cDNA nucleotide sequence and expression of a tobacco cytoplasmic ribosomal protein L2 gene

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ABSTRACT

The ribosomal protein L2 is an essential component of the ribosomal large subunit by its relation to the peptidyl transferase reaction, subunit association and elongation factor G-GTP binding. We have isolated a 937 nucleotide long cDNA encoding a cytoplasmic ribosomal L2 protein. Its deduced protein contains 260 amino acid residues and shows 65% identity with eucaryotic RL2 but only 32% identity with the chloroplast homologue. In addition, the protein presents the PROSITE signature which matches all the 50S and 60S L2 proteins and the two residues involved in the peptidyl transferase activity. The corresponding mRNA is accumulated in young plant tissues, in growing cell suspension and in germinating seeds but is not detectable in mature plant tissues, stationary cell suspension and in dry seeds. The mRNA accumulation is correlated with the growth process. Southern blot hybridization shows that cytoplasmic ribosomal protein L2 is encoded by two types of gene which could originate from each parent. Highly homologous L2 genes were also detected by Southern blots in the genomes of several monocot and dicot plant species.

INTRODUCTION

Protein biosynthesis is a complex process that takes place on the ribosomes. These are composed of a large (L) and a small (S) subunit, whose components, RNAs (r-RNAs) and proteins (rp), have been isolated and characterized by chemical, physical and immunological methods, mainly in prokaryotes, such as *Escherichia coli* (1), allowing the definition of prokaryotic ribosome architecture (2).

In plants, three kinds of ribosomes are found: the chloroplastic and mitochondrial ribosomes which are very similar to those of bacteria and are formed of 50S and 30S subunits. In contrast, the cytosolic ribosomes present in all eukaryotes are formed by two larger subunits, the 60S and 40S. Nevertheless, sequence homologies in r-RNAs and r-proteins have been found between prokaryotic and eucaryotic components.

The L2 ribosomal protein is known to be a component of the 50S ribosomal subunit and is the best studied r-protein of *Escherichia coli* and *Bacillus stearothermophilus*. Experimental evidence suggests that protein L2 plays a key role in the function of the bacterial ribosome (3,4). L2 protein binds to 23S RNA

(5–7), more precisely to the central region of the RNA chain (8,9). It is one of the eight ribosomal proteins which are involved in the binding of 5S RNA to 23S RNA (10). It is also involved in the peptidyltransferase activity of the ribosome (3,11) and amino acid residues involved in this activity have been identified (12).

Ribosomal L2 protein has been characterized at the nucleotide and amino acids levels in prokaryotes: archbacteria (15,43), bacteria (12,13,14,16,17) and cyanelle (18). Proteins homologous to L2 have been identified in plant chloroplasts (20,21,22,23,24,25,26,27) and one in microbic mitochondrion (37). In addition, cytoplasmic eucaryotic L2 proteins are present in yeast (38,39) and slime mold (19). But no nuclear encoded L2 protein has yet been isolated in higher plants.

In this paper we present the nucleotide sequence of a nearly full-length cDNA which encodes a tobacco L2 cytoplasmic ribosomal protein. It is the first identification of a cytoplasmic ribosomal L2 protein in higher eukaryotes. The cDNA was used as a probe for studying the expression of the corresponding gene in different organs of tobacco. Analysis by Southern blot showed that the cytoplasmic ribosomal protein L2 is encoded by two types of gene, probably originating from each parent. The L2 probe hybridizes with genes present in the genome of different plant species: Solanaceae and Cruciferae in dicots and Gramineae in monocots.

MATERIALS AND METHODS

Materials

Nicotiana tabacum cell suspensions were derived from pith by Jouanneau et al. (36). They were subcultured every week by 10× dilution. Three day-old cultures contain dividing cells while 11 day-old cultures consist of non-dividing cells.

