

**DEREPRESSION OF GENOME AND ALTERATION
OF DNA TEMPLATE ACTIVITY IN NONPROLIFERATING CELLS
BY MEANS OF UV AND VISIBLE LASER RADIATION**

T.I. KARU
*Laser Technology Research Center, Russian Academy of Sciences
Troitsk, Moscow Region, Russia*

1. Introduction

The irradiation with a certain wavelength of light can damage or/and activate natural components (photoacceptor molecules) of cells. In the present chapter two different mechanisms for exerting effects on cellular DNA and genome of resting (nonproliferating) cells will be considered. First, primary action after absorption of high-repetition-rate UV pulses ($\lambda=271$ nm, $f=10^4$ Hz, $\tau=10^{-8}$ s) by DNA of plateau-phase HeLa cells forms the basis of induction of replicative DNA synthesis in a subpopulation of these cells. It will be shown that UV radiation with certain parameters can have not only negative (e.g., lethal, mutagenic) but also positive effects: stimulation of colony-forming ability and enhancement of proliferation of cells.

Second, a mechanism will be considered whereby the visible light used for irradiation is not absorbed by nuclear components of a cell but probably by mitochondrial pigments like cytochrome *a/a₃* and (partial) derepression of the genome occurs as a final step after many dark reactions in a cell (photosignal transduction and amplification). After the irradiation of human peripheral blood lymphocytes with a He-Ne laser the nucleolus of these cells is subjected to short-term changes (RNA synthesis, alteration of chromatin conformation) similar to those observed after the mitogenic stimulation with lectin phytohemagglutinin (PHA). The activation of transcription of r-genes synthesizing pre-rRNA in the irradiated cells is considered as derepression of genome within the G_0 -phase of cellular cycle. The irradiation also causes in lymphocytes an accumulation of preliminary terminated protooncogene *c-myc* RNA.

2. Induction of Replicative DNA Synthesis in Plateau-Phase HeLa Cells By UV Radiation

A stream of literature data suggests that UV radiation (UVR) suppresses replicative synthesis of DNA and induces reparative synthesis of DNA in cellular cultures of mammalian origin (for review see [1-3]). Most experimental data concern mammalian cells in the exponential phase of growth (log-phase). A few investigations of UVR effects on plateau-phase or growth-arrested mammalian cells [4-10] suggest: 1) that quiescent cells have extreme resistance to high doses of UVR; 2) that lethal action of UVR is (as in case of log-phase cells) connected with pyrimidine dimers; and, 3) that there is a mechanism of DNA repair in plateau-phase cells which is absent or weakly functioning in log-phase cells [9].

Although most studies indicate that DNA-damaging agents inhibit DNA replication, a few papers suggest that some agents, UVR included, may also induce or stimulate DNA replication. A marked induction of DNA replication was observed in confluent diploid fibroblast cultures treated with low fluences of UVR ($2\text{-}20\text{ J/m}^2$) or with carcinogenic agents *N*-methyl-*N*-nitrosourea and *N*-acetoxy-2-acetylaminofluorene [11]. Another carcinogenic agent, *N*-methyl-*N*-nitrosoquinidine, stimulated DNA replication in a post-confluent culture of Syrian hamster embryo cells [12]. Gamma irradiation induced replicative synthesis of DNA in plateau-phase HeLa cultures [13].

The authors of these investigations were interested in changes in DNA replication occurring after treatment with carcinogens, such changes considered to be necessary at the initial stages of carcinogenesis. The most interesting conclusion from the experiments [11-13] is that all DNA-damaging agents, each of them predicting a different spectrum of damage (and different mutations, respectively), induced replicative DNA synthesis in a fraction of cells in plateau-phase cultures.

Recently, Peak *et al.* [14] have demonstrated enhanced (almost 2-fold) gene expression for protein kinase C following brief exposure of cultured human epithelioid P3 cells to sunlight. They demonstrated that solar radiation induced a cellular transcription response similar to that found after administration of tumor-promoting agents and ionizing radiation, which suggests that solar radiation may function as a tumor promoter. It has been shown that DNA damage induces complex mechanisms in mammalian cells allowing the cells to handle or accommodate DNA lesions. At the molecular level, these mechanisms include enhanced expression of a number of genes [15]. UVR is known to activate viral DNA sequences in transformed cells and to induce deletion of growth suppression genes [16].

