Changes in Ribonucleic Acid Fractions During Maturation of Mimosa Epicotyl Tissues

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Abstract. Total RNA and DNA of mimosa epicotyl tissues were extracted and the RNA fractionated into specific soluble RNAs (sRNAs) at different times during late germination. Epicotyls collected at each time contained qualitatively comparable meristematic and developing tissues, while mature tissues increased. Quantitative ratios of total RNA to DNA and total sRNAs to approximated ribosomal RNA (rRNA) varied consistently during development. Terminal nucleosides of sRNA did not vary in any consistent pattern through development. On the other hand, regular changes in quantitative ratios of specific sRNA groups were observed during development.

Considerable attention has been given to the possible role of transfer RNA (tRNA) in regulation of protein synthesis at the site of translation (8, 29). However, relatively little work has been done using higher plant systems. Vold and Sypherd (30) recently reported quantitative changes in some tRNAs during 48 hr of early germination of wheat seedlings. In order to minimize variability between types of tissues and study developmental changes in similar tissues, a single embryo component should be followed during development. The present study examines soluble RNA (sRNA) patterns in mimosa (Albizia julibrissin Durazzini) epicotyls over a 9-day period during late germination.

In studying quantitative distribution of tRNAs, care must be taken to avoid misinterpretation of structural changes (19, 27), such as loss of 3'-OH terminal adenosine which could give misleading evidence for true quantitative changes (20). Vold and Sypherd (30) accomplished this in part by charging (binding amino acids) the tRNAs before fractionating them quantitatively. This assumes either amino acid saturation or repeatable charging of the tRNA species being compared. To date, charging of mimosa tRNAs in this study is still too irregular in replication to permit such assumptions. Since repeatable charging was not accomplished, the efficiency and purity of sRNA extractions from epicotyls at different ages were examined. Also, tRNAs were completely discharged before fractionation, several replications were conducted, and a fractionation procedure which permits maximum separation of sRNA groups was used. The number of replications reduced misinterpretation of artifacts of extraction procedures, but not misinterpretation of in vivo structural changes which might occur in sRNAs during development (27).

In this study terminal nucleosides of sRNAs were determined at each age to avoid misinterpretation of this type of structural change. If consistent in vivo structural changes do occur, they too could play a role in regulation of protein synthesis at the level of translation.

Data collected in this study suggest consistent changes in the distribution of sRNAs during development, as well as possible evidence against consistent changes in the reversible exchange of tRNA terminal nucleosides during development. In addition, quantitative changes in total RNA, DNA, sRNA, and approximated ribosomal RNA (rRNA) during development are reported.

Materials and Methods

Mimosa seeds were germinated in vermiculite under a light intensity of 1000 ft-c during a 17-hr day, while the temperature ranged from 30° day to 25° night. Moisture was held reasonably constant.

Epicotyls were excised at 11, 14, 17, and 20 days following planting, frozen immediately in liquid nitrogen, and stored at −30°. Forty random, non-frozen epicotyls were pooled at each age to determine average fresh and oven dry weights. Sufficient numbers of epicotyls were pooled to determine quantities of total RNA, DNA, and sRNA; and to do sRNA base analyses. Three replications at each age were made to determine total RNA and DNA; 10 replications at each age to determine total sRNA and sRNA terminal nucleosides; and 5 replications at each age to determine sRNA fractionation profiles.

Based on gross and micromorphological observations, approximately 5 days elapsed between the first visible appearance of a leaf embryo and full leaf expansion (fig 1). Therefore, an estimate of meristematic or developing tissues as percentages of the

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Efficiency of sRNA extractions from epicotyls at each age was insured through double homogenization procedures, first in a Waring Blender and then in a VirTis "45" homogenizer at maximum speed for 2 min. Also, reextraction of the combined phenol and protein layers with 0.10 M tris (pH 7.6), 0.02 M MgCl₂, 0.06 M KCl, and 1.5 % (w/v) sodium lauryl sulfate was performed on several sRNA extractions from tissues at different ages. No additional sRNA was extracted in any case.