Tobacco plants were grown in a greenhouse under controlled conditions. Mature tissues were collected from 3 month-old plants: fully expanded leaves, roots, stem and cortex stele. Young tissues were collected from 2 week-old plants: developing leaves and roots. Young flower buds (one cm long) were collected. Developing seeds were taken from green fruits. Dry seeds were placed on nutritional medium and germinating seeds were collected 5 days later.

Nicotiana tabacum cell suspension cDNA library

Total RNA was extracted from frozen 7 day-old *Nicotiana tabacum* suspension cells as previously described (28). Poly(A⁺)

RNA was purified by chromatography on a oligo(dT) cellulose column. Double-stranded cDNAs were prepared using Amersham cDNA Synthesis System Plus kit, and were ligated into the vector pTZ18R. *Escherichia coli* DH5- α cells were transformed with the library by the method of Hanahan (30).

Differential screening

The pTZ18R-cDNA library was plated at a low colony density and screened by differential hybridization. Duplicate Gene-Screen nylon-membrane (NEN) replicas were hybridized with 32 P-labeled single-stranded cDNAs synthesized from Poly(A⁺) RNA isolated either from cell suspension in growth phase (3 day-old), stationary phase (11 day-old), or leaves. Hybridizations were performed according to the instructions from the manufacturer. Selected cDNA clones were isolated and conserved in 50% glycerol.

cDNA probes

The L2 double-stranded cDNA probe was obtained by digestion of the corresponding plasmid with the appropriate restriction enzymes and the 937 bp insert separated from the vector on an agarose gel. Recovery and elution of the insert were carried out using Schleicher and Schuell NA 45 paper according to the instructions of Maniatis (29). A 319 bp fragment in the coding region of the cDNA was recovered by BamHI and HindIII digestions (underlined in Fig. 1). This fragment, with a higher GC content, is used for the Southern blot. The probes were labeled by the multiprimer technique (Amersham Kit and procedures). They are noted 'L2 cDNA' for the entire probe and '319 bp fragment' for the BamHI-HindIII fragment.

The *Arabidopsis* histone H4 clone-A748-was given by Dr Claude Gigot (41). It contains 196 bp fragment in the H4 coding region, which was excised and labeled by the same multiprimer technique.

Northern-blot analysis

Total RNAs were extracted from different tissues by previously described methods (28). RNAs from radish was given to us by M. RAYNAL. The same quantity of purified RNA (10 μ g) was denatured and fractionated on denaturing gels as described by Meinkoth and Wahl (31). RNAs were transferred onto Gene-Screen membranes and prehybridized for 2 hours at 42°C with the following solution: 50% deionized formamide, 2 $\times$ Denhardt, 5 $\times$ SSC, 1% SDS, 100 μ g/ml tRNA yeast, 100 μ g/ml polyU. The membrane was hybridized in the same solution with the denatured probe at 42°C for 20 hours. The membrane was washed twice with 2 $\times$ SSC at room temperature for 10 mn, and twice at 70°C with 2 $\times$ SSC, 1% SDS for 20 mn. A stringent wash was made at 70°C with 0.5 $\times$ SSC, 1% SDS during 10 mn. Hybridization to heterologous RNAs was made at 37°C and without the stringent wash.

Southern-blot analysis

DNA was extracted from different plant species as previously described, using a CsCl-ethidium bromide centrifugation protocol (46). After digestion with appropriate restriction endonucleases and analysis on a 1% agarose gel, DNA fragments were treated and transferred onto Gene-Screen membranes under the conditions indicated by the manufacturer. Hybridization and washing conditions were the same as for Northern blots. Hybridization to heterologous DNA was at 37°C without the stringent wash.

Nucleotide sequences

Nucleotide sequences were determined by the dideoxynucleotide chain termination method (32) with double-stranded DNA templates using the Amersham Multiwell system as indicated by the manufacturer.

