TRANSIENT ACTIVATION OF POTATO CHROMATIN BY GAMMA IRRADIATION

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1. Introduction

Potatoes after harvest are dormant for about 20 days. Dormancy can be defined as the absence of bud growth due to certain chemical and physical conditions in the tuber. The parameters influencing this state are not well understood. However, recent studies by Tuan and Bonner [1] and also by Rappaport [2, 3] have shown that termination of bud rest in potatoes is accompanied by the capacity of the bud tissue to synthesise DNA. When dormancy is terminated either by passage of time or as a result of the application of gibberellic acid (GA) or ethylene chlorohydrin, the awakened buds are able to synthesise new RNA. These observations led Tuan and Bonner [1] to propose that during dormancy the genome is repressed and thus, the DNA and RNA synthesis are inhibited. These findings were confirmed by Rappaport and Wolf [3].

Gamma irradiation is employed successfully to prolong the state of dormancy in potatoes. But how this is achieved is not clearly understood. In an attempt to study the mechanism of sprout inhibition, it was found that gamma irradiated potatoes exhibited increased asparagine synthetase activity [4]. Later studies have proved that the enhancement of activity is due to de novo synthesis of the enzyme protein [5]. This activation takes place in the bud tissue also. Further evidence for the radiation induced transient activation of template activity of potato chromatin is presented here.

2. Experimental

Potatoes used in these experiments were up-to-date variety, grown locally, and were freshly harvested. Irradiation was done in a Gamma Cell 220 and the tubers received 10 Krad dose at a dose rate of 1330 rads/min. Buds were excised with a stainless steel tube, 3 mm diameter, having a sharpened edge. Non-bud region was separated using a razor blade. This block was further sliced into very fine pieces and used for experiments.

2.1. *Incorporation of [2-^{14}C]uracil into RNA and DNA*

30 buds were incubated in 3 ml reaction mixture containing 0.15 μmoles of [2-^{14}C]uracil (specific activity 137 Ci/mole) and 3 μg pencillin. The reaction mixture was incubated for 2.5 hr at 25°. Actinomycin D conc. was 10 μg/ml. RNA and DNA were isolated according to the procedure of Tuan and Bonner [1]. RNA and DNA were determined by measuring absorption at 260 nm and also colorimetric estimation using orcinol and by diphenylamine reaction described by Burton [6], respectively. Radioactivity was determined in a Beckman liquid scintillation counter.

2.2. *Effect of time lapse after irradiation on incorporation of [2-^{14}C]uracil into DNA and RNA*

The buds were excised at 0, 5, 15, 24 and 48 hr after irradiation from irradiated and control tubers. Radioactivity and content of RNA and DNA were determined as described above.

Table 1

[2-^{14}C] Uracil incorporation into DNA and RNA of irradiated potato buds.

Experiment	Specific activity (cpm/mg) RNA	DNA
Control	140	180
Control + Actinomycin D (10 μg/ml)	160	140
Irradiated	4375	2260
Irradiated + Actinomycin D (10 μg/ml)	520	1980

Buds were pre-incubated for 10 min in the presence of actinomycin D and [2-^{14}C] uracil was then added. $T_{1/2}$ pre-incubation did not in any way affect the incorporation in the experiment without actinomycin D. The experiment was commenced within 5 min after irradiation.

2.3. *Incorporation of [^{3}H] thymidine into DNA*

50 buds were incubated in 5 ml reaction mixture containing 5 μCi of [^{3}H] thymidine (specific activity 6780 Ci/mole) and 5 μg penicillin for 2.5 hr at 25°. DNA was isolated from these buds according to the procedure by Stern [7]. After preparation the DNA was dissolved in 5 ml 0.15 M NaCl containing 0.015 M sodium citrate and an aliquot was taken for counting. Mitomycin C was added to the reaction mixture 8 μg/ml level.

