Phytochemistry, 1973, Vol 12, pp 1891 to 1896 Pergamon Press Printed in England.

INHIBITION OF DNA TRANSCRIPTION BY γ-IRRADIATION

VALGENE L DUNHAM and JOE H CHERRY
Department of Horticulture, Purdue University, West Lafayette, IN 47907, U S A

(*Received* 20 *November* 1972 *Accepted* 20 *February* 1973)

Key Word Index—*Beta vulgaris*, Chenopodiaceae, sugar beet, RNA polymerase, γ-irradiation, transcription

Abstract—Exposure of sliced sugar beet root to γ-rays has been shown to inhibit the synthesis of ribosomal and possibly messenger RNA as demonstrated by decreased incorporation of ^{3}H-uridine In determining major sites of radiation damage to the transcriptional process, chromatin-bound RNA polymerase was found to be more sensitive to *in vitro* irradiation than *in vivo* irradiation RNA polymerase activity solubilized from irradiated tissue was inhibited by 60% when compared to solubilized enzyme from control tissue Control soluble RNA polymerase was inhibited by 24% when irradiated DNA was employed as template However, the enzyme from irradiated chromatin was inhibited by 60% utilizing control DNA Irradiation of the soluble enzyme *in vitro* also resulted in a severe inhibition of activity The data indicate that the inhibition of RNA synthesis observed following exposure of tissue to γ-rays may be primarily a result of inhibited RNA polymerase activity

INTRODUCTION

PREVIOUS research in this laboratory has shown that dramatic increases in respiration,[1] protein and nucleic acid synthesis[2] and the activities of various enzymes are associated with washing of sliced sugar beet root One of the enzymes whose activity increases with washing was found to be chromatin-bound RNA polymerase [1] Since the exposure of plant tissue to ionizing radiation has been shown to reduce nucleic acid synthesis,[3] an investigation was initiated to determine if the observed inhibition of RNA synthesis might be due to damaged RNA polymerase, DNA template, or both A previous report of this study[2] indicated that nucleic acid synthesis during washing of beet tissue was reduced and that no increase in chromatin-bound RNA polymerase activity occurred during washing of irradiated tissue Chromatin DNA template availability, as measured with saturating levels of *E coli* RNA polymerase, also increased during washing and was inhibited by the same dosage of irradiation but to a lesser extent than the inhibition of RNA polymerase activity Post-wash irradiation reduced chromatin-bound RNA polymerase activity less than pre-wash irradiation These data indicated that exposure of sugar beet tissue to γ-rays not only inhibited the increase in chromatin-bound RNA polymerase activity and template availability that occurred during washing, but also apparently altered the enzyme and the DNA template

There are several possible sites of radiation damage γ-Rays have been shown to alter protein conformation[4,5] as well as to break DNA strands and alter nucleic acid bases [6,7]

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To investigate some of these possibilities, studies involving *in vitro* irradiation of chromatin, solubilization of RNA polymerase from the DNA template, and *in vitro* irradiation of both the solubilized enzyme and purified DNA were initiated Data reported here indicate that ribosomal RNA and possibly messenger RNA synthesis are inhibited by γ-rays RNA polymerase activity (both chromatin-bound and soluble) was inhibited by irradiation *in vivo* and *in vitro* The activity of the enzyme was reduced when transcribing irradiated DNA, but to a lesser extent than when irradiated enzyme was employed to transcribe undamaged DNA

RESULTS

Radiation Effects on Total RNA Synthesis

As reported previously,[2] exposure of sugar beet tissue to irradiation (300 krad) followed by a 6-hr wash period and extraction of nucleic acids inhibited the incorporation of ^{3}H-uridine into total nucleic acids by 60% To determine what types of RNA were synthesized more slowly following irradiation, total nucleic acids were extracted following ^{3}H-uridine labeling and subjected to MAK column chromatography Although only a slight inhibition was observed in the 4*S* and 5*S* regions of the MAK profile, significant inhibition was observed in the light ribosomal and heavy ribosomal and DNA-like RNA regions (Fig 1) The designation of the various fractions are the same as those previously employed [8] Based on the area under the curves in the profile, light ribosomal RNA synthesis was inhibited by 20% whereas heavy ribosomal and *m*RNA (inseparable on MAK columns) synthesis was inhibited by 47% No differences were observed in the firmly bound material eluted from the column with 1 5 N NH_4OH (not included in profile)

