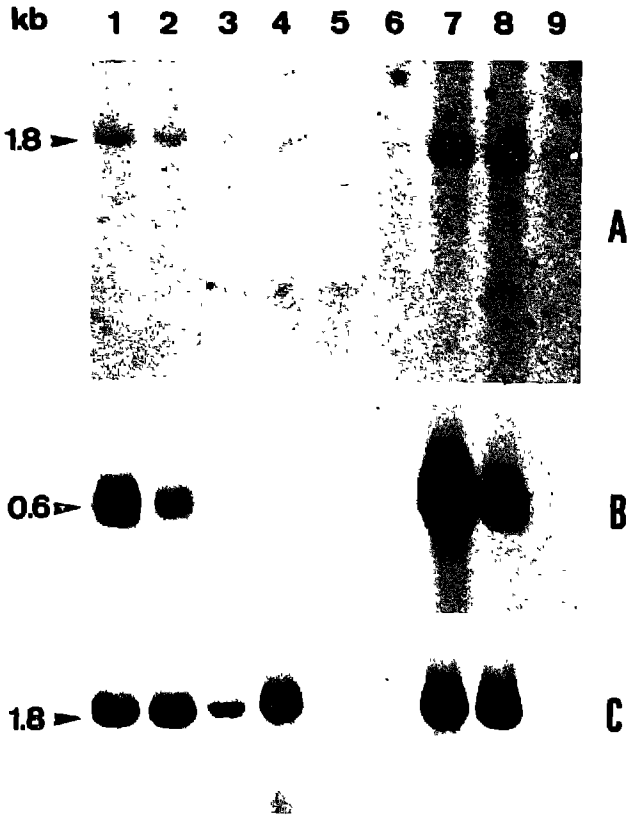

Fig. 2. Northern-blot analysis of RNA extracted (Logeman et al., 1987) from different parts of *Z. mays* plants. Plantlets (three days old) of *Z. mays* L. (inbred line W64A) were obtained upon germination of dry seeds by imbibition in water at 25°C in the dark. Total RNA (10 μg) from coleoptiles (1) and roots (2) from 3-day-old plantlets, isolated anthers (3) and flowers (4) (anthers, stamens and peduncles) from 120-day-old plants, roots (5) and leaves (6) from 2-month-old plants, maize embryos from 12 (7), 20 (8) and 50 (9) days after pollination were fractionated in a 1.5% agarose–2.2 M formaldehyde gel and blotted onto nylon membrane (Hybond, Amersham). The estimated length of the transcripts is indicated in kb. In panel A, we used probe B (3'-specific for *Tubα3*; see Fig. 1), in panel B, we used a maize histone *H4* probe (Philipps et al., 1986), in panel C we used the probe corresponding to the 3' end of maize *Tubα1* (Montoliu et al., 1989). In all three cases the probes were labelled by random priming to a 10^9 cpm/μg specific activity. The same filter (nylon membrane; Hybond, Amersham) was used with the three probes to obtain comparable results. The exposure times for each hybridization were (A) twelve days, (B) four days and (C) seven days with intensifying screens at −80°C.

codon of the α2-Tub and the first codon of the α1-Tub (Montoliu et al., 1989).

In the case of *Tubα1* and *Tubα2* it was possible to distinguish the accumulation of specific genes by using 3′ sequences. With a 836-bp probe, corresponding to the untranslated 3′-end, in the case of the *Tubα3* (probe B, Fig. 1), the accumulation of specific mRNA sequences corresponding to this gene was studied (Fig. 2). The overall level of accumulation of *Tubα3* (Fig. 2A) appears to be lower than that of *Tubα1* by a factor of 10 (Fig. 2C). The Northern blot also indicates that the transcript of the *Tubα3* is mainly accumulated in zones rich in dividing and young tissues, in a way very similar to histone H4-encoding mRNA (Fig. 2B). When the result is compared to that obtained with the *Tubα1* probe, a similar pattern of accumulation is found, except that the preferential pattern in radicular tissues, that is typical of *Tubα1* and *Tubα2*, is not found in the case of *Tubα3*. Moreover, an accumulation of the *Tubα1* in anthers and flowers from 120-day-old plants (lanes 3 and 4 in Fig. 2C), not previously studied, was detected. The RNA corresponding to both *Tubα3* and histone H4-encoding genes shows only a weak signal in these organs. In *A. thaliana* the existence of a gene specifically expressed in flowers has been reported (Ludwig et al., 1988). This pattern of mRNA accumulation prompted us to further characterize the *Tubα3* gene.