RESULTS

Selection of growth-related genes by differential screening

Genes which are preferentially expressed during the growth process were screened by differential colony hybridization to a pTZ18R-cDNA library. Clones that gave high hybridization signals with 32 P-labeled cDNA from suspension cells in the growth phase (3 day-old) and low signals with cDNA prepared from suspension cells in stationary phase (11 day-old) were selected.

These clones were rescreened by Southern hybridization: the cDNA inserts of selected clones were separated from the vector on an agarose gel and transferred to a membrane. They were successively screened with 32 P-labeled cDNA from suspension cells in the growth phase and cells in stationary phase. From this screening, eight clones were selected corresponding to genes

Figure 1. Nucleotide sequence of the tobacco 60S RL2 cDNA and the deduced amino acid sequence of the ORF. The BamHI and HindIII restriction sites are underlined.

which are strongly expressed in cell suspension in growth phase and at a lower level in stationary phase. One of these cDNAs is analysed in this paper.

Characterization of *Nicotiana tabacum* cytoplasmic L2 cDNA and the corresponding protein

The complete nucleotide sequence of a 937 bp cDNA insert was established (fig.1). The translation of this sequence shows that it contains an open reading frame corresponding to a protein of 260 amino acids. The 5' flanking region is only 7 nucleotides. The 3' untranslated region is 128 nucleotides and is followed by a 22 nucleotide polyA tract. There is no obvious polyadenylation signal upstream from this sequence. The predicted protein has 260 residues corresponding to a molecular mass of 28151 daltons. The large number of basic amino acids, 22.3%, compared with only 6.9% acidic residues indicates a predominantly basic character. It contains high levels of glycine (35 residues), arginine (26 residues), valine (22 residues), and lysine (19 residues).

A FASTA (33) search of the EMBL database shows that the nucleotide sequence has 66% identity over 750–800 nucleotides with three eucaryotic 60S cytoplasmic ribosomal protein genes—SPRKD4 and SPRPK37 of *Schizosaccharomyces pombe* and DDRPL2 of *Dictyostelium discoideum*. In addition, homologies over shorter stretches of the deduced proteins are detected with all archebacterial (15,43), eubacterial (12,14,16,18), chloroplastic (20,21,23,25,26,27) and mitochondrial (42) 50S RL2 ribosomal proteins so far published, confirming that the cDNA we have isolated encodes a eucaryotic 60S ribosomal L2 protein. Nevertheless, in spite of the high homologies between our cDNA and the three eucaryotic RL2 nucleotide sequences, the *Dictyostelium* sequence DDRPL2 presents a base deletion equivalent to position 635 of the tobacco

	METHIONINE	HISTIDINE	
	200	*	220
EUKARYOTES			240
TABACCO	NCMPKVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RLD4SSCHPO	NCMPKVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SDICDI	NNMPKVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SDICDI*	NNMPKVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RLK7SSCHPO	NCMPKVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
ARK7SSCHPO*	NCMPKVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
ARCHEBACTERIA			
RL2SMETVA	VANPKVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SHALMA	TKMNVKVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
PROKARYOTES			
RL2SECOLI	GVRETVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SEYRFS	GIRETVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SBACST	GIRETVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SMYCCA	GIRETVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SCYAPA	GIRETVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SEUGGR	GNKPTVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
CHLOROPLASTS			
RL2SMARPO	GKRETVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2STOBAC	GKRETVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SMAIZE	GKRETVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SORYSA	GKRETVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SBOYBN	GKRETVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SNICDE	---FVTVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SNICDE*	---FVTVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SSPIOL	---FVTVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
RL2SSPIOL*	---FVTVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
MITOCHONDRION			
RL2SPARTE	GTMPKVRGVAMNPDHPPHGGGN . HQHGHASTVARDAPPQQRVGLIAGRRGTGLRGQAATAAKADKA		
PROSITE	P--RGSS--NA-D		
	TT P E		
	AA		
	VV		

Figure 2. Alignment in the conserved region of all published RL2 protein sequences. The sequences noted with a star are obtained after filling the putative base deletion with the corresponding tobacco nucleotide. The PROSITE-like signature is shown on the last line.

sequence and the yeast sequence SPRPK37 shows two base deletions corresponding to positions 634 and 674 which are located within the ORF and result in a frame shift. As a consequence of these deletions, the high homologies between the nucleotide sequences are no longer present in the deduced protein sequences (Fig 2).