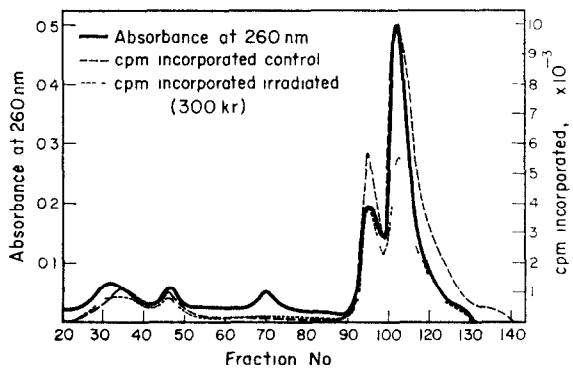

FIG 1 EFFECT OF IRRADIATION ON INCORPORATION OF ^{3}H-URIDINE INTO RNA
Tissue (15 g) was irradiated (300 krad) and washed for a total of 6 hr, the last 2 hr of this wash in the presence of ^{3}-H-uridine (2 Ci/mmol) at 10 Ci/ml of washing buffer Total nucleic acids were extracted and the MAK column eluted as described in Experimental

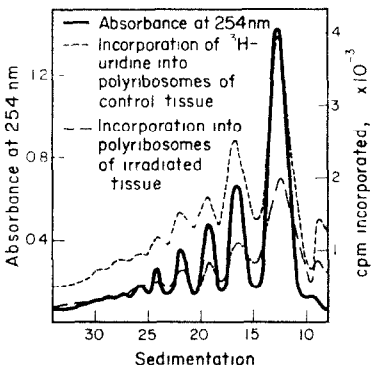

FIG 2 EFFECT OF IRRADIATION ON POLYRIBOSOME SYNTHESIS
Polyribosomes were isolated and profiles monitored as described in Experimental

Radiation Effects on Polyribosome Synthesis

It has been shown that washing of sugar beet tissue results in an increase in polyribosome synthesis [1] From an initial value of 35% polyribosomes (100% equal to monoribosomes

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plus polyribosomes) in unwashed tissue, formation of polyribosomes increased to a maximum of 60% polyribosomes after 6–8 hr of washing To determine the effect of irradiation and of the observed inhibition of ribosomal and *m*RNA synthesis on polyribosome synthesis, incorporation of ^{3}H-uridine into polyribosomes was followed Exposure of tissue to 400 krad of radiation severely inhibited (by 46%) the incorporation of ^{3}H-uridine into polyribosomes (Fig 2) In addition to inhibited ribosomal RNA and messenger RNA synthesis indicated by the above data, increased incorporation into subunits may have resulted from impaired polyribosome formation or the direct breakdown of labeled polyribosomes into subunits The specific activities of peak tubes (cpm/absorbance unit) indicate that incorporation of ^{3}H-uridine into polyribosomes was inhibited by 10% more than incorporation into monoribosomes However, this may be a function of the larger amount of monoribosomes originally present in unwashed tissue (dilution effect)

Radiation Effects on Ribonuclease Activity

Ribonuclease activity associated with the chromatin preparation was studied both during washing and as affected by γ-rays Little RNase activity was associated with the chromatin preparation (Δ absorbance 0 005–0 010 min/mg/protein) This finding is in agreement with chromatin-bound RNA polymerase time-course experiments in which no loss of RNA was observed Since ribonuclease activity was slightly inhibited in irradiated tissue, the observed inhibition of RNA polymerase activity from irradiated tissue could not be due to enhancement of ribonuclease activity

TABLE 1 COMPARISON OF *in vivo* AND *in vitro* IRRADIATION OF THE CHROMATIN SYSTEM

Dosage (krad)	% of control RNA polymerase activity *in vivo*	*in vitro*	Dosage (krad)	% of control RNA polymerase activity *in vivo*	*in vitro*
50	71	59	300	48	18
100	62	41	400	38	19
200	54	30			

In vivo irradiation preceded a 20-hr wash period after which chromatin was isolated *In vitro* irradiation followed the 20-hr wash period and isolation of the chromatin Control activity was 66 pmol ^{3}H-UMP incorporated/100 μg DNA/20 min

In vivo *and* in vitro *Irradiation of Chromatin*

The observed inhibition of chromatin-bound RNA polymerase activity by irradiation could be due to an inhibition of the synthesis of the enzyme or an alteration of either the enzyme or the template, or both To investigate this problem, chromatin was extracted following washing and then exposed to radiation Table 1 indicates a severe inhibition of RNA synthesis with increasing dosages of γ-rays beyond 100 krad Increasing dosage of γ-rays (*in vitro*) was more deleterious than *in vivo* irradiation, as measured by chromatin-bound RNA polymerase activity It would appear, therefore, that γ-rays altered the function of the enzyme or other chromatin components, such as the DNA template in RNA synthesis. Since *in vitro* irradiation was more damaging to the chromatin system, the tissue must provide protection from free radical attack

