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Differential Expression and Sequence Analysis of Ribosomal Protein Genes Induced in Stolon Tips of Potato (*Solanum tuberosum* L.) during the Early Stages of Tuberization[1]

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ABSTRACT

cDNA clones of two genes, *TUBS19* and *TUBL7*, which show a 15- to 20-fold increase in transcript level in the stolon tip during the early stages of tuberization, have been isolated by differential screening. These genes are also expressed in leaves, stems, and roots, and the expression pattern in these organs changes on tuberization. Southern analysis shows that there are similar sequences in the genome of nontuberizing wild-type potato species *Solanum brevidens* and in *Lycopersicon esculentum* (tomato). Sequence analysis reveals a high degree of similarity between the *TUBS19* cDNA and the eukaryotic *S19* ribosomal protein gene. *TUBL7* cDNA shows similarity to another eukaryotic ribosomal protein gene, *L7*.

Tuberization results in the differentiation of a specialized shoot, the stolon, into a storage organ, the tuber. Histological studies have defined the changes in cell type, shape, and growth pattern that occur during tuberization in potato (reviewed in ref. 24). However, the molecular mechanisms that bring about these changes remain to be clarified and present some challenging questions regarding plant storage organ formation.

A wide range of plant hormones and environmental factors have been shown to be involved in the tuberization stimulus (reviewed in refs. 20, 21, and 27). These include photoperiod, temperature, nitrogen supply, ABA, cytokinins, ethylene, gibberellins, and a jasmonic acid derivative. The action and possible interactions of these stimuli are far from clear. In particular, specific genes whose expression is influenced by these factors remain to be characterized.

Changes in the pattern of gene expression in the early stages of tuberization have been observed from analysis of proteins extracted from stolon tips and tubers (9, 11, 25). In vitro translation of RNA extracted from the same stages of development supports the idea that some changes in gene expression occur early in tuberization (11, 25). In this study, our aim was to identify these changes. Of particular interest were genes other than those involved in storage polymer biosynthesis, because these may have a role in morphogenesis or in the regulation of tuberization.

A number of genes have been isolated by differential screening of a stolon tip cDNA library. The expression of these genes increases dramatically during the early stages of tuberization. A detailed characterization of the expression pattern of two such genes, *TUBS19* and *TUBL7*, is described. *TUBS19* shares sequence similarity with the eukaryotic *S19* gene, which encodes a ribosomal protein. *TUBL7* shows sequence similarity to another ribosomal protein gene, the eukaryotic *L7* gene. The expression pattern in tissues in addition to the tuberizing stolon tip is described.

MATERIALS AND METHODS

Growth of Plant Material

Seed tubers of potato (*Solanum tuberosum* L.) cv Record were grown as described previously (25). Plants were harvested between 21 and 60 d after planting to obtain material at various developmental stages. For stage A (Fig. 1), stolons were harvested from plants soon after the emergence of leafy shoots (approximately 21 d after planting). There was a complete absence of swelling stolons on any of the plants from this stage. Nonswelling stolons (stage B, Fig. 1) were taken from plants on which some stolons had started to tuberize. The stolons selected from this stage, however, did not show any signs of swelling (harvested 28–35 d after planting). Swelling stolons (stage C, Fig. 1) were selected from plants in which the diameter of the swelling stolon was less than twice the diameter of the stem. Small tubers (up to 1 g fresh weight) were harvested after approximately 42 d (stage D, Fig. 1) and 25-g tubers were harvested at 60 d after planting (stage E). Roots, internodal green stem segments, and leaves were harvested from plants in which there were no visible signs of tuberization (21 d after planting) and from plants that had started to tuberize (42 d after planting).

RNA Isolation and Blot Analysis

Total RNA was extracted from approximately 2 g of fresh weight stolon tips (apical 5 mm), whole leaves, roots, and green stems using Qiagen-pack 100 cartridges (Qiagen Inc.,

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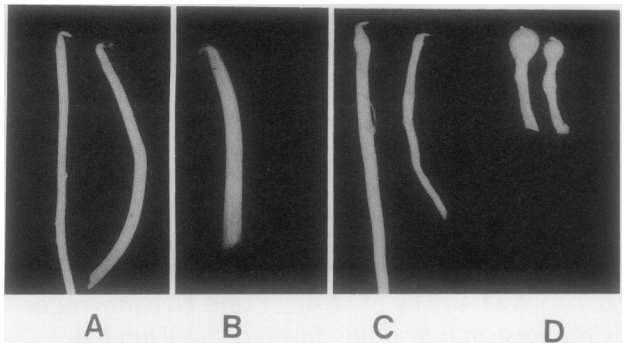

Figure 1. Stages in stolon development. A, Nonswelling stolons harvested 21 d after planting; B, nonswelling stolons harvested 35 d after planting; C, swelling stolons also harvested 35 d after planting; D, small tubers up to 1 g fresh weight.