Expression of the tobacco cytoplasmic L2 gene

Expression in 'in vitro' cultivated cells. According to the differential screening, the transcription of the cytoplasmic L2 gene is higher in cell suspension during the exponential phase than during the stationary phase. Ten μ g of total RNA from growth and stationary phase cell suspension were separated on agarose gel and blot-hybridized to the L2 cDNA probe. The corresponding mRNA, approximately 1.2 kb, accumulates abundantly in growing cells but is present at a low level in mature cells (Fig. 3, first two lanes).

Expression in tobacco tissues and organs. Total RNA was extracted from tissues and organs of adult and young tobacco plants. Seeds at different stages during formation, dessication and

Figure 3. Detection of tobacco cytoplasmic L2 mRNA in *in vitro* tobacco cultivated cells and in different tissues. Ten μ g of total RNAs extracted from cell suspension in growth phase or in stationary phase (two first lanes), from different tissues and organs of adult and young tobacco plants were separated on an agarose-formaldehyde gel electrophoresis and transferred onto nylon membranes. The 32 P-labeled tobacco L2 cDNA and Arabidopsis histone H4 cDNA fragment probes were successively hybridized to the Northern blot.

Figure 4. Detection of radish cytoplasmic L2 mRNA in seeds. Ten μ g of total RNAs extracted from seeds at different developmental stages were analysed on an agarose-formaldehyde gel electrophoresis and transferred onto nylon membranes. The 32 P-labeled tobacco L2 cDNA and Arabidopsis histone H4 cDNA fragment probes were successively hybridized to the Northern blot.

germination, were also collected. The tissues were chosen as a function of their developmental state: on the one hand, tissues which contain dividing cells such as young leaves, flower buds, young roots, germinating and developing seeds, and on the other hand, tissues with no cell division such as adult roots, adult leaves, stem stele, stem cortex and dry seeds.

After electrophoresis and transfer, the corresponding Northern blot was first hybridized with an *Arabidopsis* H4 histone probe used as a control to indicate the tissues in division, then hybridized with the tobacco L2 cDNA probe. As expected, H4 histone transcripts are more abundant in young tissues, in which cell division is known to occur, than in fully expanded organs (Fig. 3). Expression of the L2 gene is found in young tissues and germinating seeds. In contrast, L2 mRNAs were detected only at a low level in adult tissues, at the late stage of seed formation and in dry seeds (Fig. 3).

These results, compared with the expression pattern of the *Arabidopsis* H4 histone gene, indicate that the gene encoding the cytoplasmic L2 ribosomal protein identified here shows specificity of expression in growing tissues whose cells divide.

Expression during formation and germination of seeds. There are two main stages in seed formation. The first, in which the embryo is developing corresponds biologically to cell division. In the second, after division stops, storage proteins are accumulated. Biosynthesis of proteins takes place during both stages. These different steps in developing seeds have been well characterised in radish (44). We tested ribosomal L2 protein expression at different stages of radish seed development to study its correlation with cell division. Total RNA was extracted from seeds during development (stages 3 to 5 correspond to cell division and stages 6 and 7 correspond to storage protein accumulation), germination (12 to 48 hours) and dessication. The ribosomal L2 cDNA probe was hybridized to the resulting Northern blot (Fig. 4). RL2 mRNA is accumulated during the

first stages (3,4 and 5) of seed formation, is undetectable during the last stages (6 and 7), and in dry seeds, but reappears early during germination. These results suggest that the expression of the cytoplasmic ribosomal protein L2 gene is correlated with cell division.