(b) Protein and genomic structure of *Tubα3*

Sequencing of the MG19/15 genomic and MG19/1 cDNA clones, in the region hybridizing with the *Tubα* probes and its surrounding zone, was carried out (Fig. 3). The α3-Tub sequence (450 aa long) was deduced assuming that the gene coded for a protein with high homology to the known α-Tubs. In fact, a 93% homology was found when comparing α3-Tub with α1-Tub (92% with α2-Tub) from maize. The differences in sequence are concentrated in defined regions of the protein and, in particular, in the hypervariable acidic C terminus. The homology with the two α-Tub sequences reported from *A. thaliana* is also high: 85% with α1-Tub (Ludwig et al., 1988) and 88% with α3-Tub (Ludwig et al., 1987). The homology of the maize α3-Tub with the algal α-Tubs (*Chlamydomonas reinhardtii* or *Volvox carteri*) is 89% (Silflow et al., 1985; Mages et al., 1988). A typical mammal α-Tub sequence, such as the isotype K1 from man, showed the lowest (but still high) homology (80%) (Cowan et al., 1983). The homology of the nt sequence, within the coding regions, of *Tubα3* (1350 bp) compared with those from maize *Tubα1* or *Tubα2* was 85%, in accordance with the high similarity observed in the aa sequences.

To obtain an α-Tub sequence from the clone MG19/15 the presence of three introns has to be supposed. These are found at the same positions as those of *Tubα1* and *Tubα2*. The length of the introns is 846, 87 and 98 bp, respectively. In all cases, the 5′ and 3′ ends share homology with the consensus splicing junctions. It is interesting to note that the second intron is placed in the same position as the corresponding intron of *Tubα* genes from *A. thaliana* (Silflow et al., 1988). When the nt sequences of the introns, the 3′-untranslated zone or the flanking 5′ or 3′ ends of the *Tubα3* were compared with both *Tubα1* and *Tubα2*, no significant homology was found.

The 974-nt cDNA sequence (MC19/1) is identical to genomic sequence from nt positions 2747–3721 of MG19/15 genomic clone (except the third intron), where the poly(A) tail is found. There is no clear polyadenylation signal between the stop codon and the observed poly(A) site. Nevertheless, a TGTAA sequence, located 22 bp upstream from the end of the cDNA clone, which has been cited to represent the putative polyadenylation signals of the *Tubα* genes from *V. carteri* (Mages et al., 1988) and *C. reinhardtii* (Silflow et al., 1985), is found and could act as the polyadenylation signal in this case (Fig. 3, boxed). Other presumptive polyadenylation signals that depart in only one bp from the consensus AATAAA, located at 218, 225 and 328 bp downstream from the TGA stop codon, in the MG19/15 genomic sequence, are present (Fig. 3) Heterogeneity at the 3′ end of the genes is not rare and, for instance, the maize *Tubα1* gene has two polyadenylation signals which seem to be active (unpublished results).

The *Tubα3* gene has a consensus TATA box at −148 bp from the ATG start codon, a distance similar to that found for *Tubα1* and *Tubα2* (−149 and −152 bp, respectively). Upstream from the TATA box, at −191 bp from the ATG, a nonamer sequence: CGCGCCATC (marked by asterisks in Fig. 3), sharing high similarity with the 'histone box' (CGCGGATC), and placed at the same position in the reported histone-encoding genes from maize and wheat (Chaubet et al., 1986) is found. This fact could be correlated to the similar accumulation of the mRNA from the two genes in tissues rich in dividing cells (Fig. 2). In addition, the expression of both *Tubα* and histone H4-encoding genes is also regulated during cell cycle in animals (Cleveland and Sullivan, 1985). The major peak of transcription for these two genes occurs, simultaneously, in late G2 phase, as it has been demonstrated in *Physarum polycephalum* (Carrino and Laffter, 1986).