In vivo *Irradiation Effects on Soluble RNA Polymerase*

Because of the differences in sensitivity to *in vitro* and *in vivo* irradiation of chromatin, the possibility existed that the enzyme was not inhibited *in vivo* and that the observed inhibition was due to altered DNA or other chromatin factors Experiments were performed in which sugar beet tissue was irradiated (300 krad), chromatin extracted, and the enzyme solubilized The enzyme activity extracted from irradiated tissue was reduced to about 40% of the control

Soluble RNA Polymerase Activity from in vitro *Irradiated Chromatin*

The major problem associated with these studies is the elucidation of the site(s) of radiation damage This problem has been approached by solubilizing the RNA polymerase from chromatin and measuring changes in activity after exposure of the enzyme or DNA to irradiation RNA polymerase preparations from irradiated chromatin transcribed control or irradiated sugar beet DNA less efficiently than did control enzyme transcribe irradiated DNA (Table 2) It would appear from these data that the observed inhibition of RNA synthesis following irradiation is primarily a result of reduced RNA polymerase activity, and, secondarily a loss in template function Furthermore, maximal inhibition was obtained when irradiated enzyme was employed, regardless of whether the DNA template was irradiated

TABLE 2 EFFECTS OF *in vitro* IRRADIATION ON SOLUBLE RNA POLYMERASE ACTIVITY

Enzyme	DNA	% inhibition	Enzyme	DNA	% inhibition
Control	Control	0	Irradiated	Control	59
Control	Irradiated	24	Irradiated	Irradiated	57

Sugar beet DNA was isolated by a procedure[1,2] which included pronase and ribonuclease treatment followed by hydroxylapatite column chromatography The enzyme was solubilized from control or irradiated chromatin as described in Experimental Control activity was 200 pmol ^{3}H-UMP incorporated/mg protein/10 min

In vitro *Irradiation of Soluble RNA Polymerase*

If the observed inhibition of soluble and chromatin-bound RNA polymerase following *in vivo* irradiation is not an artifact resulting from a change in the solubility of the enzyme following treatment, then it would appear that damage to the RNA polymerase molecule is one of the causes of decreased RNA synthesis To further substantiate these findings, the enzyme was extracted from control tissue and irradiated *in vitro* RNA polymerase activity was inhibited with increasing levels of irradiation in a manner similar to that found with the chromatin-bound enzyme On comparison with control activity (100%), 100 krad reduced activity to 68%, 200 krad to 50% and 300 krad to 48%

DISCUSSION

Inhibition of the synthesis of ribosomal RNA and possibly also of messenger RNA following γ-ray treatment is indicated in the present work One of the consequences of such inhibition may be the observed reduction of polyribosomes in irradiated tissue As reported earlier,[2] γ-rays inhibit protein synthesis as measured by ^{3}H-leucine incorporation into

protein It would appear that this reduction in protein synthesis might be due, at least in part, to the presence of fewer polyribosomes Irradiation may also affect the synthesis or conformation of numerous factors that have been shown to be involved in translation [9] These possibilities, together with the above data, indicate that irradiation of plants prior to or immediately following a stress situation (wounding), or seed germination (processes involving increases in polyribosome synthesis) may inhibit growth or responses by an inhibition of ribosomal RNA synthesis

An argument might be made that differential extractability of the enzyme following treatment would result in the observed inhibition of RNA polymerase In addition to the data presented here, we have found no differences in extractability of either protein or enzyme activity from either tissue or chromatin following irradiation treatment

We conclude that γ-rays inhibit RNA polymerase activity (both chromatin-bound and soluble) *in vivo* and *in vitro* Recently several groups have shown that multiple RNA polymerases exist in eukaryotic cells [10,11] It is expected, therefore, that the consequences of irradiation might vary according to the concentration or activity of the type(s) of RNA polymerase present Based on other experiments,[12] about 85% of the total RNA polymerase activity solubilized from sugar beet chromatin is not sensitive to α-amanitin, a specific inhibitor of RNA polymerase II [13] RNA polymerase I, about 70% of the total activity (based on inhibitor studies), is not sensitive to α-amanitin and has been shown to be of nucleolar origin and to transcribe ribosomal cistrons [10] Therefore, based on the enzyme data presented elsewhere[12] and the fact that ribosomal RNA synthesis is inhibited, it appears that one of the critical sites involved in irradiation damage is the alteration of RNA polymerase I resulting in a reduction of ribosomal RNA synthesis However, the data do not eliminate the possibility that RNA polymerase II is also inhibited, resulting in a reduction of messenger RNA synthesis Such an inhibition could contribute to the reduced incorporation of ^{3}H-uridine into polyribosomes and into the heavy ribosomal-*m*RNA region of the MAK profile