Genomic organization of the L2 genes in the genome of tobacco and different plant species

Genomic DNA from tobacco (*Nicotiana tabacum*) was digested with *EcoRI* and *EcoRV* which have no recognition sites in the cloned cDNA, and with *HindIII* (underlined in Fig. 1) which has a recognition site before the stop codon of the cDNA. The DNA was electrophoresed on an agarose gel and transferred to a Nylon membrane. The 319 bp fragment probe was hybridized to the resulting Southern blot (Fig. 5). In each digest, the probe hybridizes to two fragments. The hybridizable *EcoRI* bands are weaker than those with *EcoRV*, certainly because DNA digestion with *EcoRI* is incomplete. The size of hybridizable fragments is respectively 2.5 and 2.9 Kb for *EcoRI*, 2.7 and 3.6 Kb for *EcoRV* and 2.0 and 2.9 Kb for *HindIII*. The copy number of the L2 gene was estimated in the allotetraploid genome of *Nicotiana tabacum* (7.8 pg of DNA—45). One, two, five and ten times 10^5 copies of 937 bp L2 cDNA were electrophoresed on an agarose gel. Genomic DNA from *Nicotiana tabacum* was digested with *EcoRV*, and one (0.39 μ g), two (0.78 μ g), five (1.95 μ g) and ten (3.9 μ g) times 10^5 copies of haploid genome are simultaneously electrophoresed on the same gel. The gel was transferred onto a nylon membrane and hybridized to the 319 bp fragment probe (Fig. 6). The copy number of cytoplasmic L2 gene was established as one gene of each type per haploid genome. These results suggest that *Nicotiana tabacum* contains two types of cytoplasmic L2 genes.

The conservation of this gene in other plant species, monocots and dicots, was analysed by Southern blot hybridization. Genomic

Figure 5. Southern blot hybridization of tobacco genomic DNA with the L2 cDNA probe. Ten μ g of genomic DNA was digested by *EcoRI*, *EcoRV* and *HindIII*, separated on an agarose gel and transferred onto nylon membrane. The tobacco 319 bp L2 cDNA probe was hybridized to the resulting genomic blot.

Figure 6. Evaluation of L2 gene number. Genomic DNA from *Nicotiana tabacum* was digested with *EcoRV*. Amounts of digested DNA corresponding to one, 2, 5 and 10 times 10^5 copies of the allotetraploid genome and one, 2, 5 and 10 times 10^5 copies of the 937 bp L2 cDNA were separated on an agarose gel, and transferred onto Nylon membrane. The 319 bp L2 cDNA probe was hybridized to the corresponding Southern blot.

DNA from different dicots, rapeseed (*Brassica napus* var. Darmor and Furax), radish (*Raphanus sativus*), *Arabidopsis thaliana*, and from one monocot, rice (*Oryza sativa* var. Columbia), were digested with EcoRI. After electrophoresis and transfer, the corresponding Southern-blot was hybridized with tobacco 319 bp fragment probe (Fig. 7). All the species show hybridizable fragments. *Arabidopsis* and radish, which have diploid genomes, show only one hybridizable fragment. In contrast, the allotetraploid genomes of rapeseed lines show two hybridizable fragments. These results are in agreement with those obtained with *Nicotiana tabacum*. The hybridization pattern with the monocot rice DNA also shows two bands. The high hybridization with other dicots and a monocot suggest that the nucleotide sequence of this ribosomal protein is conserved.

DISCUSSION

Identification of cytoplasmic ribosomal protein L2. This is the first identification of a cytoplasmic L2 ribosomal protein in higher eucaryotes. Three eukaryotic L2 genes have been identified: two in *Schizosaccharomyces pombe* (SPRKD4, 38 and SPRPK37, 39), and one in *Dictyostelium discoideum* (DDRPL2, 19). The nucleotide sequence of our clone presents fairly good homologies with the eucaryotic (66–67% identity over the entire sequences) and archeobacterial (56% identity over 450 nucleotides) sequences. Similarities with some, but not all, procaryotic or chloroplastic RL2 nucleotide sequences are detectable over short stretches, for example, a 50 nucleotide stretch with the tobacco chloroplastic RL2 gene.