The *Tubα3*-specific probe (probe B, Fig. 1) was used in Southern blots to assess whether other DNA bands could be detected with it (Fig. 4A). The bands showing the more intense signal correspond with fragments present in the genomic clone, MG19/15 (Fig. 1): 8.4 kb in *Sac*I lane, 6.9 kb in *Eco*RI lane, 2.5 kb in *Hin*dIII lane and 9.8 kb in *Bam*HI lane. These bands were also detected when using a probe corresponding to the conserved part of the α1-Tub sequence (Montoliu et al., 1989). However, other less

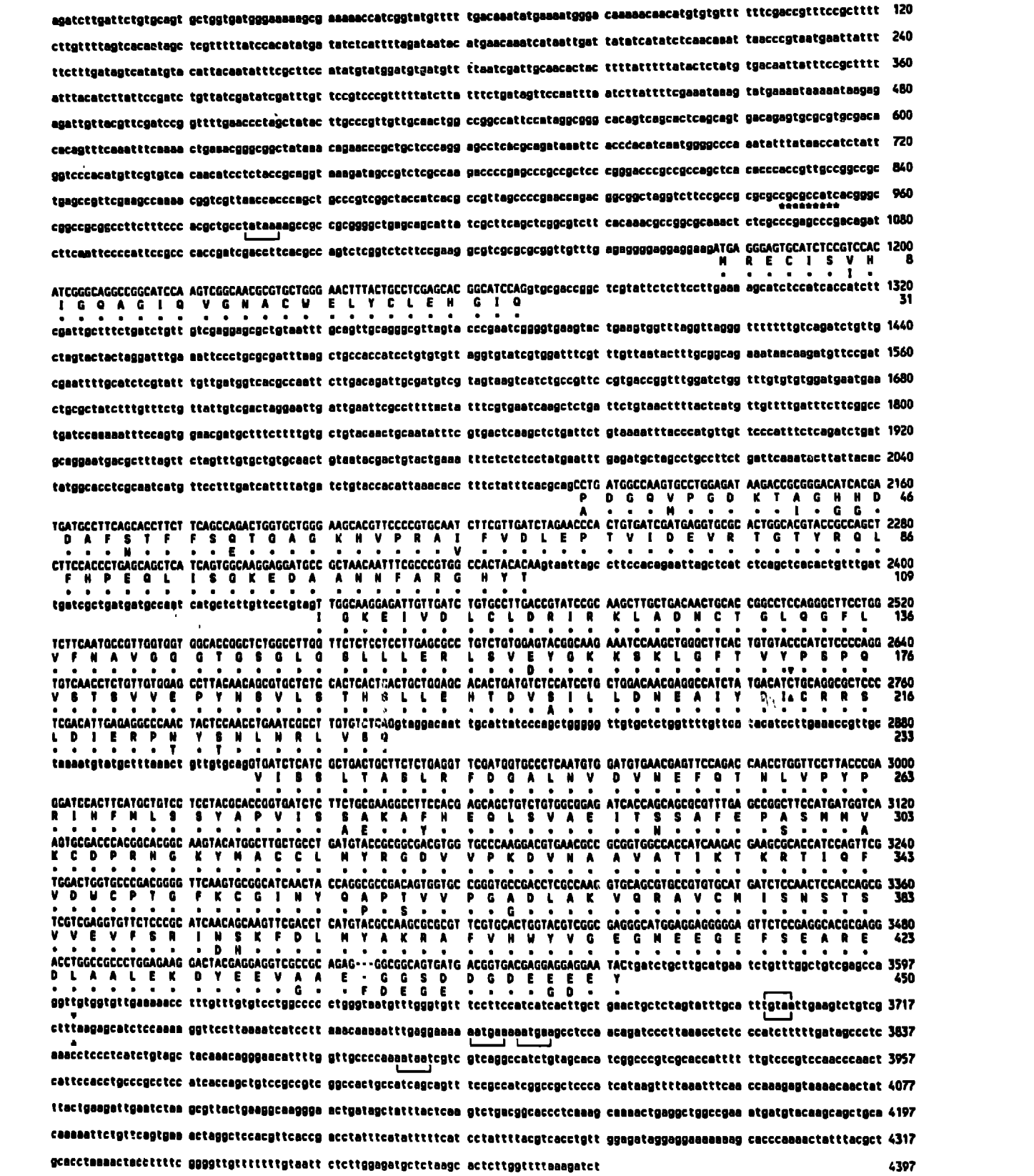

Fig. 3. Nucleotide sequence of the *Tubα3* of *Z. mays* (4397 nt long) corresponding to the *Bgl*II fragment of the MG19/15 genomic clone shown in Fig. 1. The nt sequence was obtained by the dideoxy method (Sanger et al., 1977) as described by Montoliu et al. (1989). Sequencing of pUC18 clones, using 7-deaza-dGTP (T7-deaza kit, Pharmacia), was also carried out, when necessary, to reduce compression (Mizusawa et al., 1986). The deduced coding regions are presented in upper-case letters. Putative introns and flanking sequences appear in lower-case letters. The sequence of MC19/1 cDNA clone (974 nt long) extends from nt 2747–3721, excluding intron 3, indicated by triangles. The deduced aa sequence (450 aa long) of the α3-Tub (single-letter

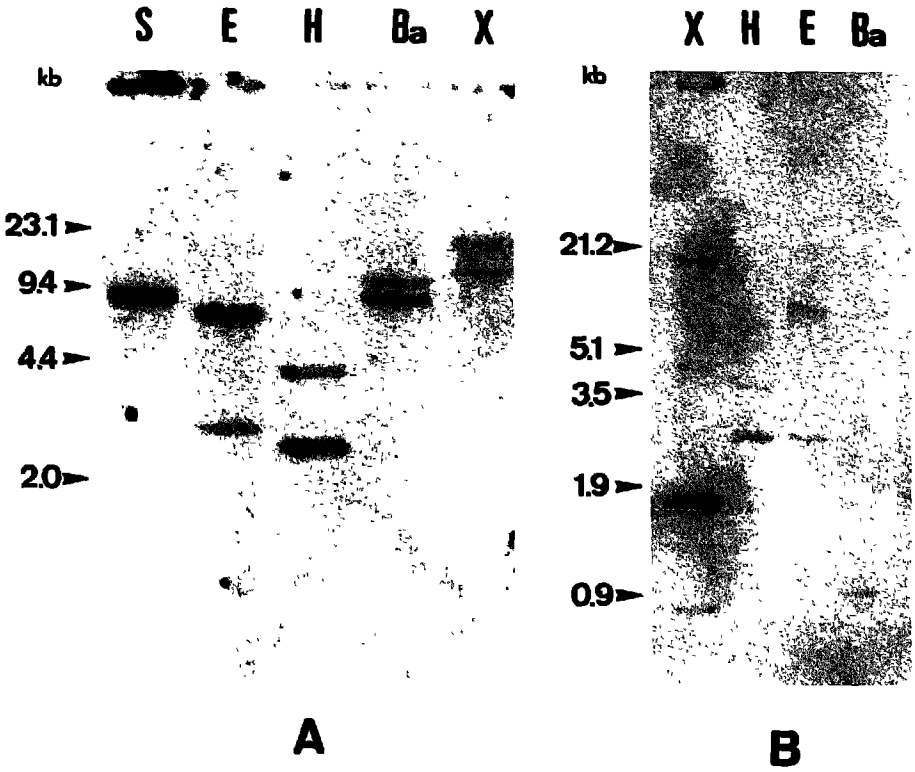

