Trakya Üniversitesi Tıp Fakültesi
Dergisi: 4 (2-3): 3-7. 1987..

THE EFFECT OF ULTRAVIOLET MICROBEAM IRRADIATION ON THE SALIVARY GLAND CHROMOSOMES

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ÖZET

Radyasyonun kromozomlar üzerine etkisi üzerinde çokça çalışılan konulardan biridir. Özellikle U.V. Microbeam ile yapılan tecrübelerin tümünde mitoz fazındaki hücreler ışınlandırılmışlardır. Chironomus tükrük bezi kromozomları, hem mitoz fazında olmadıkları hem de kromozomlar üzerinde farklı yapıları ışınlandırmaya elverişli olduklarından konuya derinlik kazandırmak için bu çalışmanın konusunu teşkil etmişlerdir.

SUMMARY

Several investigators have reported on the effect or irradiating Chromosomal DNA with an ultraviolet (U.V.) microbeam. All the studies, have been done on cells in mitosis when the chromosomal material is clearly visible Uretz et. al. (1954); Zirkle and Uretz (1960); Bloom and Özarslan (1965). Certain organisms have giant salivary gland chromosomes which are much longer, exhibit different types of structure a long their length, and are clearly visible in interphase cells. (Şengün 1982).

The purpose of this work is to investigate the effect of Ultraviolet microbeam irradiation on different parts of the giant chromosomes of salivary glands of Chironomus plumosus larvae.

MATERIAL AND METHODS

The Chironomus plumosus larvae were collected from pools. Larvae stored in the refrigerator at 4–6°C were satisfactory for using several days. To prepare the salivary glands for irradiation they mounted in hemohymph of their own, surrounded by a small drop of liquid paraffin and covered with a quartz cover–slips.

Five different parts of the salivary gland cells which could be clearly identified under the microscope were chosen irradiation. The three of them were regions of the giant chromosomes, namely: (a) euchromatic regions or the A bands, (b) the interbands and (c) the heterochromatic regions. The other two targets were; (d) the nuclear membrane and (e) the nucleolus of the cell.

The Ultraviolet microbeam is based on the desing of Uretz and Perry (1957). The output from a 500 W high pressure mercury arc lamp is filtered

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through 5 cm $NiSO_4$/ $CoSO_4$ solution Kasha (1948) to provide heterochromatic U.V. light with a small component of visible green light. A 74 × reflecting objective demagnifies the light passing through a primary aperture of 200 μm to a the teoretical spotsize of about 2,7 μm. Because of the thickness of the specimen and focusing problems, the effective minimum diameter of the spot is about 3,5 μm.

RESULTS

The reaction of different part of a salivary gland cell when irradiated with the U.V. microbeam is not the same:

a) Euchromatic region or the A bands are effected like the mitotic chromosomes, mainly a clear paling* was observed on them (Fig. 1a–1b). However the degree of paling varied from one cell to another (Compare Fig. 1b and Fig. 2b). The results are summarized in Table: 1. In some cases a different and unclearly visible paling is observed. (Fig. $2b_2$).