Figure 7. Southern blot hybridization of DNA from different plant species with tobacco L2 cDNA probe. 10 μ g of genomic DNAs from *Arabidopsis*, rapeseed, radish and rice were digested with EcoRI, separated on an agarose gel and transferred onto nylon membrane. The tobacco 319 bp L2 cDNA probe was hybridized to the resulting genomic-blot.

The protein deduced from the nucleotide sequence presents clear homologies with all RL2 proteins ranging from 65,3% identity for the eucaryote *Schizosaccharomyces pombe* (38) to 32,5% identity for *Nicotiana tabacum* chloroplast (23). The homology is particularly high between amino acids 199 to 216 which correspond to the conserved region of the previously published RL2 sequences. The tobacco protein contains Met (204 of our tobacco sequence) and His (209 of our tobacco sequence) residues corresponding to those which are related to peptidyl transferase activity(12,35). In addition, the sequence matches the RL2 PROSITE pattern PXXRG[STAV]2XN[AP]X[DE] in the highly conserved region. Procaryotic RL2 proteins (270–280 amino acids) are larger than those of eucaryotes or archeobacteria (235–255 amino acids).

All these facts indicate that the clone encodes a ribosomal L2 protein. The much greater homology of the eukaryotic 60S RL2 protein than to the 50S prokaryotic one, the size of the protein and the presence of a 22 nucleotide polyA tract at the 3' end of the cDNA show that this clone encodes a cytoplasmic 60S ribosomal L2 protein.

Nevertheless, in the carboxyterminal part of the molecule, the high homology to the eucaryotic 60S ribosomal nucleotide sequences DDRPL2 and SPRPK37 is not conserved when the deduced proteins are compared. We observed that the *Dictyostelium* sequence, DDRPL2, presents a one base deletion equivalent to position 635 of the tobacco sequence and that the yeast sequence SPRPK37 shows two base deletions corresponding to positions 634 and 674 resulting in a frame shift. A similar observation apply to RL2\$NICDE and RL2\$SPIOL if compared with the other chloroplast sequences. By filling the deletions with the corresponding nucleotides of the tobacco sequence, the deduced proteins (noted with a star in Fig 3) show homologies with other eukaryotic and archeobacterial L2 proteins over the entire sequence. These results allow us to suggest that the published sequences, RL37\$SCHPO and RL2\$DICI, may be in part artefactual. In addition it appears that the region of high homology between 50S and 60S RL2 extends over a far longer region than previously suspected. Particularly the pattern HPHGGG (aminoacids 211 to 218 of the tobacco sequence) is present in all RL2 sequences.

Expression of cytoplasmic L2 genes. Cytoplasmic L2 mRNAs are accumulated differentially in plant tissues and in cell suspension according to their developmental stage. The accumulation of L2 mRNA was compared with that of H4 histone mRNA whose expression is much higher in meristematic than in non-proliferating tissues. H4 mRNAs and L2 mRNAs are abundantly accumulated in growing cells, young tissues (leaves, flower buds, roots), germinating seeds and suspension cells in growth phase (Fig. 2,3). They are expressed at a very low level in developing seeds and are almost undetectable in mature tissues (leaves, stele, roots, stationary cells) and dry seeds. These results suggest that the cytoplasmic L2 expression is correlated with the growth state of cells. These results confirm those of Lebrun et al. (39) who observed an higher accumulation of maize cytoplasmic S11 ribosomal protein transcripts in growing tissues (4-day old shoot and root seedlings) than in expanded leaves (3 week old). Moreover, we found an abundant accumulation of the mRNA in the first stages of radish seed in formation, which correspond to the cell division phase, but no trace during the last stages corresponding to synthesis and accumulation of storage proteins. These results suggest that transcription of the

cytoplasmic L2 ribosomal protein gene occurs mostly when cells are dividing. While the hybridizable band in radish seeds is of the same length as that detected in tobacco, we cannot entirely exclude the presence of other RL2 mRNAs detected in the lower stringency conditions used.