A new interest into UVR-induced stimulative processes has arisen recently in connection with the findings that 254-nm UVR or sunlight can induce a human immunodeficiency virus (HIV) promoter and stimulative growth of the complete virus in human cells [17,18]. UVR-sensitive sites in RNA transcripts of hepatitis delta virus and potato spindle fiber viroid have been found [19]. In these cases, the DNA damage was found to be a prerequisite for UVR-induced activation of the genes.

Last but not least, one should remember that a number of reports appear from time to time indicating that UVR stimulates cell division [20-25]. Most of this work has been done with microorganisms, except that of Walicka and Beer [22]. Two strains of murine leukemic lymphoblasts, L 5178Y-S and L 5178Y-R, were exposed to various fluences of 254-nm UVR, ranging from 1.1×10^2 to $5.35 \times 10^3\text{ J/m}^2$, and cultured for up to 70 days after irradiation. UV-exposure stimulated growth in about 70% of the cultures; growth started immediately after postirradiation growth disturbances and lasted several tens of generations. Proliferative activity was more greatly enhanced for L 5178Y-R cells (a less x-ray-sensitive culture) than for the L 5178Y-S strain: maximal shortening of the mean doubling time for the L 5178Y-R strain was 43%, whereas it was 20% for L 5178Y-S cells [22]. The most important result of this investigation was that growth stimulation also occurred at later stages of postexposure development, demonstrating UVR-induced heritable changes.

In the experiments where the plateau-phase HeLa cells were irradiated with various types of pulsed UV laser radiation [26], it appeared that irradiation with high repetition rate pulses (HRRP) at 271 nm induced replicative DNA synthesis in a fraction (subpopulation) of the plateau-phase HeLa cultures. The cells irradiated at low fluences not only retained the capacity for further growth but their proliferative capacity was stimulated. The wavelength of HRRP (2nd harmonic of a copper vapor laser, $I_{\text{peak}}=1.2 \times 10^4\text{ W/m}^2$, $I_{\text{average}}=5\text{ W/m}^2$, $\tau_{\text{pulse}}=10^{-8}\text{ s}$, $\tau_{\text{dark}}=10^4\text{ s}$, $f=10^4\text{ Hz}$) fits to the maximum of the first absorption band of DNA (see Fig.10a for the DNA absorption spectrum). HRRP used in our experiments excite DNA by the single-quantum mechanism following classical laws of photobiology. One should notice that laser sources emitting HRRP are new to photobiologic studies.

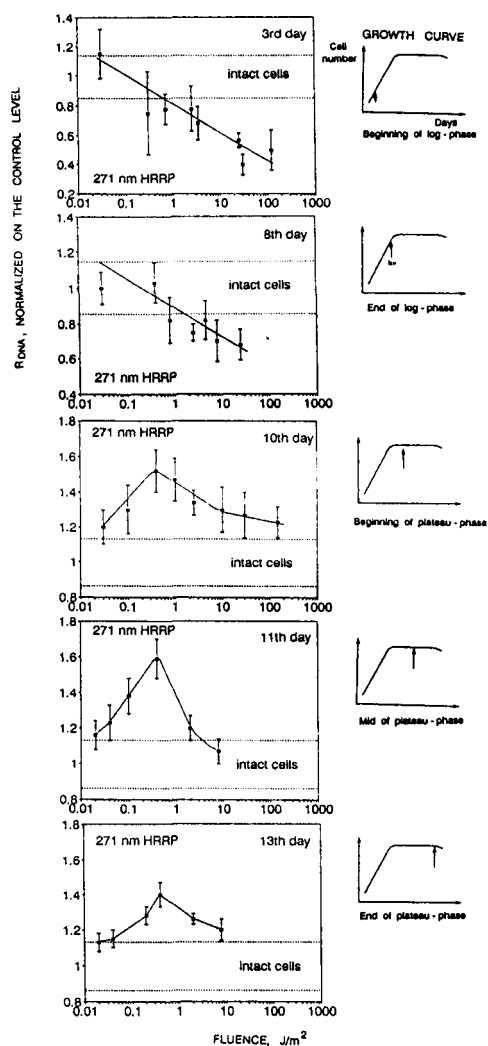


Fig. 1. Incorporation of $[^3\text{H}]$ thymidine during 20-min pulse labelings measured 2.5 h (starting point of the pulse label) after the exposure of HeLa monolayers to HRRP at different days of cultivation. The schemes on the right illustrate the growth phase of the population at the moment of irradiation. Experimental details as in [29].

