RNA-DNA HYBRIDIZATION COMPETITION STUDIES ON SENESCING BARLEY LEAVES

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SUMMARY

No detectable differences in RNA populations from 7-day-old (young) and 17-day-old (senescent) first seedling leaves of barley were found by RNA-DNA hybridization competition experiments. It is suggested that enzymatic changes during senescence may result from minor changes at the transcription level or alterations at the translation level.

INTRODUCTION

Excised leaves floated on water senesce rapidly (Srivastava, 1967b). In barley the first seedling leaf is also induced to senesce when the plant is about 7 days old and the second leaf starts to grow. Senescence in an excised or intact leaf is characterized by declines in the amounts of chlorophyll, nucleic acids and proteins and by alterations in the rate of incorporation of precursors into nucleic acids and proteins (Atkin and Srivastava, 1970; Srivastava, 1967b; Srivastava and Atkin, 1968). Since these studies as well as others (Osborne, 1965) suggested that senescence may involve changes at the transcription level the population of RNA's in young (7-day-old) and senescent (17-day-old) first seedling leaves of barley was examined by RNA-DNA hybridization competition experiments. The results of this work are described in this paper.

MATERIALS AND METHODS

Barley (Hordeum vulgare L. var. Wolfe) plants were grown in soil in pots on greenhouse benches and given 16 hours of light per day. Leaves used for RNA labelling were sterilized (Srivastava and Atkin, 1968) by treatment with 10% Clorox (3.5 minutes) and then rinsed extensively with sterile distilled water. Leaf sections (0.5 cm) from 10 g of first seedling leaves from 17-day-old plants were repeatedly infiltrated for 20 minutes in 100 ml of the sterile incubation medium (1 mC of Na$_2$H$^{32}$PO$_4$ in 100 ml of 0.1 mM ammonium citrate, pH 6.0) and subsequently incubated on a shaker for 220 minutes. Leaf sections from only 6 g of leaves from 7-day-old plants were incubated in 60 ml of medium containing 1 mC of $^{32}$P. Rapidly labelled RNA has been found (Srivastava and Atkin, 1968) to be predominantly labelled under these conditions. About 20 g leaves from 7- or 17-day-old plants were used for the isolation of unlabelled (cold) nucleic acids.

The nucleic acids were isolated by a phenol-sodium lauryl sulphate-bentonite procedure and were fractionated by sucrose-gradient centrifugation (Srivastava and Atkin, 1968). Fractions 1–10, 11–20, 21–32 and 42–52 (from the bottom of the tube) were pooled to give RNA fractions A (heavier than 23 S RNA), B (23 S region), C (16 S RNA), and D (16 S RNA).
region) and D (t-RNA region) respectively (Srivastava and Atkin, 1968). Fractions 33–41 containing DNA were discarded. The RNA fractions pooled from several gradients were precipitated with ethanol and the RNA precipitates dissolved in 5 x SSC (SSC, Standard Saline Citrate—0.15 M NaCl, 0.015 M Na citrate, pH 7.0).

If total RNA free from DNA was needed, the nucleic acid preparation was not subjected to density gradient centrifugation but was treated with crystalline deoxyribonuclease (400 µg in 40 ml of 0.02 M tris-HCl, pH 7.6 containing 1 mM MgCl₂) for 30 minutes at 37°C. The deoxyribonuclease was removed by deproteinization with phenol and RNA precipitated with ethanol and dissolved in 5 x SSC. Total RNA or the RNA fractions were dialysed against 5 x SSC and after determining the O.D.₂₆₀ₐₚ and the trichloroacetic acid precipitable radioactivity on aliquots of RNA fractions they were used for RNA–DNA hybridization.