Purity of sRNA extractions from epicotyls at each age was determined using MAK columns according to Mandell and Hershey (21). Total sRNA preparations, as well as individual sRNA fractions from reverse phase columns, were analyzed with OD₃₀₀ peaks appearing only in the sRNA region of column eluates (as compared with MAK column fractions of total RNA preparations (4))]. In addition, ³²P-tagged epicotyl sRNA preparations (from ³²P-tagged mimosa seedlings) were combined with non-tagged total epicotyl RNA (4) and fractionated on MAK columns. Optical density₃₀₀ profiles were typical, including appropriate sRNA, DNA, and rRNA peaks. However, ³²P radioactivity peaks corresponded precisely with only sRNA OD₃₀₀ peaks.

Terminal nucleosides of all sRNA samples were determined using a 0.5 N KOH-hydrolytic procedure at 37° for 20 hr followed by a 2-dimensional paper chromatographic separation (22, 32). Chromatographic locations of spots were compared to standard nucleoside and nucleotide separations, and spectra from 220 μₐ to 300 μₐ of eluted spots were used to identify and quantify ³'OH terminal nucleosides from nucleotides.

Soluble RNA samples were discharged at 37° for 15 min in a 0.2 M glycine buffer, pH 10.1, and fractionated using reverse phase chromatography as suggested by Kelmers et al. (15). The linear NaCl eluting gradient was changed to 0.35 M to 1.05 M. All optical density peaks and shoulders were quantified using their average OD₃₀₀ times their total volume. Each fraction was then expressed as a percentage of the overall average OD₃₀₀ times total volume eluted (approximately 95 % of the original sRNA applied to each column was recovered).

All variables were analyzed statistically using an analysis of variance with age (percent mature tissues) as the independent variable. Significant differences between ages for each dependent variable were located using Duncan's Multiple Range Test.

Results and Discussion

The average percentages of mature tissues with respect to the total epicotyl (by fr wt) at each age are presented in table I. Table I also illustrates average fresh weights and dry weights per epicotyl and total RNA, DNA, sRNA, and approximated rRNA [total RNA minus total sRNA since the bulk of total RNA other than sRNA is probably rRNA (6)] as percentages of total fresh weights at each age.
age. While total RNA, DNA, rRNA, and sRNA all showed significant (5% level) declines between 11 and 14 days, the percentages of sRNA with respect to either total RNA or rRNA increased with age. This trend may suggest a greater degree of quantitative stability of sRNA than other RNA species in mature tissues (1, 13, 17, 24, 25). Baguley and Ralph (1) observed higher sRNA to rRNA ratios in Bacillus subtilis growing at decreased rates. The increasing percentages of mature tissues in mimosa epicotyls would be comparable to a net decrease in total cellular growth rates.

The significant declines in all types of nucleic acids between 11 and 14 days suggest a decline in rate of growth and differentiation during this period. However, this period did not coincide with either an initial appearance of mature tissues nor with the period of greatest increase in percentages of mature tissues. Consequently, these early declines followed by relative quantitative stability of nucleic acid fractions (particularly sRNA) are real phenomena, but are not explainable in this study.

Three-OH terminal groups of sRNA at each age were observed. Each of the 4 major possible 3'-OH terminal nucleosides were expressed as a percentage of total 3'-OH terminal nucleosides. Large variations from 100% adenosine to as low as 25% adenosine with varying quantities of cytidine, guanosine, and uridine were found between sRNA samples. However, no consistent or significant trends with age were found. This suggests that either the degree of reversible addition of terminal nucleosides (2, 5, 7, 26) does not vary with age in vivo, or that the extent of loss of terminal nucleosides is an artifact of extraction procedures (12).

Figure 2 illustrates apparent and repeatable differences in quantitative distribution of specific sRNA fractions with age or percentages of mature tissues. Significant differences (5% level) were found for the OD260 fraction numbers 5 and 6 between 11 and 14 days). OD260 fraction number 7 (increased between 11 and 20 days), and successive disappearance of OD260 fraction numbers 5 and 6 between 11 and 20 days. Based on preliminary, but not quantitative, identification of the specific tRNAs involved in each OD260 fraction, the OD260 fractions reported each represent a group of specific tRNAs.