Genomic organization of cytoplasmic L2 ribosomal gene. The results suggest that *Nicotiana tabacum* contains two types of cytoplasmic L2 genes. The copy number was established as one gene of each type per haploid genome. Since *Nicotiana tabacum* is an allotetraploid genome, each gene type could originate from each parent, *Nicotiana tomentosiformis* and *Nicotiana sylvestris*.

According to the stringent hybridization conditions, the heterologous cytoplasmic L2 genes belonging to monocots and dicots present 80% homology to the tobacco sequence. This sequence conservation between the plants analysed is higher than between tobacco and *Schizosaccharomyces pombe* (65% of identity). This suggests that the nucleotide sequence of cytoplasmic L2 ribosomal genes is well conserved in all plant species.

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REFERENCES

- Wittman, HG. (1982) *Ann. Rev. Biochem.* **51**, 155–182.
- Wittman, HG. (1983) *Ann. Rev. Biochem.* **52**, 35–65
- Hampel, H., Schulze, H. and Nierhaus, KH. (1981) *J. Biol. Chem.* **256**, 2284–2288
- Auron, PE. and Fahnestock, SR. (1981) *J. Biol. Chem.* **256**, 10105–10110
- Stöffler, G., Daya, L., Rak, KH. and Garrett, RA. (1971) *Mol. Gen. Genet.* **114**, 125–133
- Garret, RA., Müller, S., Spierer, P. and Zimmermann, RA. (1974) *J. Mol. Biol.* **88**, 553–557
- Marquardt, O., Roth, HE., Wystup, G. and Nierhaus, KH. (1979) *Nucleic Acids Res.* **6**, 3641–3650
- Spierer, P., Zimmermann, RA. and Mackie GA. (1975) *Eur. J. Biochem.* **52**, 459–468
- Chen-Schmeisser, U. and Garret, RA. (1976) *Eur. J. Biochem.* **69**, 401–410
- Röhl, R. and Nierhaus, KH. (1982) *PNAS USA*, **79**, 729–733
- Fahnestock, SR., Erdmann, V. and Nomura, M. (1973) *Biochemistry* **12**, 220–224
- Kimura, M., Kimura, J. and Watanabe, K. (1985) *Eur. J. Biochem.* **153**, 289–297
- Kimura, M., Mende, L., Wittmann-Liebold, B., (1982) *FEBS lett.* **149**, 304–312
- Zurawski, G., and Zurawski SM., (1982) *Nucleic Acids Res.* **13**, 4521–4526
- Arndt, E., Kroemer, W. and Hatakeyama, T. (1990) *J. Biol. Chem.* **265**, 3034–3039
- Gross, U., Chen, JH., Kono, DH., Lobo, JG. and Yu, DTY. (1989) *Nucleic Acids Res.* **17**, 3601–3602
- Ohkubo, S., Muto, A., Kawauchi, Y., Yamao, F. and Osawa, S. (1987) *Mol. Gen. Genet.* **210**, 314–322
- Evrard, JL., Kuntz, M. and Weil, JH. (1990) *J. Mol. Evol.* **30**, 16–25
- Singleton, CK. (1989) *Nucleic Acids Res.* **17**, (7989)
- Fukuzawa, H., Kohchi, T., Sano, T., Shirai, H., Umesono, K., Inokuchi, H., Ozeki and H., Ohyama, K. (1988) *J. Biol. Mol.* **203**, 333–351
- Christopher, DA., Cushman, JC., Price, CA. and Hallick, RB. (1988) *Curr. Genet.* **14**, 275–286