It was found that the stimulation of DNA synthesis by HRRP was specific for plateau-phase cells [28]. Measurement of DNA synthesis rate as a function of the fluence of HRRP was done on the 8th, 10th, 11th, and 13th days of cultivation. In other words, the cells were irradiated at the end of the log-phase and at the beginning, in the middle, or at the end of the plateau-phase, as

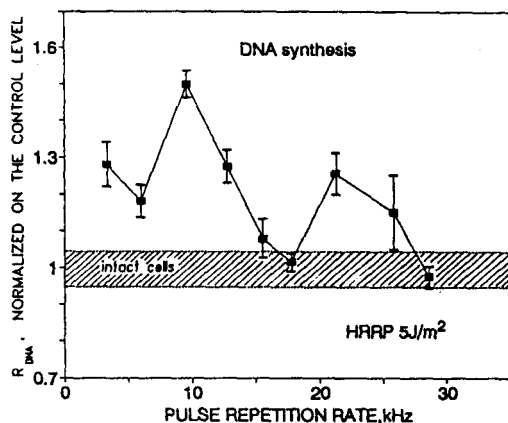


Fig. 2. Incorporation of [^3H] thymidine into DNA during 20-min pulse labelings measured 2.5 h (starting, point of the pulse label) after the exposure of plateau-phase HeLa cells to HRRP at a fluence of 5 J/m^2 as a function of pulse repetition rate. Experimental details as in [26].

illustrated by the schemes of the right of Figure 1. As seen in Figure 1, all plateau-phase cultures (10th, 11th, and 13th days of cultivation) responded to irradiation with increasing ^3H -thymidine incorporation. No inhibition of DNA synthesis was observed as the fluence was increased.

Also, the colony-forming ability (number of colonies as well as their diameters) was increased after the irradiation with HRRP at the same fluences [26].

The stimulation of DNA synthesis depended on the pulse repetition rate of the radiation (Figure 2). As seen in Fig. 2., two bands of pulse repetition rates (near 8-12 kHz and 19-25 kHz, with maxima at 10 kHz and 21 kHz) had an effect on DNA synthesis. This result indicates that the action of HRRP on plateau-phase HeLa cells connected with DNA damage is specific and characteristic of this type of radiation.

One way to distinguish between replicative and reparative synthesis of DNA is by counting

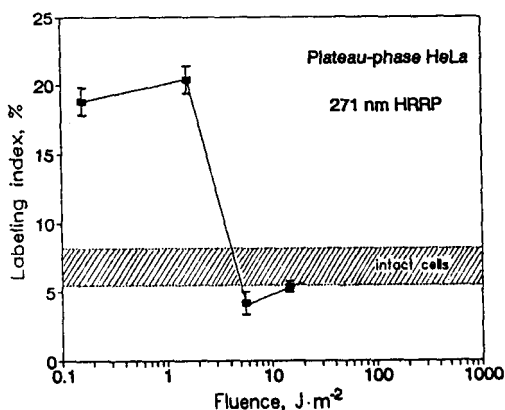


Fig. 3. Labeling index (percentage of cells incorporating [^3H] thymidine) 2.5 h after the exposure of plateau-phase HeLa cells to 271-nm HRRP at various fluences (radioautographic measurements are described in [26]).

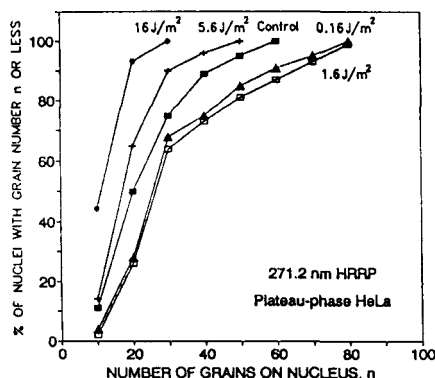


Fig. 4. Silver grain counts in control and 271-nm HRRP-irradiated plateau-phase HeLa cells after 20-min pulse labeling with [^3H] thymidine (radioautographic measurements). The respective labeling indices are shown in Fig.3.

silver grains in autoradiographs. This method is used to determine the percentage of DNA-synthesizing cells (labeling index). Also, by counting the number of grains above every nucleus, the proportions of replicative and reparative DNA synthesis can be determined (cells undergoing replicative synthesis are heavily labeled; cells undergoing reparative DNA synthesis are only weakly labeled).

