Short Communication

Modification of Abscission by UV-Induced Alteration in RNA and Protein Metabolism

Richard M. Klein and Pamela C. Edsall The New York Botanical Garden, Bronx, New York 10458

Received December 28, 1966.

Low irradiances of 254 nm UV photoreversibly interfere with the capacity of plant cells properly to utilize auxin for cell division, enlargement and differentiation (3, 8, 17). This UV-induced auxinrecalcitrance does not seem to be caused by activation of auxin oxidase or by alteration of auxin metabolism, penetration, or translocation. In view of the role of nucleic acid and protein metabolism in auxin utilization (1, 7), a study of the interactions of UV and antimetabolites seemed warranted.

The experiments reported here were based on the hypothesis that if UV induces auxin-recalcitrance by interfering with nucleic acid or protein synthesis involved in auxin-induced growth, there should be a synergistic interaction of UV and a test antimetabolite visualized by increased abscission. The requirements for establishing synergistic interactions have been elucidated by Lockhart (10) and are fulfilled in these experiments.

Repression of abscission of explants of the pulvinus zone of primary bean-leaf petioles is dependent upon auxin and growth (5). Pulvinus explants (2) were incubated in agar containing 10 μ M IAA at 20°; under these conditions, 50% of the explants absciss in 72 hours (9). Irradiation of explants at zero time with 5 mJ/cm² UV had no effect on abscission with this concentration of auxin, although higher irradiances increased the percentage abscission (8).

Concentration-response experiments with sclected antimetabolites and antibiotics presented at zero time were conducted to select a concentration which had little or no effect on abscission: higher concentrations invariably increased abscission. The abscission-increasing effect of at least 1 antimetabolite from each group was reversible by the appropriate metabolite. All experiments were repeated 3 times with about 25 explants per variable: results are presented as the averaged percent abscission.

There was no interaction between UV and any tested anti-thymine (table I). This finding, plus the fact that treatments which split or negate thymine dimers (temperature shock, addition of thymidine, etc.) were ineffective in ameliorating UV- induced auxin recalcitrance, indicates that $DN\Lambda$ synthesis is not obligatorily related to auxin-recalcitrance. On the other hand, all tested anti-uracils interacted synergistically with UV in increasing the percentage of abscission.

Three of the 4 tested anti-amino acids also caused synergistic increases of abscission. Although β -2-thienyl alanine did not interact with UV, it increased the percentage abscission. These results suggest that suppression of protein synthesis is a factor in UV-induced auxin recalcitrance.

Puromycin and cyclohexamide, which interfere with protein synthesis (1, 6), actinomycin D, which interferes with RNA synthesis (7), and mytomycin C, which interferes with DNA-dependent RNA and ribosome synthesis (4, 15), all interacted synergistically with UV in increasing abscission, but chloramphenicol was inactive (11).

The failure of anti-thymines to interact with UV in abscission, and the synergistic interactions noted with anti-uracils, RNA antibiotics, and antimetabolites of protein synthesis all indicate that UV serves to modify the RNA and protein metabolism necessary for auxin action in the maintenance of the integrity of the abscission zone (12). It is possible that the lack of interaction between antithymines and UV may be due to a proportional increase in resistance of DNA to UV as increasing numbers of thymine sites are occupied by antithymines, but the lack of UV-anti-thymine synergism argues against this possibility.

Since UV radiation can cause dimer formation in uracil and uridine (14) and can directly inactivate RNA (13, 16) our results give presumptive evidence (but not proof) that UV-induced auxin recalcitrance is caused by a modification in that RNA (and hence protein) synthesis involved in auxin utilization. This conclusion serves as a basis for more detailed studies of the phenomenon.

Acknowledgments

This study was supported by NSF grant GB 4964. Actinomycin D was generously supplied by Merck, Sharp, and Dohme.