- Bowman, CM., Barker, RF. and Dyer, TA. (1988) *Curr. Genet.* **14**, 127–136
- Shinozaki, K., Ohme, M., Tanaka, M., Wakasugi, T., Hayashida, N., Matsubayashi, T., Zaita, N., Chunwongse, J., Obokata, J., Yamaguchi-Shinozaki, K., Ohto, C., Torazawa, K., Meng, BY., Sugita, M., Deno, H., Kamogashira, T., Yamada, K., Kusuda, J., Takaiwa, F., Kato, A., Tohdo, N., Shimada, H. and Suguria, M. (1986) *Embo J.* **5**, 2043–2049
- Zurawski, G., Bottomley, W. and Whitfield, PR. (1984) *Nucleic Acids Res.* **12**, 6546–6558
- Kavousi, M., Giese, K., Larrinua, IM., McLaughlin, WE. and Subramanian, AR. (1990) *Nucleic Acids Res.* **18**, 4244–4244
- Hiratsuka, J., Shimada, H., Whittier, R., Ishibashi, T., Sakamoto, M., Mori, M., Kondo, C., Honji, Y., Sun, CR., Meng, BY., LI, YQ., Kanno, A., Nishizawa, Y., Hirai, A., Shinozaki, K. and Sugiura, M. (1989) **217**, 185–194
- Spielmann, A., Roux, E., Von, Allmen, JM. and Stutz, E. (1988) *Nucleic Acids Res.* **16**, 1199–1199
- Grosset, J., Marty, I., Chartier, Y. and Meyer, Y. (1990) *Plant Mol. Biol.* **15**, 485–496
- Maniatis, T., Fritsch, EF. and Sambrook, J. (1990) *Molecular Cloning: A Laboratory Manual*. Cold Spring Harbor University Press, Cold Spring Harbor.
- Hanahan, D. (1983) *J. Mol. Biol.* **166**, 557–580
- Meinkoth, J. and Wahl, G. (1984) *Anal. Biochem.* **138**, 267–284
- Sanger, F., Nicklen, S. and Coulson, AR. (1977) *Proc. Natl. Acad. Sci. U.S.A.* **74**, 5463–5467
- Pearson, WR. (1990) *Methods Enzymol.* **183**, 63–93
- Bairoch, A. (1990) *EMBL CD-ROM Release 6.0*
- Auron, PF, Erdelsky, KJ. and Fahnestock, SR. (1978) *J. Biol. Chem.* **253**, 6893–6900
- Jouanneau, JP. and Tandeau de Marsac, N. (1973) *Exp. Cell. Res.* **77**, 167–174
- Pritchard, AE., Seilhamer, JJ., Mahalingam, R., Sable, CL., Venuti, SE. and Cummings, DJ. (1990) *Nucleic Acids Res.* **18**, 173–180
- Teletski, C. and Käufer, NF. (1989) **17**, 10118
- Nischt, R., Gross, T., Gattermann, K., Swida, U. and Käufer, NF. (1987) *Nucleic Acids Res.* **15**, 1477–1492
- Lebrun, M. and Freyssinet, G. (1991) *Plant Mol. Biol.* **17**, 265–268
- Chaboute, ME., Chaubet, N., Philipps, G. and Gigot, C. (1987) *Plant Mol. Biol.* **8**, 179–191
- Gausling, K. (1977) *J. Mol. Biol.* **115**, 335–354
- Köpke, J. and Wittmann-Liebold, B. (1989) *Can. J. Microbiol.* **35**, 11–20
- Raynal, M. (1989) Thesis, University of Perpignan, France.
- Bennett, MD and Smith, JB (1976) *Phil. Trans. R. Soc. Lond. B.*, 227–273
- Delcasso-Tremousaygue, D., Grellet, F., Panabières, F., Ananiev, ED. and Delseny, M. (1988) *Eur. J. Biochem.* **172**, 767–776