2.4. *Template activity of potato chromatin*

Chromatin was isolated and purified from excised buds according to the method described by Huang and Bonner [8]. RNA polymerase was prepared from *E. coli* using the procedure of Chemberlin and Berg [9]. Fraction 3 was used in these experiments. The reaction mixture for assay consists of 0.1 μmole each of UTP, CTP, GTP and [8-^{14}C] ATP (specific activity 4 Ci/7.5 moles). 0.25 μMoles Tris-HCl pH 8.0, 100 μmole $MgCl_2$, 0.25 μmole $MnCl_2$, 3 μmole β-mercaptoethanol, RNA polymerase 30 μl, chromatin or calf thymus DNA 0.1 ml, 25 μg DNA and water to 0.5 ml. The incubation was done for 10 min at 37°. The reaction was stopped with 5% TCA and the precipitate washed well and dissolved in 2 N NH_4OH. The final volume was adjusted to 0.5 ml. Aliquots were taken for counting. A blank without RNA polymerase or chromatin was always run parallel and blank counts were subtracted. Without DNA the enzyme showed 0.5 μmoles nucleotide incoporation.

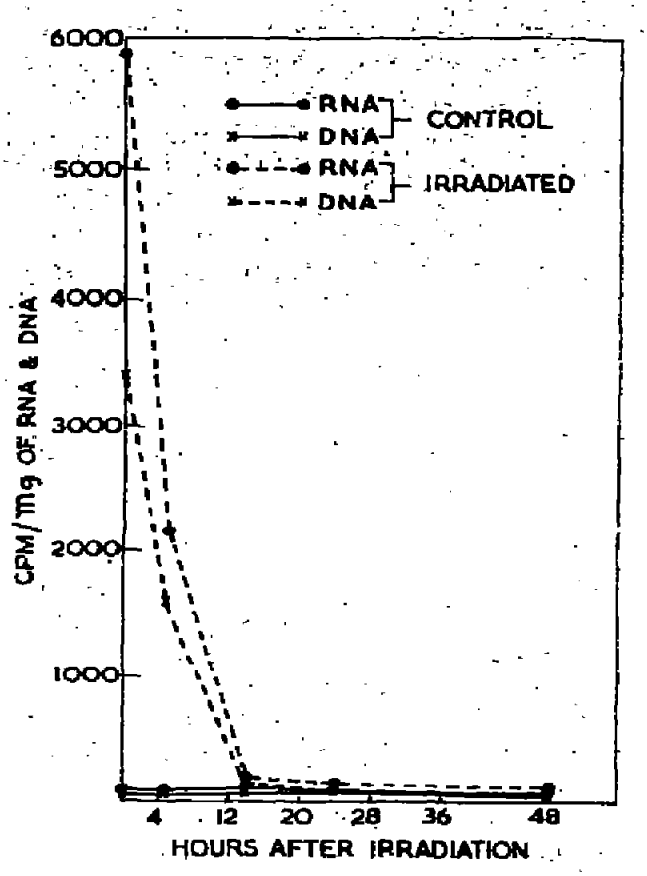

Fig. 1. Effect of time lapse after irradiation on the incorporation of [2-^{14}C] uracil into DNA and RNA. Details are described under Experimental.

For determining the changes in template activity of the potato chromatin after gamma irradiation chromatin was isolated and purified from buds at different time intervals viz., 10, 30, 60, 120, 180, 240 min after irradiation and their template activity was determined.

3. Results

Dormant potato buds have very limited capacity for both DNA dependent RNA synthesis and DNA replication [1]. Irradiation at sprout inhibiting dose enabled them to synthesise both RNA and DNA (table 1). Supplementation of actinomycin D at 10 μg/ml level in the reaction mixture inhibited RNA synthesis by 90% and DNA synthesis by about 10%. The RNA

Table 2

[^{3}H] Thymidine incorporation into DNA of potato buds.