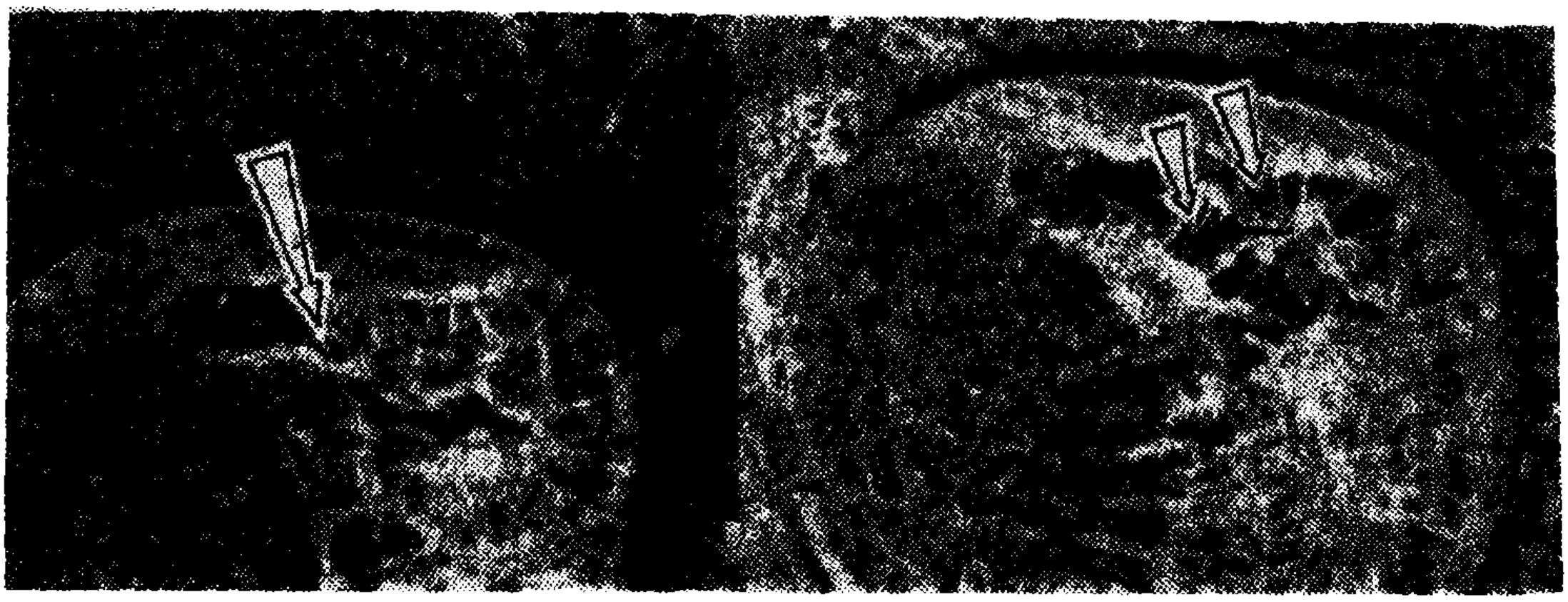

Fig 1. 3 minutes irradiation a) Before irradiation. b) After irradiation
Olympus (Faz-contrast, 200 x).

Table 1. Paling of A bands in polytene chromosomes irradiated with a U.V. microbeam.

Exposure time	Number of sites irradiated	Paling Reaction		
		Strong	Weak or uncertain	None
2 mins	61	32 (53 %)	13 (21 %)	16 (26 %)
3 mins	28	16 (57 %)	5 (18 %)	7 (25 %)
4 mins	24	18 (75 %)	2 (8 %)	4 (17 %)
Total	113	66	20	27

* Paling is that changing in refractive index at sites irrediated with an ultraviolet microbeam irradiation.