It is interesting to note that the dose response curve for the *in vitro* irradiation of RNA polymerase is curvilinear This has recently been shown to be true also for purified *E coli* RNA polymerase [14] The curvilinear nature of the curve was considered to be a result of preferential inactivation of the σ-factor of the bacterial enzyme

Although the data indicate that irradiation of RNA polymerase results in a severe inhibition of activity, the DNA template is also altered (Table 2) Such damage may be of just as much consequence to the cell as an inhibited enzyme Previous reports have shown that exposure of DNA to γ-rays has resulted in strand lesions and chemical alteration or destruction of the bases, especially thymine [15] Therefore, the RNA transcribed from damaged template may result in altered proteins, mutations (if damage remains non-repairable) and perhaps cell death

EXPERIMENTAL

Material Sugar beet root tissue was cut into small pieces, rinsed and washed in sterile phosphate buffer, pH 6 5, as described previously [2] All tissue or preparations (chromatin, enzyme, DNA) were irradiated in a

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^{60}Co source at an intensity of 8 3 krad/min Beet tissue and preparations were maintained at 4° during irradiation

Nucleic acid synthesis To determine the effects of γ-rays on the synthesis of various species of RNA, 15 g of chopped tissue were irradiated (300 krad) and washed for a total of 6 hr, the last 2 hr of this wash in the presence of ^{3}H-uridine Total nucleic acids were extracted[16] from the tissue following the washing period, repeatedly rinsed with buffer containing unlabeled uridine, and then subjected to MAK (methylated albumin kieselguhr) column chromatography RNA was eluted from the column with a linear gradient of NaCl (0 2–1 0 M) and the fractions monitored at 260 nm Aliquots of each fraction were precipitated with 10% TCA (trichloroacetic acid) and 0 1 mg of herring sperm DNA The ppts were collected on glass filters (Whatman GF/A), washed with 5% TCA repeatedly and the radioactivity determined

Polyribosome isolation Chopped sugar beet tissue (20 g) were frozen in liq N_2 and ground in a pestle and mortar (pre-cooled to −20°) in the presence of acid-washed sand Polyribosomes were isolated as described [1] All solutions, which contained 2-mercaptoethanol and deoxycholate, were prepared just before use Exposure of tissue to γ-rays (400 krad) preceded an 8-hr wash, the last 2 hr of this wash in the presence of ^{3}H-uridine, as described above The final sucrose gradient (10–34%) was monitored at 254 nm Fractions were collected and radioactivity determined as described above

Chromatin preparation and solubilization of RNA polymerase Chromatin was prepared as described previously,[2] made up to 50% glycerol and stored in liq N_2 without significant loss of activity until use Preliminary experiments involving *in vitro* irradiation of chromatin solutions indicated that 50% glycerol negated the effects of irradiation Therefore, subsequent experiments in which chromatin was irradiated were performed immediately after isolation and in the absence of glycerol RNA polymerase was solubilized from the chromatin [12] Irradiation experiments involving the enzyme and calf thymus or sugar beet DNA were performed in the absence of glycerol Assays of chromatin-bound RNA polymerase[2] and soluble RNA polymerase[12] were as described

Ribonuclease studies Nuclease activity associated with chromatin and soluble RNA polymerase preparations were assayed employing a modification of a procedure described by Anfinsen *et al* [17] Aliquots of chromatin or enzyme preparations were incubated at 37° for 30 min with 2 mg RNA (yeast-type XI, Sigma) The reaction (2 5 ml-total vol) was stopped by the addition of 0 25 ml of 2 5 M perchloric acid containing 0 75% uranyl acetate The tubes were chilled and then centrifuged The clear supernatants were read in a spectrophotometer at 260 nm All assays were performed at the pH of the chromatin and soluble RNA polymerase preparations (pH 7 9) and other known nuclease pH optima All chemicals used were reagent grade ^{3}H-UTP and ^{3}H-uridine were purchased from Schwarz Bioresearch, Orangeburg, N Y DNA determination was by the diphenylamine colorimetric procedure of Burton,[18] and protein determination was by the Lowry[19] method

Acknowledgements—We wish to thank Mrs Jane Thurman for her skilled technical assistance and Dr Lidia Wasilewska for the preparation of sugar beet DNA This work was supported by A E C contract 000-1313-33 This report is journal paper 4771 of the Purdue Agriculture Experiment Station

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