Table I. Interaction of UV Radiation plus Anti-uracils, Anti-thymines, Anti-amino acids, or Antibiotics on BeanPulvinus Explant Abscission

Anti-uracils mJ/cm²		5-Nitrouracil		5-Fluorou r acil		2-Thiouracil		6-Azauracil		
UV	Control	0.5	1.0	0.01	0.05	0.1	0.5	0.1	0.5	
0	51	57	60	52	55	51	57	49	52	
5	55	73*	81*	78*	7 9*	73*	80*	75*	77*	
Anti-thym	ines									
mJ/cm²			apto- iracil	5-Iododeoxy- uridine		2-Thiothymine		6-Azathymine		
UV	Control	1000	100	0.5	0.1	0.1	0.5	0.1	1.0	
0	50	56	53	51	53	52	57	53	55	
5	53	54	53	54	54	53	50	52	54	
Anti-amine	o acids									
		Fluorophenyl-		β -2-Thienyl		Meth	ionine			
mJ/cm²		alanine		alanine		sulfoxide		Tryptazan		
UV	Control	50	100	0.05	0.1	0.5	1.0	0.5	1.0	
0	52	55	57	61	64	55	59	59	56	
5	52	80*	83*	61	67	71*	84*	72*	81*	
Antibiotics										
mJ/cm²		Cyclohexamide		Actinomycin D		Miton	Mitomycin C		Puromycin	
UV	Control	0.001	0.01	0.0001	0.0005	0.01	0.05	0.0001	0.0005	
0	51	51	68	50	68	61	68	52	52	
5	58	81*	83	73*	83	88*	100*	65	75*	

Concentration of antimetabolites in μM ; concentrations of antibiotics in $\mu g/ml$.

Statistically-significant synergistic interaction.

Literature Cited

- CARPENTER, W. J. R. AND J. H. CHERRY. 1966. Effects of protein inhibitors and auxin on nucleic acid metabolism in peanut cotyledons. Plant Physiol. 41: 919–22.
- CHATTERJEE, S. K. AND A. C. LEOPOLD. 1964. Kinetin and gibberellin actions on abscission processes. Plant Physiol. 39: 334–37.
- GARAY, A. S. AND F. SÁGI. 1962. Effects of UVradiation on the auxin-auxin oxidase-phenol complex and on the sensitivity of plant tissues to indoleacetic exid. Physiol. Plantarum 15: 194-99.
- 4. IYER, V. N. AND W. SZYBALSKI. 1963. A molecular mechanism of mitomycin action; linking of complementary DNA strands. Proc. Natl. Acad. Sci. U. S. 50: 355-60.
- JACOBS, W. P., M. P. KAUSHLIK, AND P. G. ROCH-MIS. 1964. Does auxin inhibit the abscission of *Colcus* leaves by acting as a growth hormone? Am. J. Botany 51: 893-97.
- KEY, J. L. 1966. Effect of purine and pyrimidine analogues on growth and RNA metabolism in the soybean hypocotyl -- the selective action of 5fluorouraci¹. Plant Physiol. 41: 1257-64.
- KEY, J. L. AND J. C. SHANNON. 1964. Enhancement by auxin of ribonucleic acid synthesis in excised soybean hypocotyl tissue. Plant Physiol. 39: 360–68.
- 8. KLEIN, R. M. 1967. Influence of ultraviolet radia-

tion on auxin-controlled plant growth. Am. J. Botany (in press).

- KLEIN, R. M. 1967. Effect of ultraviolet radiation on auxin-controlled abscission. Ann. N. Y. Acad. Sci. (in press).
- LOCKHART, J. A. 1965. The analysis of interactions of physical and chemical factors on plant growth. Ann. Rev. Plant Physiol. 16: 37-52.
- LYON, J. L. AND F. T. ADDICOTT. 1965. Abscission responses to inhibitors of protein synthesis. Plant Physiol. 40: xxvi.
- SCOTT, P. C. AND A. C. LEOPOLD. 1966. Abscission as a mobilization phenomenon. Plant Physiol. 41: 826–30.
- SIBATANI, A. AND N. MIZUNA. 1963. Synthesis of ribonucleic acids in *Escherichia coli* irradiated with ultraviolet light. Biochem. Biophys. Acta 76: 188-200.
- SMITH, K. C. 1963. Photochemical reactions of thymine, uracil, uridine, cytosine and bromouracil in frozen solutions and in dried films. Photochem. Photobiol. 2: 503–17.
- SUZUKI, W. AND W. W. KILGORE. 1964. Mitomycin C: Effect on ribosomes of *Escherichia coli*. Science 146: 1585–86.
- 16. SWENSON, P. A. AND R. B. SETLOW. 1964. β -Galactoside: Inactivation of its messenger RNA by ultraviolet irradiation. Science 146: 791–94.
- ultraviolet irradiation. Science 146: 791–94. 17. DEZEEUW, D. AND A. C. LEOPOLD. 1957. The prevention of auxin responses by ultraviolet light. Am. J. Botany 44: 225–28.