The chromatin from first seedling leaves (200 g) of 7-day-old plants was prepared according to the procedure of Huang and Bonner (1962) and the DNA prepared from the chromatin by deproteinization with phenol. The DNA was finally purified by chromatography on a methylated albumin coated Hyflosupercel column (Srivastava, 1967a).
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The denaturation of DNA, its immobilization on nitrocellulose filters, the formation of RNA–DNA hybrids and the post-annealing ribonuclease treatment were carried out as described by Gillespie and Spiegelman (1965). Fifty micrograms of denatured DNA per filter were used. Usually, 3–4 DNA filters and one blank were incubated (18 hours at 67° C) in each scintillation vial containing increasing amounts of radioactive RNA in 2 ml

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**Fig. 2.** Saturation curves for $^{32}$P–RNA fractions A, B, C and D from 7- and 17-day-old leaves. Fifty μg DNA filters and increasing amount of $^{32}$P–RNA were used. Cpm μg RNA for A, B, C and D respectively from 7-day-old leaves were 453, 134, 123, 275 and from 17-day-old leaves were 1895, 1054, 528, 1647.

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**Fig. 3.** Competition by unlabelled RNA fractions from 7-and 17-day-old barley leaves in hybrid formation between $^{32}$P–RNA fractions (A, B, C and D) from 7-day-old leaves and barley DNA. Fifty μg DNA filters were used. Specific activity of $^{32}$P–RNA fractions as in Fig. 2.
of 5 x SSC. Retention of DNA on nitrocellulose filters during immobilization was 90% with no measurable loss of DNA during annealing. In hybridization competition experiments, 50 μg DNA filters were incubated with fixed amounts of radioactive RNA and increasing amounts of unlabelled RNA in 2 ml of 5 x SSC. All radioactivity measurements were made in a Nuclear Chicago 720 Counter, using toluene based scintillation fluid (Srivastava and Atkin, 1968).

RESULTS AND DISCUSSION

The data in Fig. 1 (left) show that the saturation of barley DNA with radioactive RNA from 7- or 17-day-old barley leaves was not complete with RNA/DNA ratios up to about 6:1. This behaviour suggests that the labelled RNA contains a wide variety of molecular species many of which are present in very small proportions. Fig. 1 (right) shows that the RNA populations isolated from 7- or 17-day-old leaves compete equally well with the labelled RNA from 7- or 17-day-old leaves whereas yeast t-RNA did not show any competition.

Since competition experiments on different fractions of RNA may be more sensitive than those on total RNA, the experiments on RNA fractions A, B, C and D were conducted. As found with total RNA, saturation of DNA and RNA fractions was not achieved except possibly with fraction C (Fig. 2). The data in Figs. 3 and 4 show that the unlabelled RNA fractions A, B, C and D from 7- or 17-day-old leaves competed equally well against the respective labelled RNA fraction from 7- or 17-day-old leaves.

The above results show no detectable differences in the populations of RNA between 7-day-old (young) and 17-day-old (senescent) leaves which agrees with the similar 32P-nucleotide composition of RNA fractions (Srivastava and Atkin, 1968) and nearly similar
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template activity of chromatin (Srivastava, 1968) from 7- and 17-day-old leaves. These results of hybridization experiments should, however, be interpreted with caution. The existence of repeated sequences in DNA of higher plants and animals has been demonstrated (Britten and Kohne, 1968). Under the conditions used for hybridization the rapidly labelled RNA reacts primarily with the redundant fraction of the genome and unique DNA base sequences do not contribute to a very large proportion of the duplex formation (Walker, 1968). Hybridization competition experiments provide only minimal estimates of the extent of differences among various populations of RNA molecules and a lack of discrimination does not necessarily imply identity (Church and McCarthy, 1968a, b). The results of this study, therefore, do not prove that all RNA molecules synthesized in 17-day-old leaves are also produced in 7-day-old leaves but they do indicate that major differences between RNA populations from young and senescent barley leaves do not exist. Increase of chromatin-associated nuclease activity in barley leaves during senescence has been reported (Srivastava, 1968) and it is possible that either minor changes at the transcription level or alterations at the translation level are involved in the onset of senescence. The control of senescence at the translation level seems specially attractive since cytokinins which occur in certain t-RNA's (Skoog and Armstrong, 1970) can retard senescence and changes in t-RNA species in soybean cotyledons (Bick et al., 1970) and t-RNA methylation in barley leaves (unpublished data) during senescence have been found.

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REFERENCES


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