Figure 3 presents the labeling index 2.5h after irradiation of plateau-phase HeLa cells with HRRP at various fluences. The number of cells incorporating [^3H] thymidine increased and exceeded the control level by about 3 times at fluences of 0.16 or 1.6 J/m^2 . After increasing the fluence (5.6 and 16 J/m^2), the number of DNA-synthesizing cells decreased and dropped to near the control level. The stimulative fluences here (0.16 and 1.6 J/m^2) coincide with those determined by radiometric measurements of [^3H] thymidine incorporation (Fig.1).

Figure 4 shows the distribution of silver grains above the nuclei of DNA-synthesizing cells. The shift of the cumulative curves to the left from the control curve (intact cells) when the cells were irradiated at 5.6 and 16 J/m^2 , points to a reduced number of cells with heavily labeled nuclei, i.e., to suppression of replicative DNA synthesis. With lower fluences (0.16 and 1.6 J/m^2), the cumulative curves shift to the right, i.e., there is a greater number of cells with heavily labeled nuclei, which indicates activation of replicative DNA synthesis.

When the cell population immediately after the irradiation is treated with [^3H] thymidine and continuously incubated with it for several hours, the fraction initially labeled represents cells in the S phase at the moment of irradiation, while the subsequent increase in the labeling index (percentage of DNA-synthesizing cells) reflects the flow of cells into the S phase during the interval studied. It is obvious from Fig.5a that the percentage of labeled cells increased in the both control and irradiated cultures; however, the irradiated cells underwent a rapid increase in the number of labeled cells during the first hour after treatment.

By counting the number of silver grains above every nucleus it is possible to draw some conclusions about the rate of DNA synthesis. We divided the cells into three groups: unlabeled cells (less than 8 grains per nucleus), weakly labeled cells (8 to 29 grains), and heavily labeled cells (more than 30 grains). The third group includes HeLa cells undergoing normal replicative synthesis of DNA and the second group includes the cells undergoing reparative synthesis of DNA [27].

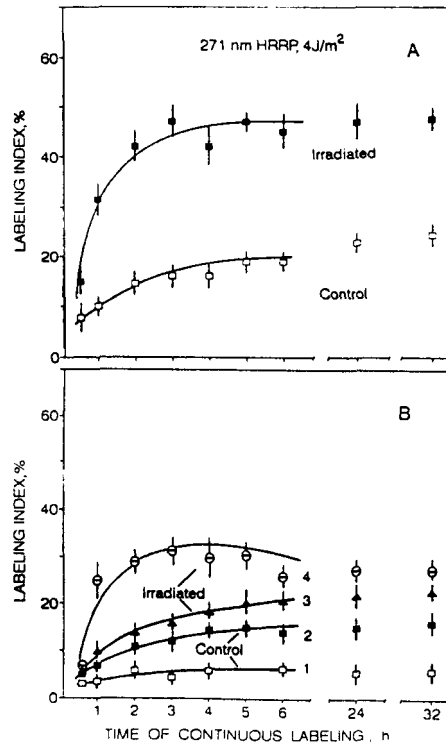


Fig. 5. Labeling index percentage of DNA-synthesizing cells) after the exposure of plateau-phase HeLa cells to 271-nm HRRP at 4 J/m^2 , measured by the autoradiographic continuous-labeling technique: (A) percentage of labeled cells in irradiated and control cultures, and (B) percentage of cells with highly labeled (curves 2 and 3) and weakly labeled nuclei (curves 1 and 4) in control (curves 1 and 2) and irradiated (curves 3 and 4) cultures. Experimental details the same as in Ref. [26].

As seen in Figure 5b, the percentage of labeled cells in the irradiated cultures increased mainly due to the number of weakly labeled cells (curve 4). A lesser increase was seen in the number of heavily labeled, irradiated cells (curve 3). The data obtained show that [³H] thymidine incorporation into DNA of plateau-phase HeLa cells after irradiation with HRRP in a certain fluence range ($0.1\text{-}3 \text{ J/m}^2$ (Fig. 1) and at certain pulse rates (near 10 and 21 kHz, Fig.2) is mainly due to reparative DNA synthesis (Fig.5b). A lesser [³H] thymidine uptake is accomplished via replicative DNA synthesis (Figs.4 and 5b). Under our experimental conditions, HRRP is able to induce both types of DNA synthesis, replicative as well as reparative.