Experiment	Total activity (cpm)	DNA content (μg per bud)
Control	130	2
Irradiated	2800	11
Irradiated + mitomycin C (8 μg/ml)	90	1.5
Irradiated (2.5 hr)	250	10

The incubation period was 2.5 hr. Irradiated (2.5 hr) indicates that buds were excised from potatoes 2.5 hr after irradiation and then incubated with [^{3}H] thymidine.

synthesised in potato buds due to irradiation, represented, therefore, the transcription of genetic material of potato genome. Since the function of irradiation was to keep the tissue in a quiescent state for a longer time, this activation of the genome may not be expected to last long. An experiment was designed to study this aspect. The buds were excised from potatoes at varying time intervals after irradiation and their ability to incorporate [2-^{14}C] uracil into their DNA and RNA was tested. The incorporation of uracil radioactivity was maximal within 2 hr after irradiation and a fast decline in synthesis was observed (fig. 1) afterwards. The buds from control tubers did not show much incorporation. In order to confirm that irradiation also activated DNA synthesis for a short time incorporation of [^{3}H] thymidine into DNA was determined (table 2). Irradiated buds were capable of incorporating thymidine into their DNA whereas control or irradiated buds treated with mitomycin C, a known inhibitor for DNA replication [10], did not exhibit any incorporation. When buds were isolated 2.5 hr after irradiation very little incorporation was observed, although the DNA per bud showed an increase.

The increase in RNA synthesis might be attributed to a number of factors like increase in RNA polymerase activity, increase in pool size of nucleotides or derepression of the genetic material by irradiation. The chromatin, when isolated from buds excised immediately after irradiation, was highly active in the synthesis of RNA (table 3). On comparison with calf thymus DNA, the chromatin showed 85% activity.

Since the buds were unable to incorporate uracil

Table 3

Effect of gamma irradiation on the template activity of potato chromatin.

Source of DNA (25 μg)	(nmoles of [^{14}C] ATP incorporated into RNA)
Calf thymus	5.5
Chromatin from	
a) Unirradiated potatoes	0.0
b) Irradiated potatoes	4.5

Details of the experiments are given under Experimental.

Table 4

Template activity of the chromatin isolated at varying time intervals after irradiation.

Time after which chromatin was isolated (min)	(nmoles of [^{14}C] ATP incorporated into RNA)
10	4.7
30	4.5
60	1.8
120	0.0
180	0.0
240	0.0

Amount of DNA in all cases was 25 μg.

efficiently into RNA and DNA 2 hr after irradiation, the template activity of the chromatin isolated from buds at varying time intervals was examined (table 4). The synthetic activity was completely impaired at 2 hr. This suggested that chromatin lost its ability to act as template for RNA synthesis, therefore, it was repressed again.

4. Discussion

Potatoes are in a state of active metabolism during a short time interval after irradiation. The respiratory rate as well as metabolic activity of the potatoes increase within 24 hr after irradiation [11]. During this period the tubers are capable of de novo synthesis of asparagine synthetase [5]. This activity reaches a maximum in 5 hr in the excised bud tissue, which corre-

sponds well with the general increase in protein synthesis after irradiation. Thus it is clear from the studies described here that potato genome is capable of synthesising messenger type RNA and specific proteins. Many workers during the fifties believed that interference with DNA synthesis is one of the primary effects of radiation [12–14]. On the contrary there are reports of an increase in DNA content of mammalian cells on X-irradiation [15, 16]. More recently an accelerated DNA synthesis in onion root after X-irradiation is observed by Das and Alfer [17]. Their findings show that DNA replication continues after irradiation and that the genetic material can be activated by irradiation. This activation can lead to a sequence of events which terminates in the formation of mRNA and new proteins. On the other hand, there is evidence, that higher doses, i.e. 50 Krads and above, can inhibit the template activity of chromatin from sugar beet tissue [18].

After irradiation of potatoes with sprout inhibiting dose of 10 Krad, active synthesis of proteins ensues within 3 to 4 hr and continues till 6 to 8 hr [5], whereas the [^{14}C]uracil incorporation starts almost immediately and terminates within 2.5 hr. It is possible that some of these proteins may have specific function and act as repressor for the synthesis of key enzymes in the sprouting process. In recent years there is mounting evidence for the hypothesis that proteins play the part of regulators [19, 20] of gene activity. Many workers [21–23] give importance to histone as regulators. The nucleohistone complex of chromatin in which DNA is fully complexed with histone is inert in supporting RNA synthesis. The transient activation of potato chromatin and the consequent synthesis of RNA and protein is of interest in the sense that it is an ideal system to study the regulation of enzyme synthesis.

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