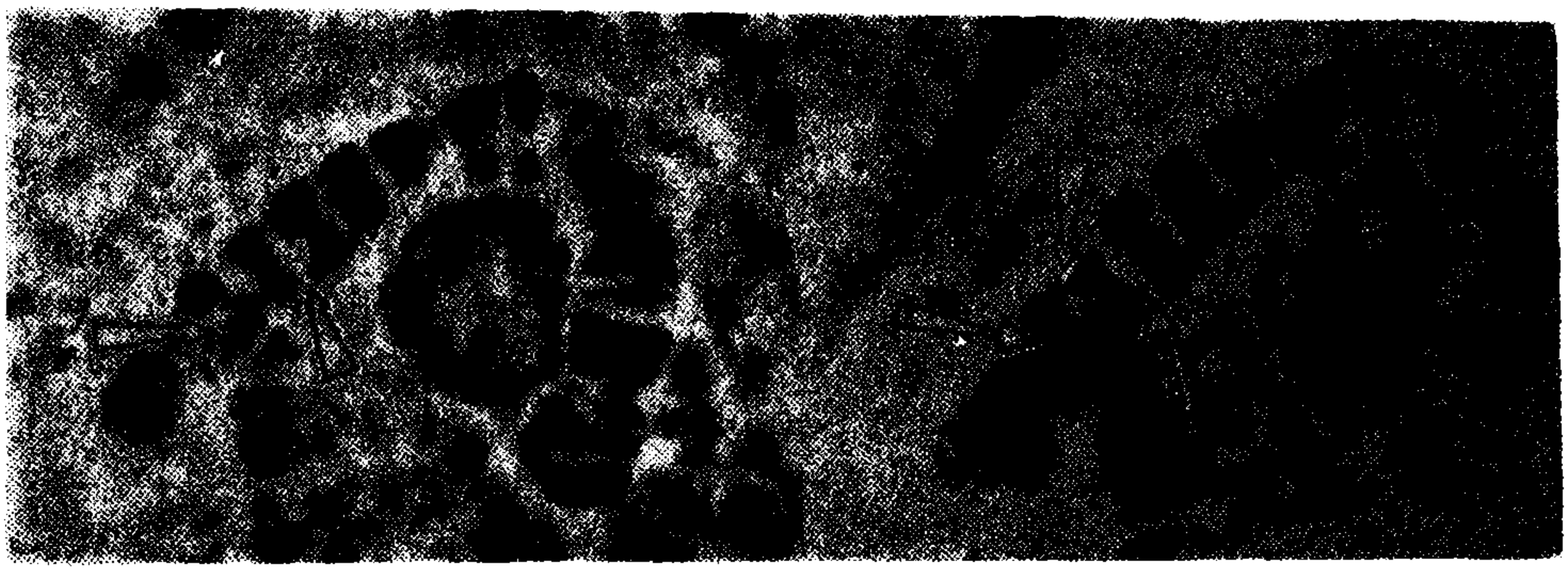

Fig 2. 4 minutes irradiation a) Before irradiation b) After irradiation Olympus (Faz-contrast, 400 x)

b) The interband regions are usually too small to irradiate, even with a microbeam but larger interbands are observed as a result of strectching of the giant chromosomes sometimes. When these larger clear areas are irradiated, some changes in its color is observed (Table: 2).

Table 2. Darkening of interbands in polytene chromosomes irradiated with a U.V. microbeam. These changes occur less frequently then for the A - bands.

Exposure time	Number of sites irradiated	Reaction		
		Strong	Weak or uncertain	None
2 mins	12	—	1 (8 %)	11 (92%)
3 mins	10	—	—	10 (100%)
4 mins	27	2 (7%)	5 (19%)	20 (74%)
Total	49	2	6	41

c) The smallest chromosome of Chironomus plumosus larvae is associated with the nucleolus and has a different types of chromatins (euchromatin and functional heterochromatin) at each end. If these heterochromatic regions are irradiated paling is again observed (Table: 3).

Table 3. Paling of heterochromatin region in polytene chromosomes irradiated with a U.V. microbeam.

Exposure time	Number of sites irradiated	Paling Reaction		
		Strong	Weak or uncertain	None
2 mins	20	7 (35 %)	4 (20 %)	9 (45 %)
3 mins	26	8 (31 %)	6 (23 %)	12 (46 %)
4 mins	30	14 (47 %)	7 (23 %)	9 (30 %)
Total	76	29	17	30

d) The U.V. microbeam had no visible effect of the nuclear memrane of a salivary gland cell even when it was irradiated for 8 mins.

e) No visible alteration was noted in nucleoli irradiated for 2, 3 or 4 mins but in 3 out of 14 nucleoli irradiated for 8 mins. Weak paling may have occured. However, the very large nucleolus in a cell of the salivary gland of Chironomus mostly has several dark and light regions with uncertain borders. Therefore it was not possible to decide whether the observed light regions in irradiated samples corresponded to typical paling or not.