This research suggests that the induction of DNA replication by low doses of damaging agents is not a rare phenomenon. The various damaging processes have some common qualitative and quantitative characteristics. First, the variation of replicative DNA synthesis in plateau-phase cellular cultures with fluence of UVR, dose of γ -radiation, or concentration of carcinogenic chemicals is qualitatively similar: a bell-shaped curve with a rather sharp maximum, the effect rapidly decreasing to the control level at the higher doses of the agents [12, 28, 29, 11, 13]. Similar

bell-shaped fluence dependencies were also established for HIV-1 promoter induction in HeLa cells by UVR [18]. As established by Conn *et al.* [11], the bell-shaped fluence dependence of replicative DNA synthesis was quite different from the curve for reparative DNA synthesis. Reparative DNA synthesis continued to increase up to a fluence of 20 J/m² and reached a plateau between 20 and 40 J/m².

Second, optimal UVR fluences (3 J/m², [II], 0.5 J/m², [29]; Fig.1) and magnitudes of effects (1.6, [II], 1.4-1.6 [28,29]; Fig.1) have been found to be very similar.

Third, replicative synthesis of DNA is induced only in a relatively small subpopulation of plateau-phase cells. Cohn *et al.* [11] established this fraction to be ≈6% in plateau-phase cultures and =18% in serum-arrested cultures. We did not perform detailed measurements, but an analysis of autoradiographic data and clone size distributions allows us to estimate this fraction to be 10-15%. Cohn *et al.* [11] found that the number of morphological transformants correlated with the percentage of cells in the carcinogen-responsive subpopulation which incorporated [³H] thymidine.

Under certain experimental conditions, the stimulation of [³H] thymidine incorporation into plateau-phase cells is a specific cellular response to HRRP; the continuous wave (CW) UVR administered at practically the same wavelength, the same fluence, and during the same postirradiation period had no effect [26] or was inhibitive (Fig.6c). In these experiments, the measurements were made during the first 5h of postirradiation. With HRRP, the first point with increased [³H] thymidine incorporation was 2 h after exposure (Fig.6a), which is a very rapid response of plateau-phase cells compared to the responses of these cells to UVR at 254 nm and to γ -radiation. With UVR at 254 nm, replicative DNA synthesis started to increase only after 12 h and was at a maximum 24 h after irradiation [11]. The incorporation of [³H] thymidine was inhibited a few hours after irradiation of plateau-phase HeLa cells with γ -radiation, and stimulated 24 h after exposure [13]. It is quite possible that after the initial inhibition which occurs during the first 2 hours after irradiation with CW UVR at 270 nm Fig.6b, there will be an increase in [³H] thymidine incorporation. Measurements to determine this were not performed in our work, principally because there are no reasons to believe that the induction of DNA replicative synthesis by HRRP at 271 nm is a specific cellular response as compared to CW UVR at 270 nm. More probably, this response is specific for particular experimental conditions, and occurs very soon after exposure. However, this point needs further clarification.

Upon reaching the plateau-phase, populations of normal cells accumulate in the early G₁ phase of the cell cycle, while most cells of transformed line, HeLa included, accumulate in a late G₁ phase [30]. Similar differences in the morphology of the G₁ phase between cells from normal tissues and solid tumors are reported [31].

It has been shown in cells fusion experiments that the rate of initiation of replicative DNA synthesis in the nuclei of HeLa cells in the G₁ phase can be very rapid (≈1.5 h). UV radiation of the quiescent cells before fusion induced decondensation of chromatin in a fluence-dependent manner in the nuclei of these cells [32]. Decondensation of chromatin must occur before reparative [33] and replicative [34] DNA synthesis can take place. It is quite possible that a subpopulation of the plateau-phase cells arrested at a late G₁ phase is responsible for the effects of HRRP described in this chapter. One can speculate that in this particular subpopulation, the damage caused by HRRP induces a chromatin template configuration promoting the beginning of replicative DNA synthesis.

