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Plant Gene Register

Nucleotide Sequence of Four Ribosomal Protein L27 cDNAs from Growing Axillary Buds of Pea¹

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The small and large subunits of the eukaryotic ribosome contain a total of three to four rRNA molecules and 70 to 80 ribosomal proteins, which are required in stoichiometric amounts (Mager, 1988). Expression of ribosomal protein genes in plants has been correlated with active growth and cell division in a variety of tissues and organs, including auxin-treated soybean hypocotyls (Gantt and Key, 1985), developing maize embryos (Larkin et al., 1989), cytokinin-treated soybean cell cultures (Crowell et al., 1990), and tomato shoot apical meristems (Flemming et al., 1993). Ribosomal protein genes in plants and other organisms generally are expressed coordinately (Gantt and Key, 1985; Mager, 1988). Some ribosomal proteins are encoded by single-copy genes (Hwang and Goodman, 1993), whereas others are encoded by small gene families. For example, the *Brassica* rpS15a (for ribosomal protein S15a) gene family contains two expressed members that encode identical polypeptides (Bonham-Smith et al., 1992). Based on Southern blot analysis, the maize rpS14 gene family may contain as many as six members, three of which have been shown to be expressed; the two rpS14 cDNA clones that have been sequenced encode peptides that differ in size and sequence (Larkin et al., 1989). We show here that the pea (*Pisum sativum*) rpL27 family contains at least five expressed members, the largest ribosomal protein gene family from any plant for which sequence data are available.

We previously isolated an rpL27 cDNA from growing axillary buds of pea (rpL27–1; Stafstrom and Sussex, 1992). When compared to a rat gene, rpL27–1 lacked a single nucleotide at the 5' end of the coding sequence (TG instead of ATG). To identify the complete coding sequence corresponding to this gene, two different cDNA libraries were screened using rpL27–1 as a probe. Four additional clones were isolated; DNA sequences of these clones were distinct from each other and from rpL27–1 (Table I). Three of these clones (rpL27–3, rpL27–4, and rpL27–5) contain an ATG at the expected position. In addition, rpL27–3 and rpL27–5 contain an in-frame stop codon at position –12. Nucleotide sequence identity among the five rpL27 clones is greater than 96%. The deduced amino acid sequences of the four (nearly) full-length clones differ from each other at one to three residues (rpL27–2, which encodes only the C-terminal 49 amino acids,

is identical with rpL27–4). All pairs of alternative residues represent "similar" amino acids. Since each of the five rpL27 cDNAs was cloned only once, it is quite likely that this gene family is considerably larger.

The closest match to the pea rpL27 clones in the GenBank data base is an rpL27 cDNA from potato (Taylor and Davies, 1994). The potato clone encodes a peptide of 138 amino acids. Maximal alignment of the pea and potato sequences includes gaps of one and four residues. The deduced amino acid sequences of rpL27–4 and the potato clone are identical at 107 positions (80%) and similar at 121 positions (90%). Eight of the similar residues involve substitution of Lys's and Arg's. The pea genes show a strong preference for Lys's, which occur at seven of these sites. The high degree of similarity among the pea clones (relative to the potato clone) probably indicates that they diverged from a single gene rather recently.

Axillary buds on intact pea plants are inhibited from growing (dormant) because of apical dominance. Following decapitation of the terminal bud, large and small buds at node-2 of Alaska pea seedlings begin to grow within 8 h (Stafstrom and Sussex, 1992). After 3 d, small buds are inhibited from growing further by apical dominance imposed by the rapidly growing large bud. These small buds become dormant again, but they can resume growing if the large bud is removed. Thus, pea axillary buds can be stimulated to undergo more than one complete growth-dormancy cycle during the course of a few days. The steady-state level of rpL27–1 mRNA was closely associated with the growing state of bud development, i.e. it reversibly increased and decreased during multiple growth-dormancy cycles (Stafstrom and Sussex, 1992). Multiple members of ribosomal protein gene families from maize and *Brassica* were shown to be expressed coordinately using gene-specific probes based on unique 3' noncoding sequences (Larkin et al., 1989; Bonham-Smith et al., 1992). Because of the high level of similarity among the 3' noncoding domains of the pea rpL27 clones, it was not possible to determine the expression pattern of each clone by this method. Instead, we are attempting to use clone-specific oligonucleotide primers and PCR amplification to determine whether they are expressed coordinately in all tissues.

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The GenBank and EMBL accession numbers for the sequences reported in this article are U10043, U10044, U10045, and U10046 (rpL27–2, rpL27–3, rpL27–4, and rpL27–5, respectively).

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Table I. Characteristics of rpl27 clones from pea

Organism	<i>Pisum sativum</i> L. cv Alaska.
Gene Product, Function:	Cytoplasmic ribosomal protein L27; function determined by homology.
Source, Method of Isolation:	Clones were isolated from two cDNA libraries, both made from poly(A ⁺) RNA isolated from growing axillary buds (24 h after decapitating the terminal bud). rpl27-1 was used as a probe. rpl27-2 was isolated from a λ -GEM4 library (Promega; Stafstrom and Sussex, 1992) and rpl27-3, rpl27-4, and rpl27-5 were isolated from a pSPORT1 library (BRL; Stafstrom et al., 1993).
Method of Identification:	Sequence similarity to rpl27-1.
Sequencing Strategy:	Dideoxy sequencing reactions were primed from SP6 or T7 sites within the cloning vectors or from internal sequences using synthetic oligonucleotides.
Characteristics of cDNAs:	Clones rpl27-2, rpl27-3, rpl27-4, and rpl27-5 contain 278, 564, 576, and 568 bases, respectively. rpl27-3, rpl27-4, and rpl27-5 contain a 405-nucleotide open reading frame that encodes a polypeptide of 135 residues. These three clones contain 5' noncoding sequences of 39, 11, and 38 bases, respectively, that are identical where they overlap. rpl27-3 and rpl27-5 contain in-frame stop codons at position -12. Because of an internal <i>Xba</i> I site not found in the other clones, the sequence of rpl27-2 begins at nucleotide position 239 and it contains a 147-base open reading frame. Nucleotide sequence identity of the coding domains of rpl27-1 and each of the four new clones is greater than 96%. rpl27-2, rpl27-3, rpl27-4, and rpl27-5 contain 3' noncoding sequences [excluding poly(A) tails] of 131, 120, 160, and 125 bases, respectively. The 3' noncoding domains of four clones are nearly identical over the first 92 bases (rpl27-1 is similar to the other clones over the first 27 bases). Pairs of clones share additional similarities in other areas of their 3' noncoding sequences.
Structural Features of Deduced Amino Acid Sequence:	The deduced amino acid sequences of the five rpl27 clones differ from each other at one to three residues, which occurred at a total of four positions: position 35 (Glu or Asp); position 49 (Phe or Tyr); position 50 (Pro or Ser); and position 116 (Ser or Ala). The deduced amino acid sequences of rpl27-4 and a <i>Solanum tuberosum</i> (potato) clone were identical at 107 of 134 positions (79.9%) and similar at 121 positions (89.6%) (Taylor and Davies, 1994).
Expression Characteristics:	Based on northern blots and in situ hybridization experiments, rpl27 mRNA accumulates in all regions of axillary buds within 1 h of decapitating the terminal bud, reaches a maximal level within 6 h, and decreases to the basal level when buds cease growing and become dormant (Stafstrom and Sussex, 1992). Gene-specific expression is being explored.

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