Fig. 4. Southern analysis of the *Tubα3*-tubulin genomic sequences. Southern blots (Zeta-Probe, BioRad) were prepared from *Z. mays* (inbred line W64A) genomic DNA, extracted from 6-day-old coleoptiles (panel **A**) or roots (panel **B**) following procedures of Dellaporta et al. (1983), were digested with *Bam*HI (Ba), *Eco*RI (E), *Hin*dIII (H), *Sac*I (S) and *Xho*I (X). The products of digestion were fractionated by electrophoresis in 0.8% agarose gels. Some of the bands of · phage DNA M_r markers digested with *Hin*dIII (A) or *Hin*dIII + *Eco*RI (B) are indicated on the left side of each blot. An estimation of the DNA fragment length from agarose gels was obtained by using described algorithms (Schaffer and Sederoff, 1981). In panel A, the 3'-specific random-primed (10^9 cpm/μg) probe for the *Tubα3* (probe B, Fig. 1) has been used with the standard conditions of hybridization (Montoliu et al., 1989). In panel B, a random-primed (10^9 cpm/μg) probe corresponding to the conserved part of tubulin sequences in the *Tubα3* (probe A, Fig. 1) has been used with lower stringency conditions (hybridizations were carried out at 60°C and 2 × SSPE (Tm-35) and final washes were at 65°C in 2 × SSC) to detect sequences homologous to *Tubα* in the genome of *Z. mays*. The exposure time was eight days in panel A and ten days in panel B with intensifying screens at −80°C.