So, a characteristic response of plateau-phase HeLa cells to pulsed UVR was described: the induction of replicative DNA synthesis in a cellular subpopulation after irradiation with high-repetition-rate UV pulses. Whether induction of replicative DNA synthesis by HRRP at 271 nm is a specific cellular response to pulsed UVR is not perfectly understood. It is certainly a specific effect under particular experimental conditions, and occurs very soon after exposure. CW UVR at

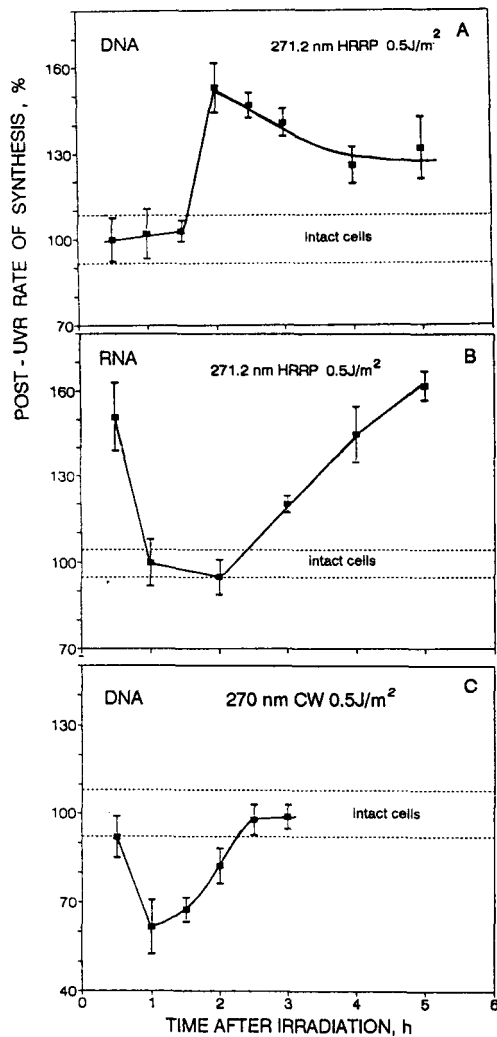


Fig. 6. Incorporation of (A,C) [^3H] thymidine or (B) [^{14}C] uridine during 20-min pulse labelings measured at different time points (time in the abscissa marks the beginning of the pulse labeling after the exposure of plateau-phase HeLa cells to (A,B) HRRP or (C) CW UVR.

270 nm might have a similar effect under other experimental conditions (e.g., with longer postexposure times).

3. Derepression of the Genome after Irradiation of Human Lymphocytes with He-Ne Laser

Circulating lymphocytes confronted with an immunological stimulus, shift from the resting state (G_0 -phase of cellular cycle) to one of rapid enlargement culminating in DNA synthesis and mitosis (blasttransformation). Characteristics of biochemical and morphological reactions in lymphocytes under the action of mitogens (agents responsible for blasttransformation, e.g.

phytohemagglutinin, PHA) are well studied [34, 35]. Cellular responses to a mitogen can be divided into short-term responses without *de novo* synthesis and occurring during the first seconds, minutes and hour after the addition of the mitogen, and long-term ones connected with the synthesis of protein and occurring from hours to days after the beginning of stimulation.

We provided parallel experiments with PHA and irradiation and compared the results for these two experimental groups with each other and with the intact control. As the short-term responses, Ca^{2+} influx, the RNA synthesis, accessibility of chromatin to acridine orange, and steady state level of c-myc mRNA were studied. DNA synthesis after 72 h was measured as a long-term response to the treatment.

3.1. SIMILARITY IN SHORT-TERM RESPONSES OF LYMPHOCYTES TO PHA AND IRRADIATION

The irradiation with He-Ne laser (56 J/m^2 , 5.6 W/m^2 , 10 s) induced short-term changes in lymphocytes from human peripheral blood, qualitatively and quantitatively similar to those caused by PHA ($2 \mu\text{g/ml}$). There was a two-to threefold increase of the intracellular Ca^{2+} concentration 2 min after the irradiation or PHA treatment (Table 1).

Also, an significant activation of the transcription function was found. Increased acridine orange binding to chromatin (reflecting the changes in the structure of the chromatin, in particular its decondensation) was found to be maximal 1.5-2 h after the treatment (Fig.7). It is well-known that this increase, about 50-70% above control values is characteristic of practically all kinds of Go cells stimulated by different ways [34]. The maxima in RNA synthesis rate were recorded 1.5-2 and 5 h of post-treatment (Figure 7).

The ultrastructural changes in nucleolus [38] and chromatin [39] were studied by electron microscopy 1 h after He-Ne laser irradiation or beginning of PHA-treatment. The nucleolus contains r-genes synthesizing pre-rRNA. Pre-rRNA is synthesized here and subsequently processed first into intermediate molecules and then into the ribosomal subunits [41]. It is also known that the morphology of the nucleolus reflects its ribosomal activity [42,43].

