NUCLEOLUS DEGRADATION AND GROWTH INDUCED
BY UV-MICROBEAM IRRADIATION
OF INTERPHASE CELLS GROWN IN CULTURE

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It has been reported that local microbeam irradiation of the nucleolus or a particular nucleolar
organizer region (NOR) of mitotic cells can cause a drastic alteration in the reformation of postmi-
totic nucleoli. These resulting nucleoli may reappear as small micronucleoli or as unusually large nucleoli (2, 1, 5, 12). These alterations are similar to those of the nucleolar mutant with deficiencies in the NOR (4, 9).

Mature nucleoli of interphase cells have been subjected to microirradiation by many investigators who have observed nucleolar material segregation (10), small changes in nucleolar size and shape (6), or local lesions (3). But no one has reported the induction of prominent nucleolar diminution or growth even in the case of inhibition of nucleolar RNA synthesis by UV-microbeam irradiation (7, 8, 11).

We have found that under certain conditions UV-microbeam irradiation can cause striking volume changes in mature nucleoli of interphase mammalian cells in culture (13). In the present communication we describe the results of a light microscope study of the phenomenon. The possible mechanism of nucleolar volume changes is discussed.

MATERIALS AND METHODS

Pig embryo kidney cells (SPEV), maintained in culture for a number of years, were grown in monolayer on a quartz cover slip mounted in the window of a sterile culture chamber. The culture medium used was 90% medium 199 and 10% fetal calf serum and antibiotics (200 IU/ml of penicillin and 100 IU/ml of streptomycin). The nucleoli of the flattened cells were locally irradiated through the cover slip by an inverted monochromatic (280 nm) UV-microbeam apparatus with UV-spot 3 μm in diameter in the focal plane. The dose rate was estimated, by using a calibrated photocell, to be \( \sim 5 \times 10^{-3} \) erg/μm²/s. The dose of irradiation was varied by varying the time of exposure to the beam within a range from 0.05 to 2 s.

Before irradiation, the progression of cells through the cell cycle was followed with phase-contrast optics. The normal cell cycle duration was about 20-24 h, with interphase taking \( \sim 18-22 \) h. The cells were irradiated at various times from 4 to 16 h after metaphase. After irradiation, changes in nucleolar areas were recorded by the ciné-micrographic method for several hours. The ciné-micrographs were used to measure nucleolar areas. 136 cells were subjected to the microirradiation.

RESULTS

Each of the interphase nuclei contained one or several nucleoli 3-6 μm in diameter. In the middle interphase, the nucleolar area increased at a rate of no more than 3-4%/h. After local UV microirradiation, we observed rapid and profound diminution of the irradiated nucleolus. This phenomenon turned out to be dependent upon the presence of one undamaged nucleolus in the same nucleus and also on the dose. The main quantitative results are presented in Table I and illustrated by Figs. 1 and 2.

In the cells with nuclei containing a solitary nucleolus, microirradiation did not produce marked changes in the nucleolar size (Fig. 2). The same was observed in the cells with nuclei containing two or several nucleoli, all of which were irradiated (Table I).

Microirradiation caused significant changes in nucleolar morphology only in nuclei with more than one nucleolus, one of which was irradiated (Fig. 1). Hence, the significant changes took place only in the presence of an unirradiated nucleolus in the same nucleus. The area of the irradiated nucleolus decreased by a factor of 2.5-3, and the nucleolar volume diminished probably by a factor of up to 4. Most of this volume change took place during the 1st h after irradiation. In 2-3 h, changes ceased and the nucleolar remnant re-

### Table I

| Nucleolar Area 3 h after Microirradiation Expressed as a Percentage of that before Microirradiation* |
|---------------------------------------------------------------|
| Nucleus with two nucleoli                                      |
|                                                               |
| Exposure (s) | One of two nucleoli irradiated | Both nucleoli irradiated | Solitary nucleolus irradiated |
| 0.1          | 36 ± 5                          | 80 ± 8                   | 85 ± 7                      |
| 2            | 176 ± 13                         | 114 ± 6                  | -                          |
| Area of irradiated nucleolus (%)                              |
| Area of unirradiated nucleolus in the same nucleus (%)         |
| Total nuclear area per nucleus (%)                            |
| Number of nuclei studied                                      |
| 18           | 10                               | 12                       | 12                           |

* The values given are the means ± SD.
† Data for nuclei with starting nucleoli equal in size are presented.
Figure 1. (a) The cell with two nucleoli, one of which (arrow) was irradiated; exposure 0.1 s. (b) The same cell 3 h postirradiation. Note the significant nucleolar volume changes. At this moment, the left nucleolus was irradiated; exposure 0.1 s. (c) The same cell 3 h after the left nucleolus was irradiated. Note that during these 3 h the left nucleolus has been reduced but the right one has been expanded.