Suggestions of changes in distribution of tRNAs have been found in bacteria and animal tissues during development and in response to changes in environmental conditions (9, 10, 14, 18, 19, 23, 27, 31, 33). Only recently have differences in quantitative distribution of tRNAs in higher plant tissues been reported (30) (Anderson, M. B. and J. H. Cherry, Translational control of protein synthesis by tRNA and aminoacyl synthetases in soybean seedlings. Paper presented at Midwest Section of American Society of Plant Physiologists, June 21, 22, 1968). However, Vold and Sypherd (30) considered whole embryos and seedlings only during the first 48 hr

## Table I. Fresh Weight, Dry Weight, Total RNA, DNA, sRNA, and Approximated rRNA During Development in Mimosa Epicotyl Tissues

<table>
<thead>
<tr>
<th>Seedling age (days)</th>
<th>Ave percentages of mature tissues in epicotyls (^1)</th>
<th>Ave fr wt per epicotyl (mg)</th>
<th>Ave dry wt per epicotyl (mg)</th>
<th>Total RNA (OD260)</th>
<th>Total DNA (OD260)</th>
<th>Total sRNA (OD260)</th>
<th>Total approximated rRNA (OD260)</th>
</tr>
</thead>
<tbody>
<tr>
<td>11</td>
<td>69.8</td>
<td>43.5</td>
<td>7.7</td>
<td>0.36±0.13(^2)</td>
<td>0.12±0.07</td>
<td>0.032±0.001</td>
<td>0.33</td>
</tr>
<tr>
<td>14</td>
<td>76.4</td>
<td>65.7</td>
<td>11.2</td>
<td>0.15±0.05</td>
<td>0.05±0.01</td>
<td>0.021±0.001</td>
<td>0.13</td>
</tr>
<tr>
<td>17</td>
<td>83.4</td>
<td>76.3</td>
<td>13.0</td>
<td>0.12±0.06</td>
<td>0.05±0.01</td>
<td>0.023±0.002</td>
<td>0.10</td>
</tr>
<tr>
<td>20</td>
<td>86.8</td>
<td>94.0</td>
<td>17.0</td>
<td>0.11±0.01</td>
<td>0.05±0.01</td>
<td>0.020±0.001</td>
<td>0.09</td>
</tr>
</tbody>
</table>

\(^1\) See text for explanations.

\(^2\) Mean ± 1 SE.

![Figure 2](https://example.com/figure2.png)

**Fig. 2.** Typical OD260 profiles representing mimosa epicotyl sRNA fractionation. The numbers assigned to each OD260 peak or shoulder are comparable to the numbers discussed in the text. A) Eleven-day seedlings. B) Seventeen-day seedlings.
of germination, while Anderson and Cherry compared tRNA distribution between different components of early germinating seedlings. The study reported herein considered only 1 plant component during later stages of germination and therefore considered development of mature tissues. The meristematic and developing tissues should have been qualitatively similar at each age based on gross and micromorphological observations (fig 1). However, the fully-developed or relatively mature tissues differed both quantitatively and qualitatively at each age (fig 1). Consequently, sRNA fractions which varied with increasing percentages of mature tissues likely represent their association with mature tissues (the opposite being true for decreases).

This study supports changes in either quantitative or qualitative characteristics of some sRNA fractions during development which could alter patterns of protein synthesis and direct growth and developmental patterns in higher plants. Further studies specifically identifying the tRNA (sRNA) fractions which change with increasing percentages of mature tissues, and characterizing these changing tRNA fractions (e.g. degree of chargability, secondary structures) are currently underway.

Acknowledgment

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Literature Cited

26. Starr, J. L. and D. A. Goldthwaite. 1963. The incorporation of nucleotides into amino acid transfer ribonucleic acid. I. The partial purification
and properties of an enzyme catalyzing the incorporation of adenylic acid into the terminal position. J. Biol. Chem. 238: 682-89.