DISCUSSION

In metaphase chromosomes the chromomeres and the interchromomeral regions are not visible separately probably as a result of spiralization. In these chromosomes, U.V. microbeam causes paling at a given region of chromosomes consisting of chromosomes and of interchromomeres where as in salivary gland giant chromosomes the bands and the interbands are large enough to be irradiated separately. Our results support the wiev that the paling is related to the existence, to its condition and to the amount of DNA, at least of the nucleic acids. The occasionally observed paling of the interbands may be due to the existences of the DNA in these regions as thin and unvisible bands functional DNA localized in interbands. If this assumption is true we may expect that the paling is the more strong the more condensed DNA is irradiated. The heterochromatin occuring at one ends of the smallest chromosome of Chironomus plumosus prepupae is flocky, swollen and not condensed. The paling is not pronounced at these flocky heterochmatic region.

In embryonic fibroblasts as well as in tumour cells including the L–Strain cells cultured regularly in our laboratory a paling of the irradiated region of the nucleolus could be observed Algüneş (1974). However it is not possible to point out the occurence of a paling in all types of cell strains although the cells were irradiated more or less under the similar conditions. But in the nucleoli of the salivary gland cells of Chironomus plumosus larvae such a paling effect could not observed regularly even though these cells were irradiated for 8 min. It may be due to the fact that the nucleoli of salivary gland cells of Chironomus have not sufficient amount of nucleic acids to observe the paling or it may be due to the existances of certain protective substances. It was possible to prevent the paling effect of U.V. irradiation on the nucleoli of cells irradiated in media which had a pH below the

range of the physiological pH, [Şengün et. al. (1973)] and AET containing media, [Özalpan et. al. (1976)]. caused a reduction of the percentage of paling effect in nucleoli. Therefore it may be throught that, the absence of paling of Chironomus salivary gland nucleoli may be due to its special function.

REFERENCES

1. **Algüneş, Ç.:** 1974. *The effect of heterochromatic ultraviolet microbeam irradiation upon DNA synthesis of these cells exhibiting normal and abnormal division.* İ.Ü. Fen Fak. Mecm (B) 38 167–169.

2. **Bloom, W. and Özarslan, S.:** 1965. *Electron microscopy of ultraviolet irradiated parts of chromosome segments.* Proc. Nat. Acad. Eci 53 (6) 1294–1302.

3. **Özalpan, A., Üçer, E., Algüneş, Ç. and Şengün, A.:** (1976). *The effect of AET upon the nucleolar paling.* Doğa (TÜBİTAK) 1, 1–3.

4. **Perry, R.P.:** (1957). *Changes in the ultraviolet absorption spectrum of parts of living cells following irradiation with ultraviolet microbeam.* Experimental Cell Research 12: 546–559.

5. **Şengün, A., Karadeniz, C., Ulakoğlu, G. and Algüneş. Ç.:** (1973). *The combined etfects of various ph values and of heterochrematic U.V. irradiation on nucleolei of mammalian cells in tissue culture.* İ. Ü. Fen Fak. Mecm. (B). Vol. 38: 167–169.

6. **Şengün, A.:** (1982). *Technical Advances in Biomedical Playsics.* Nato Summer School 14–128 Eylül 1982 İstanbul.

7. **Uretz, R.B., Bloom, W. and Zirkle, R.E.:** (1954). *Irradiation of parts of individual cells II. effect of on ultraviolet microbeam focused on part Chromosomes.* Science 120: 197–199.

8. **Uretz, R.B. and Perry, R.P.:** (1957). *An improved ultraviolet microbeam opparatus.* The review of scientific instruments. Vol. 28, No. 11, 861–866 November 1957.

9. **Zirkle, R.E., Bloom, W. and Uretz, R.E.:** (1956). *Use of partial-cell irradiation in studies of cell division.* Proc. Intern. Conf. Peaceful Uses At. Energy Cenova 1955 11: 273–282.

10. **Zirkle, R.E. and Uretz, R.B.:** (1960). *Energy relation in Chromosome "Paling" by ultraviolet microbeam.* Science 151: 1316.