Studio City, CA) following the manufacturer's protocol. Poly(A^+) RNA was prepared by affinity chromatography using an oligo(dT)-cellulose column or by using oligo(dT)-coated magnetic beads (Dynal Ltd., Liverpool, UK). RNA blotting was carried out using Hybond-N (Amersham) following the manufacturer's protocols. Total RNA (15 μg/track) was resolved by electrophoresis on 1.2% agarose gels containing formaldehyde. Probes were prepared from the *Eco*RI inserts of the putative tuberization-inducible clones by gel purification and were labeled with [α-^{32}P]dCTP to high specific activity (typically 1×10^9 cpm/μg) using random primers (6). Following hybridization (16 h at 42°C in 50% formamide), filters were washed sequentially in 2× SSC,[2] 0.5% SDS, followed by 2× SSC, 0.1% SDS, and then 0.1× SSC, 0.1% SDS for 20 min per wash at 52°C. The filters were then exposed to x-ray film at −70°C for between 24 and 96 h. The hybridization signal on RNA blots was quantified by densitometry using a Quantimet 900 image analyzer (Leica, Cambridge, UK). Equal loading of gels was verified by reprobing stripped filters with a 25S potato rRNA gene probe.

Library Construction and Differential Screening

A cDNA library was constructed from poly(A^+) RNA extracted from swelling stolon tips (stage C). cDNA was prepared from 5 μg of poly(A^+) RNA using a Pharmacia cDNA synthesis kit. *Eco*RI linkers were ligated to the termini of the cDNA, which was then ligated into the *Eco*RI site of the λ-Zap II vector (Stratagene), packaged using an in vitro packaging kit (Amersham), and plated on the XL1-Blue strain of *Escherichia coli* (Stratagene). The library contained approximately 1.5×10^6 pfu with a mean insert size of 1.2 kb. Differential screening of the stage C stolon tip cDNA library was carried out as described before (19) using labeled cDNA probes from stage A and stage C.

After hybridization (16 h), filters were washed sequentially in 2× SSC, 0.1% SDS and then 0.1× SSC, 0.1% SDS for 2 × 20 min changes in each solution at 65°C and exposed to x-ray film (Fuji) at −70°C, with intensifying screens, for 48 to 72 h. Plaques that hybridized to the probe from swelling stolon tips (stage C) but not to the nonswelling stolon tip probe (stage A) were selected and purified to homogeneity by two further rounds of plaque screening. Seven recombinants with these characteristics were isolated from screening of approximately 40,000 initial plaques. The in vivo excision protocol of Stratagene with the R408 helper phage was used to rescue putative tuberization-induced cDNAs in pBluescript SK(−) plasmids. The plasmids were purified using Qiagen-100 cartridges.

DNA Extraction and Southern Blots

Plant genomic DNA was extracted from leaves as described previously (5). Five micrograms of each DNA was digested with *Eco*RI and/or *Hin*dIII and resolved by electrophoresis on 0.8% agarose gels. DNA was transferred under vacuum to nylon membranes (Hybond-N). Filters were hybridized with the labeled inserts of the *pTUBS19* and *pTUBL7* clones prepared as described above. Filters were washed at high stringency (0.1× SSC, 0.1% SDS at 65°C) and exposed to x-ray film for 24 to 72 h at −70°C with intensifying screens.

DNA Sequence Analysis

DNA sequence was obtained from both strands of alkaline denatured plasmid by dideoxy sequencing using Sequenase version 2.0 (U.S. Biochemical Corp.). DNA sequence data were compiled and compared using the Genetics Computer Group programs for the Vax (4). Alignments of complete sequences were carried out using the GAP program.