intense bands that cannot be explained by the structure of the MG19/15 clone, are present. These bands cannot be interpreted as belonging to neither *Tubα1*, *Tubα2* or *Tubα3* but they do not appear when a shorter 3'-*Tubα3* probe (220 bp long, downstream from the *Taq*I site of probe B, Fig. 1) corresponding, approximately, to its noncoding but transcribed 3' end is used (result not shown). When the hybridization is carried out using a 672-bp probe corresponding to the most conserved part of the α3-Tub (probe A, Fig. 1), and using a lower stringency, new bands appear (Fig. 4B). They correspond in some cases to bands present also in Southern blots using probes specific for the *Tubα1*, *Tubα2* and *Tubα3*. For instance, the 1-kb band in *Bam*HI lane, as well as the 7.7-kb band in *Eco*RI lane correspond to the *Tubα2*. The three bands present in the *Hin*dIII lane (6.6, 3.7, and 2.5 kb) can be easily correlated with the known sequences of *Tubα1*, *Tubα2* and *Tubα3*, respectively. In the *Xho*I lane, a typical 0.9-kb fragment present in both *Tubα1* and *Tubα2* clearly appears. In conclusion, the *Tubα* family from maize seems to consist of at least two groups of homologous genes; one of these groups includes the tandem of *Tubα1* and *Tubα2*. The other group includes *Tubα3* with possibly another gene still uncharacterized not in tandem with *Tubα3*, at least within 10 kb.

(c) Comparison of mRNA accumulation between *Tubα* genes in maize

The different levels of mRNA accumulation of the three maize *Tubα* genes were compared, in a quantitative way, using RNA slot-blot analysis (Fig. 5). Only the results with

◀ code) is shown below the corresponding nt triplets and compared with the α1-Tub (Montoliu et al., 1989), shown below only for the variant aa. A presumptive polyadenylation signal (see Results and Discussion, section b) is boxed. The putative TATA box and other putative polyadenylation signals are displayed with horizontal brackets. A short sequence (9 bp long), starting at −191 from the ATG codon, very similar to the reported 'histone box' in the promoter region of maize and wheat histone-encoding genes (Chaubet et al., 1986) is underlined with asterisks. EMBL/GenBank/DDBJ Nucleotide Sequence Databases accession number is M36582.

