SEQUENCE AND EXPRESSION OF A MICROSPORE cDNA CLONE WITH HOMOLGY TO A RIBOSOMAL PROTEIN

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ABSTRACT

A cDNA library was made to RNA from corn anthers containing developing pollen at the uninucleate microspore stage. A randomly selected clone from this library which contained an insert (531 bp) was isolated and sequenced. An open reading frame of 330 bp was located. Computer alignments of the putative amino acid sequence with sequences from GenBank and the SwissProt protein databases indicated homology to L12, an acidic ribosomal protein. RNA blot analysis showed highest levels of this mRNA in mature pollen. The significance of this observation in light of the known biochemistry of ribosome synthesis in developing pollen is discussed.

KEY WORDS: pollen, *Zea mays*, L7/L12 protein, ribosomes.

INTRODUCTION

Expression of the precise types and sequence of genes in developing pollen is essential for the correct differentiation and subsequent function of the pollen grain. In order to gain a greater understanding of the types of genes expressed in pollen, we have been isolating and characterizing cDNA clones made to mRNAs from mature as well as developing maize pollen. Often we have used differential screening to identify clones which are up-regulated in pollen (e.g. Stinson et al., 1987), but we and others (Hodge et al., 1992) have also found it fruitful to randomly select cDNA clones with large inserts for further study. While these often do not represent tissue-specific clones, they do represent RNAs which are present in the sampled tissue and may be of full or nearly full length.

We report here the nucleotide sequence and expression pattern of a cDNA clone isolated from an immature pollen cDNA library. RNA blot analysis of this clone indicates that a hybridizing RNA of the appropriate size can be found in mature maize pollen and root, with lesser amounts observable in immature pollen and ovule. This RNA has a predicted protein product with amino acid homology to the acidic L7/L12 ribosomal protein.

MATERIAL AND METHODS

Plant tissue

Field and greenhouse grown maize plants (strain W-22) were used as the source of tissue for the library. Pollen from

the uninucleate stage was collected as previously described (Stinson et al., 1987) and flash frozen in liquid nitrogen. The frozen tissues were ground with a mortar and pestle to > 80% cell breakage at liquid nitrogen temperature.

Library preparation and clone selection

Poly(A)+ RNA was isolated from immature pollen (Turcich et al., 1994) and reverse transcribed using oligo d(T₁₂₋₁₈) priming for the construction of a cDNA library in the λ ZAPII vector (Stratagene) following the manufacturer's instructions. Clones containing inserts were identified by selecting white colonies following in vivo excision of the total phage library.

RNA blot analysis

For RNA blot analysis, RNA from the indicated tissues was isolated as previously described (Turcich et al., 1994). Equal amounts of total RNA from each tissue were electrophoretically separated in formaldehyde-agarose gels, and then transferred to Hybond-N (Amersham) nylon membranes for probing.

DNA sequencing

Both strands of the cDNA clone were sequenced utilizing the Sequenase Version 2.0 sequencing kit (US Biochemicals) using [³⁵S]-dATP. Complete sequencing of the subcloned fragment was achieved by utilizing the pSK- plasmid T3 and -20 primers.

Computer analysis

Computer analyses were performed using the Genetics Computer Group (GCG) Sequence Analysis Software, Version 7.1 for UNIX (Genetics Computer Group, 1991, Program Manual for GCG Package, Madison, Wisconsin). Protein databases were searched by FASTA and TFASTA, sequence

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	1						60
rpl12:Zm:2	.MASGELACR	YAALILSDDG	IAIT.AEKIA	TIVKAANIKV	ESYWPALFAK	LLEKRNVEDL	
ricecdna	.MSSSEVACT	LAALILHDDG	IPIT.SEKIA	TLVKAANIKV	EAYWPGLFAK	LLEHRSVDDL	
huml12	MASVSELACI	YSALILHDDE	VTVT.EDKIN	ALIKAAGVNV	EPFWPGLFAK	ALANVNIGSL	
zml12	MKFV	AAYLLAVLAG	NASPSADDLT	AILESVGCEV	DNEKMELLLS	QLSGKDITEL	
yeastl12	MKYL	AAYLLLNAAG	NT.PDATRIK	AILESVGIEI	EDEKVSSVLS	ALBKGKSVDEL	
	61						118
rpl12:zm:2	ILSVG...SG	GGAAPV.AAA	APAGGAAAAA	APAVEEKKE.	..EAKESDD	DMGFSLFD	
ricecdna	ILSVG...SG	GGAAPVAAAA	APAAGGGAAX	APXAEKKEE.	..EAKEE...		
huml12	ICNVG...AG	GPAPAAGAAP	AGGPAPSTAA	APAEEKKVEA	KKEESEESDD	DMGFGLFD	
zml12	IAAGREKFAS	VPCGGGGVAV	AAAAPAAGGA	APAAEAKKEE	KVEEKEESDD	DMGFSLFD	
yeastl12	ITEGNEKLAA	VP..AAGPAS	AGGAAAASGD	AAAEKKEE.	..EAAESDD	DMGFGLFD	

Fig. 1. Amino acid sequence comparison between the sequence described here (rpl12:Zm:2) and several published L7/L12 proteins (rice cDNA, accession number D48782; human [huml12, accession # M17886], Zea mays [Zml12, accession # X86553], yeast [yeastl12, accession # X06958]). Bold letters indicate amino acid identity, and dots indicate gaps.

alignments were generated using the "Gap" program, and multiple protein alignments were compiled with the "Pileup" program.