RESULTS

Differential Gene Expression during Tuberization

As in previous studies, the expression of patatin was used as a marker for tuberization (23). Absence of its transcript in the nonswelling stolons, selected for stage A, is evident in Figure 2, lane 1. When swelling in the subapical region of the stolon tip was observed, patatin transcripts could be detected in all stolon tips whether they were swelling or not, for example, in stolon tips from stage B (Fig. 2, lane 2). On the basis of the absence of patatin transcript, stage A was defined as noninduced. Differential screening of 40,000 plaques resulted in the isolation of seven clones that were differentially expressed between nonswelling (stage A) and swelling stolons (stage C). Two of the seven (*TUBS19* and *TUBL7*) were selected for further analysis.

Expression levels of *TUBS19* and *TUBL7* were determined by RNA blot analysis (Fig. 3, A and B, respectively). In common with patatin, there was virtually no expression of either of these genes in stolon tips from noninduced plants (Fig. 3, A and B, lanes 1). In stolon tips from induced plants, the expression level increased dramatically (20-fold for *TUBS19* [Fig. 3A] and 15-fold for *TUBL7* [Fig. 3B]). Transcript level of both these clones was maximal in stolon tips from induced plants before any visible changes in morphology (stage B, Fig. 3A, lane 2; Fig. 3B, lane 2). The transcript levels then declined as the tuber increased in size, the level decreasing progressively to 8% of the maximal level in tubers of 25

[2] Abbreviations: SSC, standard sodium citrate; pfu, plaque-forming unit.

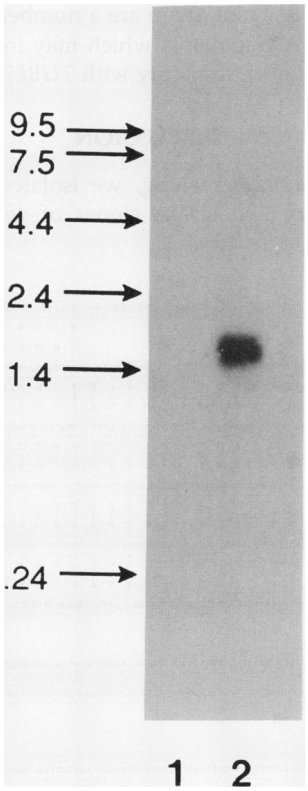

Figure 2. RNA blot analysis of patatin expression. Hybridization of patatin probe to 15 μg of total RNA extracted from stolon tips from stage A (lane 1) or stage B (lane 2). Arrows indicate the position of end markers in kilonucleotides.

g fresh weight for *TUBS19* (Fig. 3A, lane 5) and 20% for *TUBL7* (Fig. 3B, lane 5). Essentially the same expression pattern was observed for three independent sets of plant material.

Filters were stripped and hybridized with a potato 25S rRNA probe. An approximately equivalent hybridization signal (densitometer readings varied by less than 10% between tracks) was detected for RNA extracted from tissue at all stages. Figure 3C shows the data for the filter used in Figure 3A (the *TUBS19* blot). Similar data were obtained for the *TUBL7* and patatin blots (not shown).

Expression of *TUBS19* and *TUBL7* in Other Parts of the Potato Plant

RNA blot analysis of RNA extracted from leaves, roots, and stems of induced and noninduced plants was also carried out using the labeled inserts of *pTUBS19* and *pTUBL7* as probes (Fig. 4). Both clones were expressed in all tissue types. Figure 4A shows the expression pattern for *TUBS19*. In general, there was an increase in transcript level in all tissue types taken from induced plants compared with those from noninduced plants (Fig. 4A, lanes 4–6 [induced] compared with lanes 1–3 [noninduced]). The levels in all these tissues was lower than in induced stolon tips. For *TUBL7*, the transcript level in leaf, stem, and root was also greater in tissue from induced plants compared with noninduced (Fig. 4B, lanes 4–6 [induced] compared with lanes 1–3 [noninduced]). Again, the filters were stripped and hybridized with a potato ribosomal probe to confirm equal loading of lanes (Fig. 4C).

Sequence Analysis of *TUBS19* and *TUBL7*

The sequences of the cDNA clones of *TUBS19* and *TUBL7* are shown in Figure 5, A and B, respectively. The size of the insert in *pTUBS19* is 741 bp. The size of the transcript was estimated to be 900 to 1000 bases from Figure 3A, by comparison with the RNA size markers. A potential long open reading frame from the nucleotide position 59 to the the TGA stop codon at nucleotide 460 was identified (Fig. 5A). The deduced amino acid sequence of the *TUBS19* open reading frame was compared with known sequences using the Genetic Computer Group programs for the Vax (4). There was significant similarity with the eukaryotic *S19* gene, the best similarity being with the *Xenopus laevis* sequence (1). Shown in Figure 5A is the global alignment of the potato and *X. laevis* sequences, which show 75% similarity and 65% identity.