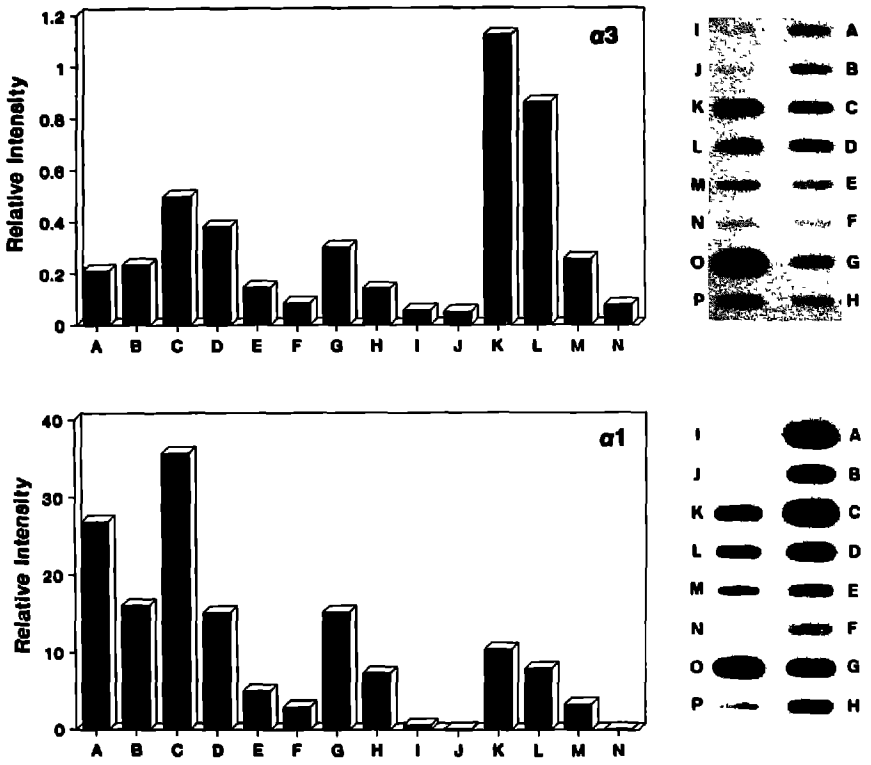

Fig. 5. RNA-slot blot analysis of transcript accumulation of *Tubα3* and *Tubα1* in different organs of *Z. mays*. Three equivalent filters (nylon membrane Zeta-Probe, BioRad) were prepared using the BIO-DOT-SF (BioRad) apparatus with the same amount (10 μg) of total RNA, in each slot. The RNAs were extracted, as described in Fig. 2, from: (A) roots and (B) 3-day-old coleoptiles; (C), (D) and (E) roots from 6-day-old plantlets were manually dissected in three zones, rich in dividing, elongating and maturing cells, respectively, as described (Ludevid et al., 1990); (F), (G) and (H) 6-day-old coleoptiles were fractionated in three parts: shoot, node and plumule, respectively, as reported (Stiefel et al., 1988); (I) roots and (J) leaves from 2-month-old plants; (K), (L), (M) and (N) maize embryos 14, 16, 20 and 50 days after pollination, respectively. Finally, two more slots (O) and (P) containing 100 and 10 pg of the probe were used, in each case, as a reference value. The same amount of DNA (50 ng) was used to obtain random-primed probes in each case. Hybridizations were performed with the same amount of label (5×10^7 cpm) and volume of solution (15 ml). Conditions for hybridization were as described by Khandjian (1986) except that dextran sulfate was omitted. Final washes were in 0.1 × SSC at 50°C twice for 15 min. The relative intensities, referred to the intensity of the signal of the 10-pg slot (upon densitometer scanning), are plotted with bars. The measurement was carried out after exposure of the filters during 18 h for *Tubα1* and 50 h for the *Tubα3*, respectively.

Tubα3-specific probe (probe B, Fig. 1) and *Tubα1*-specific probe (MR19a; Montoliu et al., 1989) are shown. The histograms represent the values of the densitometric analysis of the autoradiographed slots related to the intensity of 10 pg of the corresponding probe in each case. Parallel experiments were carried out with *Tubα2* that showed a similar pattern of mRNA accumulation to *Tubα1*, but with an overall level up to 100-fold lower, depending on the organ compared. Incubation of different amounts of the three specific probes, used under the same stringency conditions as the slot-blot analysis, showed no detectable cross-hybridization between them and with the carrier plasmid (data not shown). The results obtained in this experiment indicate that the general level of *Tubα3* mRNA, in the organs studied, is approx. ten- to 100-fold lower than that of *Tubα1*. The accumulation of *Tubα3* mRNA is around 100-fold lower than that of *Tubα1* in the case of 3- or 6-day-old roots and coleoptiles (Fig. 5, lanes A–H), whereas their respective relationships during maize embryogenesis and adult plants (Fig. 5, lanes I–N) show a factor of 10. *Tubα3* is highly accumulated in young embryos with a pattern correlating with cell division, i.e., decreasing upon aging. This is also the case when different parts of the young root and coleoptiles, having different amounts of meristematic and nonmeristematic tissues, are analyzed. A basal level of mRNA is found in adult organs, as roots and leaves. The preferential accumulation in the radicular systems, observed for *Tubα1*, is not found in this case.

(d) Conclusions

(1) A gene, *Tubα3*, encoding a protein having a high homology with α-Tub has been identified in maize. The structure of the protein and the gene shares a high degree of homology with the two other genes encoding the same protein already characterized in maize (Montoliu et al., 1989).

(2) The *Tubα3* has an overall level of mRNA accumulation between ten- and 100-fold lower, depending on the

organ, than the one observed in *Tubα1*. Its mRNA accumulates in organs rich in dividing cells, a fact that correlates with the homology observed in the 5'-flanking region with histone-encoding gene boxes. It does not show a preferential mRNA accumulation in any of the analyzed tissues, having the highest mRNA level in young embryos.

(3) The *Tubα* family of *Z. mays* consists of at least two groups of genes. Each one would have a maximum of two or three members. One of the groups would include the *Tubα1-Tubα2* tandem and the other one the *Tubα3*.

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