RESULTS AND DISCUSSION

Isolation and sequencing of one of the randomly selected clones from the maize immature pollen cDNA library of a size of 531 bp, revealed an open reading frame of 330 bp. Sequence comparisons of the putative translation product showed significant homology to the eukaryotic ribosomal protein L7/L12 (Fig. 1). The ATG start of translation is at bp 67 and the termination at bp 394. The entire coding sequence is contained within the clone, but the entire 3' UTR does not seem to be present in this clone.

Comparison of the putative amino acid sequence reported here with sequences available on Genbank shows a high degree of similarity to L12 genes from various organisms (Fig. 1). Several partial rice cDNA sequences show extremely high homology, but interestingly a previously known maize L12 sequence (Zml12) is less homologous to the new maize L12 sequence reported here than are L12 genes from other more distantly related plants.

Fig. 2. RNA blot analysis showing tissue dependent expression of L7/L12. Equal amounts of total RNA (5 µg) were added to each lane.

RNA blot analysis to study the expression of this gene in maize shows not only that it is expressed in uninucleate pollen as expected, but it is also expressed in leaves, silks, and in high quantities in mature pollen and root (Fig 2).

The presence of high levels of the L12 mRNA in mature pollen is unexpected because in the plants that have been studied the ribosomal RNA (rRNA) genes appear to be transcriptionally inactive during the terminal stages of pollen maturation and during pollen tube growth (Mascarenhas, 1975, 1990; Mascarenhas and Bell, 1970; Peddada and Mascarenhas, 1975; Steffensen, 1966; Tupy et al, 1983). Moreover, no ribosomal proteins are synthesized in pollen tubes of lily (Steffensen, 1971). The rRNA genes are, however, actively transcribed in the microspore prior to microspore mitosis. Although no new ribosomes are synthesized in the mature pollen grain and during pollen tube growth, the mature pollen grain is equipped with a large store of ribosomes. These ribosomes are utilized in the protein synthesis that occurs during germination and that is required for pollen tube growth (reviewed in Mascarenhas, 1990).

L7 is an N-terminal acetylated form of L12; however, the two are functionally indistinguishable. L7/L12 are present in ribosomes as a pair of dimers which constitute a morphological feature of the ribosome known as the L7/L12 stalk (Möller and Maassen, 1986). This structure is essential for ribosomal interactions involving translational factors and GTP hydrolysis, and removal or readdition of the proteins results in concomitant loss or restoration of ribosomal activity (Möller and Maassen, 1986). It has been noted that this is a major example of ribosome function where proteins, as opposed to RNA, play a clearly defined and perhaps dominant role (Oleinikov, et al., 1993). If the L12 protein turned over during protein synthesis, the new synthesis of L12 protein could potentially be an effective way of regulating the rate of translation during pollen tube growth. Protein synthesis is activated very early during pollen germination (Linskens, 1967; Mascarenhas and Bell, 1969) and the rate of protein synthesis continuously decreases during pollen tube growth (Mascarenhas and Mermelstein, 1981). The L12 dependent regulation of protein synthesis is merely speculation at this point, since relatively little is known about the turnover of L7/L12 during protein synthesis and we do not know whether the L12 mRNA is translated during pollen germination.

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The nucleotide sequence data reported in this paper will appear in the EMBL, GenBank and DDBJ Nucleotide Sequence Databases under the accession number U40147.

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SEKWENCJA I EKSPRESJA KLONU cDNA Z MIKROSPORY HOMOLOGICZNA DO BIAŁEK RYBOSOMALNYCH

STRESZCZENIE

Sporządzono bibliotekę sond cDNA dla RNA z pylników kukurydzy zawierających jednojądrowe mikrospory. Wyselekcjonowano przypadkowe klony z tej biblioteki zawierające insert 531 bp i zsekwenconowano go. Zlokalizowano otwartą ramkę odczytu długości 330 bp. Metodą komputerową ustalono domniemaną sekwencję aminokwasów i wskazano na sekwencję kwaśnych białek rybosomalnych homologiczną do białka L12 z baz danych GenBank i SwissProt. Najwyższy poziom tego samego mRNA wykryto w dojrzałych ziarnach pyłku. Przedyskutowano znaczenie tej obserwacji w świetle dotychczasowej wiedzy na temat biochemii i syntezy rybosomów w rozwijających się ziarnach pyłku.

SŁOWA KLUCZOWE: pyłek, *Zea mays*, białko L7/L12, rybosomy.