The size of the insert in *pTUBL7* is 1195 bp. The size of the *TUB7L*-specific transcript was estimated to be 1300 bases from the RNA blot analysis shown in Figure 3B. The largest open reading frame runs from the methionine initiation codon at position 266 to the TAG termination codon at nucleotide 992. This open reading frame encodes a polypeptide of

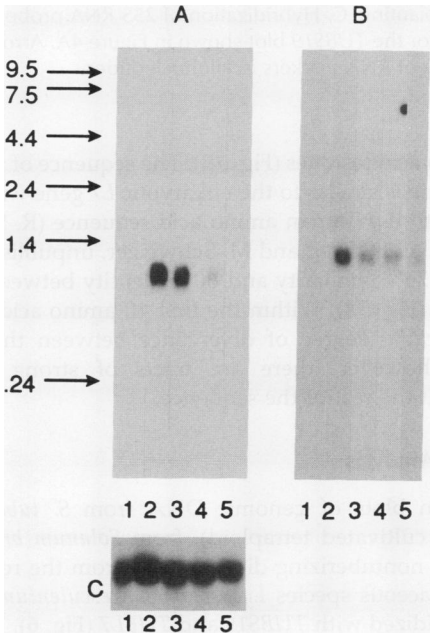

Figure 3. RNA blot analysis of *TUBS19* and *TUBL7* expression. A, Hybridization of *TUBS19* to 15 μg of total RNA extracted from stolon tips from stage A (lane 1), stolon tips from stage B (lane 2), stolon tips from stage C (lane 3), small tubers from stage D (lane 4), and tubers (25 g fresh weight) (lane 5). B, Hybridization of *TUBL7* to 15 μg total RNA (lanes as in A). C, Hybridization of 25S rRNA probe to stripped filter used in Figure 3A (lanes as in A). Arrows indicate the position of RNA markers in kilonucleotides.

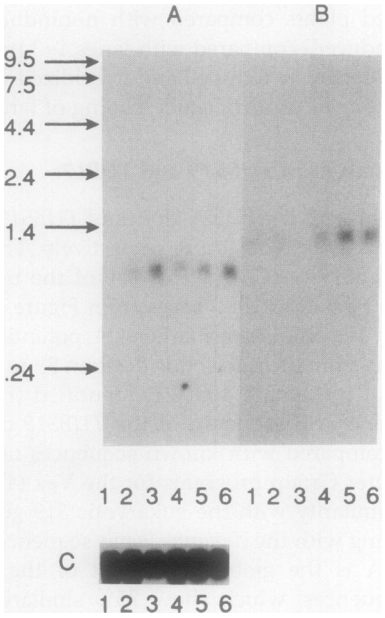

Figure 4. RNA blot analysis of *TUBS19* and *TUBL7* in leaves, stems, and roots. A, Hybridization of *TUBS19* to 15 μg of total RNA extracted from leaf, root, and stem (lanes 1–3, respectively) from plants harvested 21 d after planting (nontuberizing); lanes 4 to 6, RNA extracted from leaf, root, and stem, respectively, of plants harvested 42 d after planting (tuberizing). B, Hybridization of *TUBL7* to 15 μg of total RNA extracted from leaf, stem, and root (lanes 1–3, respectively) from plants harvested 21 d after planting; lanes 4 to 6, RNA extracted from leaf, stem, and root from plants harvested 42 d after planting. C, Hybridization of 25S RNA probe to stripped filter used for the *TUBS19* blot shown in Figure 4A. Arrows indicate the position of RNA markers in kilonucleotides.

241 amino acid residues (Fig. 5B). The sequence of the *TUBL7* cDNA clone is similar to the eukaryotic *L7* gene with highest similarity to the human amino acid sequence (R. Schneider, H. Herzog, L. Heffrer, and M. Schweiger, unpublished data). There are 75% similarity and 60% identity between the two sequences (Fig. 5B). Within the first 40 amino acids, there is a considerable degree of divergence between the two sequences, however, there are tracts of strong similarity throughout the rest of the sequence.