The diminution in the irradiated nucleolus was always accompanied by a concomitant enlargement of the unirradiated nucleoli. This enlargement compensated for the loss in volume of the irradiated nucleolus, and the total nucleolar area per nucleus did not change appreciably. The cinematographic data showed that the unirradiated nucleolus enlarged primarily on the side facing the irradiated one.

The effect of microirradiation turned out to be reversible, to some extent, through the subsequent microirradiation of the previously unirradiated nucleoli in the nucleus. After this second irradiation, the enlarged nucleolus decreased in size and the previously diminished nucleolus enlarged. These changes restored the nucleoli to almost their original size before the first microirradiation (Fig. 1c).

As expected, the effect of microirradiation demonstrated a dependency upon the dose of radiation. But, unexpectedly, this dependency manifested an inverse character. At higher exposures (1–2 s), the diminution of the irradiated nucleolus and the enlargement of the unirradiated one were less than at lower exposures (Table 1). The largest effect was recorded after the lowest exposure for 0.1 and 0.05 s.

DISCUSSION

The results show that in contrast to routine whole cell irradiation local UV-irradiation can stimulate significant diminution as well as expansion of mature nucleoli. But the local irradiation by itself does not cause the nucleolar diminution (Fig. 2). A significant nucleolar volume change takes place only in the presence of an unirradiated nucleolus in the same nucleus (Fig. 1). This fact suggests that the diminution, as well as the expansion, results from an interaction between damaged and undamaged nucleoli. Such an interaction suggests competition with a predominance of unirradiated nucleoli. This radiation-induced predominance is not irreversible (Fig. 1c).

At least two hypotheses may be advanced concerning the mode of the observed nucleolar volume changes. The first assumes that nucleolar volume depends on the level of nucleolar material synthesis at the NOR. Nucleolar volume changes would therefore result from inhibition of this syn-

Figure 2. (a) The cell with a solitary nucleolus, which was irradiated; exposure 0.1 s. (b) The same cell 3 h postirradiation. The nucleolar volume did not change appreciably. Phase optics. Scale marker, 10 μm. Total magnification × 1,500.
thetic activity at the irradiated NOR and from a compensatory response at the unirradiated one. This explanation is attractive, but there are difficulties in accepting it. There is considerable evidence that UV-microbeam irradiation can cause appreciable inhibition of nucleolar RNA synthesis in mammalian cells (7, 8, 11). Nevertheless, no one has yet reported prominent nucleolar diminution in relation to this effect. Moreover, we did not record a prominent nucleolar volume change when a high dose of UV irradiation was delivered to the nucleoli. The most significant effect was recorded at a lower dose (less than $5 \times 10^{-3}$ ergs/nucleolus) when a lower level of nucleolar inactivation could be expected (8).

The alternative hypothesis may be advanced that the observed phenomenon is not due to synthesis of new nucleolar material, but rather to migration and redistribution of pre-existent nucleolar material between competitive NORs. It may be supposed that the low doses of local irradiation do not seriously damage the bulk of the nucleolar material and produce only a gentle stimulus for dispersion and migration of this material out of the injured region toward the undamaged and, therefore, more competitive NOR. Thus, migration would take place only in the presence of undamaged and more competitive nucleoli in the same nucleus. Nucleolar changes would not be detected within a nucleus having a solitary nucleolus (Fig. 2) or within a nucleus after whole cell irradiation or after microbeam irradiation of all nucleoli. As a result of migration, the total amount of nucleolar material within the nucleus would not change. If the phenomenon is due to migration, it is not surprising that the unirradiated nucleolus enlarges primarily on the side facing the flow of migration. It may be supposed that the high doses of microirradiation are able to damage the bulk of nucleolar material and to destroy its natural ability to migrate, thereby preventing significant changes in the nucleolar size of the irradiated as well as the unirradiated nucleolus.

The above speculations seem to show that the ideas about migration and redistribution of pre-existent nucleolar material are sufficient to explain all the peculiarities of the experimental data presented here. These ideas are not new and have been discussed many times since the work of McClintock (9), mostly in relation to nucleolar reformation during mitosis. We believe that these ideas will be useful to explain some of the features of the behavior of mature nucleoli in the interphase cell.

**SUMMARY**

In contrast to total cell irradiation, local UV-microbeam irradiation can stimulate a significant diminution in the irradiated mature nucleoli in interphase mammalian cells in culture. This diminution is accompanied by the concomitant expansion of the unirradiated nucleoli within the same nucleus, and the total nucleolar volume per nucleus does not change appreciably. It is suggested that these nucleolar volume changes are the result of the dispersion, migration, and redistribution of the nucleolar material between competitive nucleolar organizer regions of the interphase nucleus.

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