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Short Communication

Hormonal Control of Senescence of Bean Endocarp: Auxin-suppression of RNase[1]

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Time course studies indicate that auxin prevents senescence of excised bean endocarp (*Phaseolus vulgaris*, L., var. Kentucky Wonder pole beans) through an effect on metabolism of RNA. Firstly, during aging of tissue sections in absence of auxin degradation of RNA precedes degradation of DNA and protein; these degradations are prevented by auxin (4, 5, 7). Secondly, during short (2-hr) incubations of tissue sections auxin enhances synthesis of RNA before enhancement of protein synthesis can be detected (Sacher, unpublished results). Additionally, during incubations of 15 to 24 hr auxin causes a substantial net synthesis of both RNA and protein, which is inhibited by actinomycin D (6). That net synthesis is manifested in each of 6 subcellular fractions (8) indicates that auxin controls synthesis of all cellular RNA and protein.

As a result of an auxin-induced net synthesis of RNA and a loss of RNA in absence of auxin, there is a large difference in RNA content between water- and auxin-aged tissue. Recent studies (with Dr. Ann Matthysse, unpublished) indicate that this difference cannot be accounted for when chromatin is prepared from endocarp aged 15 hr with and without auxin, and assayed for template activity in presence of *E. coli* RNA polymerase. In 3 experiments with separate batches of tissue there was no difference in template activity between water- and auxin-aged tissue, notwithstanding auxin-aged tissue having 30 to 80 % more RNA than water-aged tissue.

In this paper are presented results showing that auxin causes a substantial decrease in RNase during aging of tissue sections. This action of auxin is consistent with hitherto observed effects of auxin on RNA and protein synthesis during aging of bean endocarp.

Bean endocarp sections were prepared and used for assay of RNase and content of RNA and protein at zero time and after aging for 15 hr with and without auxin (α-naphthaleneacetic acid, NAA). The sections were washed in running tap water for 20 min, blotted and replicate batches (1 g fr wt) weighed and then aged in darkness in Petri dishes on filter paper wetted with water or a solution of 10 μg/ml NAA. The tissue samples were then washed in water for 20 min, blotted, homogenized, the homogenate centrifuged at 30,000*g* at 1° for 20 min or 105,000*g* for 90 min and the supernatant fraction used as the enzyme solution. Reaction mixtures contained enzyme and 2 mg/ml yeast RNA (Calbiochem) adjusted to pH 5.0 with acetate buffer. Incubation was at 40° for 60 min. The reaction was stopped by addition of an equal volume of a solution of 0.25 % uranyl nitrate and 2.5 % trichloroacetic acid (3). After centrifugation the supernatant fraction was assayed for nucleotides at 260 mμ, and corrected for acid soluble nucleotides in the enzyme solutions. Total RNA and protein were extracted from other replicates of tissue and assayed as described previously (6).

During aging of sections of bean endocarp auxin caused a 36 to 40 % decrease in RNase activity as compared with water-aged tissue (table I). Correlated with this difference in RNase there was 25 % more RNA in auxin- than in water-aged tissue, which is owed to a loss of RNA in absence and an increase in RNA in presence of auxin. As is usual for this tissue (6, 8), an increase in protein attends the auxin-induced increase in RNA, and in water-aged tissue there is no degradation of protein until a substantial degradation of RNA has occurred (table I).

There is variability in RNase content of water-aged tissue as compared with the zero time controls. The results of 4 other experiments show an average decrease in RNase of 42 % during water-aging of tissue sections for 15 to 20 hr. In these experiments there was a 45 % greater loss of RNase in auxin-aged than in water-aged tissue. Among 3 other experiments there was an increase in RNase in water-aged tissue in 2 and no change in RNase in the third. Auxin, however, consistently causes a substantial decrease in the amount of RNase irrespective of whether an increase or decrease occurs in RNase in absence of auxin.

Assays of RNase in 0.1 M NaCl extracts of pre-

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Table I. *Effect of Auxin on Soluble RNase Activity of Bean Endocarp Tissue Sections During Aging for 15 Hr*

Duplicate 1 g fr wt samples of endocarp sections incubated for 15 hr in 20 μg/ml streptomycin sulfate with and without 10 μg/ml NAA. Enzyme extraction and assay procedures described in text.

Expt	Treatment	μg RNA[1] hydrolyzed per g fr wt per hr	μg RNA[1] per g fr wt	μg protein[1] per g fr wt
I	Zero time	4505 ± 40	443 ± 6	3169 ± 16
	H_2O	3936 ± 140	413 ± 3	3267 ± 17
	NAA	2535 ± 245	506 ± 2	3673 ± 0
II	Zero time	3710 ± 200	547 ± 2	...
	H_2O	3780 ± 50	475 ± 4	...
	NAA	2254 ± 14	602 ± 26	...

[1] Means and SE's of duplicate samples. RNase activity assayed in 105,000*g* supernatant fraction.

cipitate fractions of homogenates from water- or auxin-aged endocarp show that auxin causes a similar decrease in particle-bound RNase and soluble RNase. These results are consistent with the interpretation that the auxin-induced decrease in soluble RNase is not owed to auxin causing inactivation of RNase by mediating its reversible binding to cellular particles. Secondly, auxin (0.1–10 μg/ml) has no direct effect on RNase activity of homogenates during an exposure of 2 hr, which includes the grinding and enzyme assay periods. This, however, does not exclude the possibility that auxin inhibits RNase by interacting directly with RNase specific m-RNA or RNase *per se in vivo*. Thirdly, assays of RNase activity in mixtures of enzyme solutions from water- and auxin-aged tissues show no evidence of an inhibitor of RNase in the enzyme solution from auxin-treated tissue, as the activity of the mixture was equal to that of the 2 enzyme solutions. The possibility is being investigated that auxin affects RNase turnover at the level of either transcription or translation.

It may be concluded that auxin suppresses the amount or activity of RNase, which is correlated with auxin causing an increase in both RNA and protein and preventing senescence. We have not determined yet whether auxin also suppresses the amount of other hydrolases (*e.g.*, DNase, phosphatase, protease), but a general suppression of enzymes by auxin is unlikely in view of the large increase in soluble protein caused by auxin in this tissue (8). Also, assays of 3 enzymes during aging of endocarp tissue sections showed that auxin caused an increase of 22 % in 2 of them and maintained the level of the third (4). Other plant hormones affect turnover of specific hydrolases during senescence. For barley endosperm gibberellin enhances specifically a large synthesis of α-amylase, protease and RNase without affecting synthesis of bulk protein, and this synthesis is inhibited by abscisin (2). Kinetin suppresses an increase of soluble and particle-bound RNase in excised leaves of *Avena* (11), and of RNase and other spherosome-bound hydrolases in tobacco leaves (1) and accelerates degradation of nucleases in excised barley leaves (10).

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