Southern Analysis

Southern blots of genomic DNA from *S. tuberosum* cv Record (a cultivated tetraploid), from *Solanum brevidens* (a wild-type, nontuberizing diploid), and from the related diploid Solanaceous species *Lycospersicum esculentum* (tomato) were hybridized with *TUBS19* and *TUBL7* (Fig. 6). With both these probes the greatest number of hybridizing DNA fragments was observed with the tetraploid cv Record (lanes labeled A). Strongly hybridizing DNA fragments were also detected with the *S. brevidens* (B) and tomato DNA (C). Therefore, *TUBS19* and *TUBL7* or similar sequences are not confined to tuberizing plants. In both *S. brevidens* and *L. esculentum* the copy number of both *TUBS19* and *TUBL7* appears to be low, and the genes may be present as a single copy. In the *TUBL7* blot, there are a number of more weakly hybridizing DNA fragments, which may indicate that other genes share sequence similarity with *TUBL7*.

DISCUSSION

Using differential screening, we isolated from a potato stolon tip library two cDNA clones of genes that encode

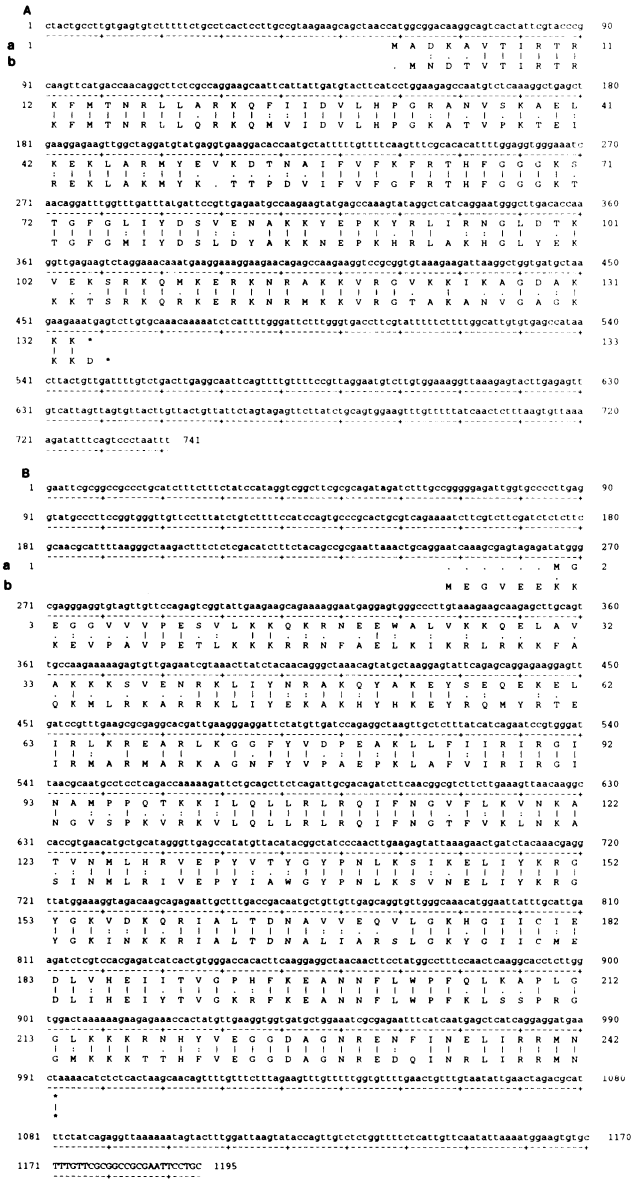

Figure 5. Nucleotide and deduced amino acid sequences of the cDNA clones of *TUBS19* (A) and *TUBL7* (B). In A, the deduced amino acid sequence (labeled a) is compared to the *S19* sequence from *X. laevis* (labeled b). In B, the deduced amino acid sequence (labeled a) is compared to that of the human *L7* gene (labeled b). Solid lines show the alignment of identical amino acids; dots show the alignment of related amino acids (one dot indicating a greater evolutionary distance than two as defined using the default values in the GAP program).

