The Effects of Ultraviolet and X-Radiation Upon the Centrochromatin of *Saccharomyces**

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Comprehensive analyses of the effects of low doses of ultraviolet or X-rays on the retardation of mitosis by Carlson¹ and Carlson and Hollaender² revealed that the nuclei in grasshopper neuroblasts are most susceptible to irradiation from interphase through late prophase, during periods, presumably, of intensive synthesis of desoxyribonucleotides. Maximal susceptibility to X-rays occurs at late prophase while greatest sensitivity to ultraviolet occurs at early prophase. Since both ultraviolet and X-rays block formation of desoxyribonucleotides at dosage levels insufficient to affect protein synthesis,^{3,4} the findings of Carlson and Hollaender suggest (1) that some disturbance of the mechanism of chromatin formation underlies the depression of mitotic activity effected by radiation and (2) that ultraviolet and X-rays act upon different phases of chromatin formation.

The differential effects of ultraviolet and X-irradiation on the centrochromatin of *Saccharomyces* were studied using a tetraploid culture of known resistance to ultraviolet and X-irradiation.^{5,6} The genetics and cytology of this organism have also been described.⁷ A single twenty-four hour culture was held at 4°C to provide inocula during the experiments. Samples were prepared for irradiation by introducing approximately 1×10^7 cells into 25 ml of glucose, peptone, yeast-extract broth contained in a 250 ml flask. After 24 hours growth at 30°C, more than 95 per cent of the cells were in the non-budding interphase state, and haematoxylin staining revealed the characteristic spherical centrochromatin. The cells were harvested, washed twice with M/15 KH_2PO_4 and diluted to a concentration of about 5×10^5 cells per ml. Thirty ml of the suspension were transferred to sterile petri dishes for irradiation. During irradiation the suspensions were agitated with a magnetic stirrer. X-radiation was delivered at the rate of 1000 r/min. from a tungsten target tube operating at 20 ml 180 KVP. The ultraviolet dosage was delivered at the rate of 5.9 ergs/mm²/sec. (measured at the surface of the dish) by a Hanovia quartz envelope lamp emitting 95 per cent its ultraviolet energy at wave length 2537 Å. Five ml samples were removed from the dishes exposed to X-rays and two ml of triple strength glucose, peptone, yeast-extract agar were added and the samples incubated at 30°C. Ultraviolet irradiated samples were treated similarly.

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The centrochromatin⁸ in the yeast cell exists in interphase as a spherical body in joint contact with the nuclear vacuole and the spindle (Fig. 1A). In interphase cells which have been irradiated with lethal doses of X-rays (i.e. doses which preclude formation of macroscopic colonies upon plating) crescent formation occurs, apparently normally, while in similar cells exposed to sublethal doses of ultraviolet irradiation, crescent formation is delayed for from two to three hours.

The disruption of mitochondria reported by Sarachek and Townsend⁹ is observed in cells with conspicuous mitochondria grown under special conditions. The treated cells described above were prepared under conditions in which the mitochondria are quite inconspicuous (without heavy fat deposits) and no observable disruption occurred.

The following tabulation presents data on the effects of different doses of radiation upon the delay in formation of the crescent centrochromatin in *Saccharomyces* in comparison with the degrees of cellular inactivation and retardation of budding produced by comparable amounts of irradiation.

Dosage	Percentage Inactivation	Percentage Retardation of Budding	Delay in Crescent Formation
ergs/mm ² /sec			
U.V. (59	0	0	2-3 hrs
(885	55	85	—
(1475	99	100	18-20 hrs
r/min			
X-Ray (500	0	0	None
(1800	55	35	None
(60000	99.8	60	None

Unirradiated control cells commence budding after 90 minutes and complete the development of at least one mature bud at the

Fig. 1. The normal formation of the centrochromatinic crescent during transition from interphase through prophase. Cells are stained with Heidenhains haematoxylin. A. Interphase: a Spindle. b Sphere of centrochromatin. c Vacuole. B. Early prophase. Crescent formation beginning. C. Budding cell. Crescent complete.

end of three hours; crescent formation is completed ten to fifteen minutes before the onset of budding. The haematoxylin stain revealed that cells exposed to the maximal X-ray dosage are capable of producing crescents as readily as control cells, while those exposed even to the minimal ultraviolet dose fail to effect crescent formation within normal time. The effect of the ultraviolet is only temporary; eventually all cells form crescents.

In an attempt to reverse the action of ultraviolet, irradiated cell suspensions were placed in 25 ml flasks held at 30°C and illuminated from below for 90 minutes by a 500 Watt Fresnel Spot Lamp at a distance of 15 cm. Three cm of 0.03 CuCl₂ were interposed between the flasks and the light source. Though a marked increase in cell survivals resulted, no reduction of the inhibition of crescent formation was detected.

The formation of the crescent of centrochromatin preceding budding is accompanied by duplication of the cellular DNA.¹⁰ It has been established, by Kelner, that low doses of ultraviolet radiation immediately suspend synthesis of DNA in micro-organisms, and that this inhibition is readily removed by reactivating light.³ Since the ultraviolet induced inhibition in *Saccharomyces* is not photoreversible and the inactivating doses of X-ray (which should prevent DNA synthesis) fail to affect the progress of crescent formation, the effects of radiations upon the prophase arrangement of centrochromatin in *Saccharomyces* may not be related directly to their effects upon DNA synthesis.

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