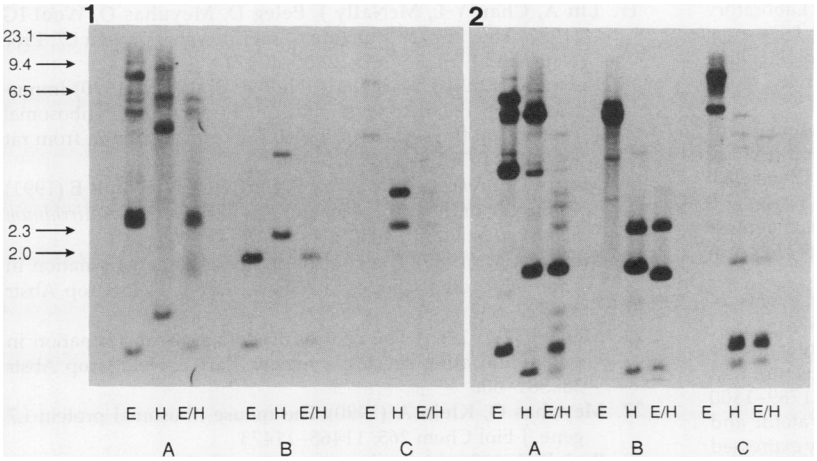

Figure 6. Southern blots of genomic DNA extracted from *S. tuberosum* cv Record (lanes labeled A), *S. brevidans* (B), and *L. esculentum* (C) probed with the labeled inserts of *pTUBS19* (panel 1) or *pTUBL7* (panel 2). Five micrograms of DNA was cut with *Eco*RI (E), *Hin*dIII (H), or both (E/H). DNA size markers were λ digested with *Hin*dIII and their positions are indicated by arrows (sizes in kb).

ribosomal proteins. Both cDNA clones share sequence similarity with eukaryotic type ribosomal protein genes. It seems likely, on the basis of their eukaryotic traits, that *TUBS19* and *TUBL7* encode cytoplasmic ribosomal proteins. Previously, there have been relatively few reports of plant cytoplasmic ribosomal genes. These include the *L3* genes from *Arabidopsis thaliana* (12), the *S11* genes from *Arabidopsis* (7), soybean (7), and maize (15), and *S14* genes from maize (16).

The inserts in *pTUBS19* and *pTUBL7* both contain the entire coding regions of their respective genes as deduced by comparison with other *S19* and *L7* genes (Fig. 5, A and B). The *TUBS19* open reading frame encodes a polypeptide of 133 amino acids, similar to the 132 amino acids in the *Xenopus* S19 polypeptide (1) and the same as in the highly similar S24 protein from rat (3). The *TUBL7* open reading frame, encoding a polypeptide of 242 amino acids, is of a similar size to the human L7 polypeptide (248 amino acids [R. Schneider, H. Herzog, L. Heffrer, and M. Schweiger, unpublished data]) and the rat and mouse *L7* gene products (258 and 270 amino acids, respectively [17, 22]). In *TUBS19* the ATG methionine codon (A at position 59) is in optimal context for initiation (ACCATGG [13]). The ATG initiator codon in *TUBL7* occurs in the context GATATGG, which deviates from the optimal but does have a G at position 4. In common with cDNA clones encoding other ribosomal proteins, there is no typical polyadenylation sequence (AATAAA) downstream of the stop codon in either *TUBS19* or *TUBL7*, supporting the suggestion that plants can use other criteria as a polyadenylation sequence (2).

The function of individual eukaryotic ribosomal proteins remains to be fully elucidated. It has been shown that mammalian protein S24 (equivalent to S19) can be cross-linked to initiation factor eIF-3, which is necessary for the binding of mRNA to ribosomes (26). This suggests that S19 is located at the site where the initiation factor binds, where translation of mRNA is initiated. Consistent with this is the observation that the S19 protein is located on the surface of the 40S ribosomal subunit (18). Similarly, the L7 protein has been localized on the surface of the 80S ribosome by biotinylation (16).

The control of eukaryotic ribosome assembly is a complex process that involves the equimolar accumulation of four RNA molecules and more than 70 different ribosomal protein species (22). Ribosomal gene expression is regulated at both the transcriptional and translational levels (8, 10). In this study, it has been demonstrated that there is a 15- to 20-fold increase in the steady-state level of the *TUBS19*- and *TUBL7*-specific transcripts in the early stages of tuberization. Similar changes in the levels of ribosomal protein gene transcripts during developmental processes have not been reported. There is no corresponding decrease in the ratio of total RNA to rRNA (Fig. 3C) during tuberization, and as yet, it is difficult to speculate on the reason for the increase in ribosomal protein gene expression. It will be interesting to determine whether or not other ribosomal gene transcripts exhibit similar expression patterns. Also of interest will be factors that control the transcription of these genes. Expression of *TUBS19* and *TUBL7* may be related to one or more components of the tuberization stimulus. Therefore, it will be interesting to isolate and analyze the promoters of these genes and to determine the precise expression pattern in the different cell types of the tuberizing stolon tip.

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