The ring-shaped nucleoli of circulating human lymphocytes investigated in our experiments [38] are characterized by low activity of r-genes. They turned out to be good objects for revealing were the effects of He-Ne laser irradiation. One hour after the irradiation of human circulating lymphocytes with He-Ne laser (56 J/m^2 , $t=10 \text{ s}$) or adding PHA ($2 \mu\text{g/ml}$) to the cell suspension, significant changes in the nucleoli of the treated lymphocytes were found as compared with the intact cells. The changes were considered to be similar for both experimental groups: appearance of a FC (abbreviations see in caption of Figure 8) of irregular form, fragmentation of a single FC to 2 or 3 smaller FC's, increase in the total area of DFC, increase in the number of RNP-granules, and appearance of small vacuoles which are absent in the nucleoli of the intact lymphocytes.

TABLE 1. Concentration of cytosolic calcium ($[\text{Ca}^{2+}]$, in nM) in lymphocytes from blood of three donors after irradiation of the lymphocytes with He-Ne laser (56 J/m^2) or treatment with PHA ($2 \mu\text{g/ml}$) [36].

Time	2 min			3 min			6 min		
	Intact control	PHA	He-Ne	Intact control	PHA	He-Ne	Intact control	PHA	He-Ne
I	124±6	254±3	422±8	125±3	195±6	82±2	122±9	150±1	92±8
II	135±4	297±4	319±6	137±2	208±8	121±4	131±3	133±7	129±4
III	124±3	263±8	270±4	123±6	173±3	115±9	122±5	132±3	119±6

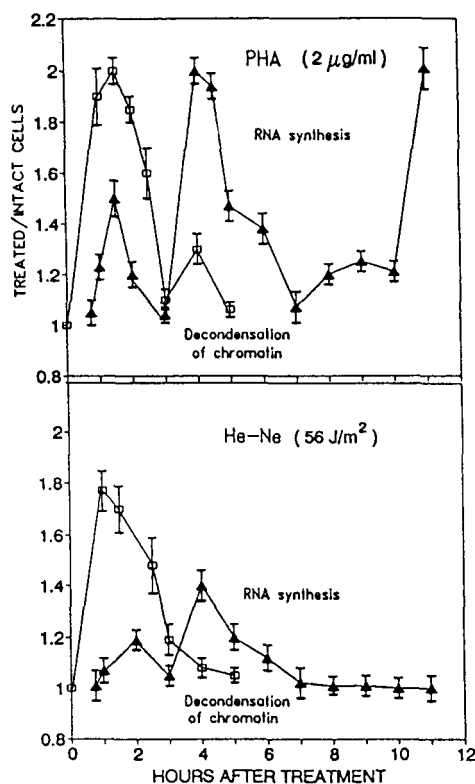


Fig. 7. Comparison of the kinetics of two activation events stimulated by PHA or He-Ne laser radiation in human lymphocytes. Experimental details can be found in [36, 37].

Figure 8 presents general rearrangements of a lymphocyte nucleoli one hour after the beginning of PHA-treatment, as it was first proposed in [40]. We found that the irradiation caused similar changes in the nucleoli [38]. In this scheme, number 1 presents an intact ring-shaped nucleolus characterized by a low activity of r-genes. Irradiation as well as PHA-treatment caused the appearance of two main modifications in the nucleolus (numbers 2 and 3). Based on the current knowledge about the activational rearrangement of the nucleoli of Go cells, one can propose the transformation from the typical ring-shaped nucleolus (1) to a ring-shaped nucleolus with an irregular FC (2), followed by the transformation to a strand-like nucleolus with several FC's and enlarged DFC and GC (3). The direct transformation 1→3 is also possible.

A possible functional interpretation of the changes observed in the nucleoli of irradiated cells is following. First, the lengthening of the outer FC contour (an enlargement of its irregular shape and an increase in the number of FC's) can be interpreted as an expression of new r-genes because actively transcribed rRNA is situated (as described above) in the periphery of FC's [44]. There also exists a correlation between the number of FC's and the level of transcription of r-genes [43]. Second, the increase in the total area of the DFC in the irradiated cells might reflect an enhancement of pre-rRNA production. It is known that DFC consists of newly synthesized pre-rRNA molecules [42, 45]. Third, the increase in the number of RNP-granules observed in our

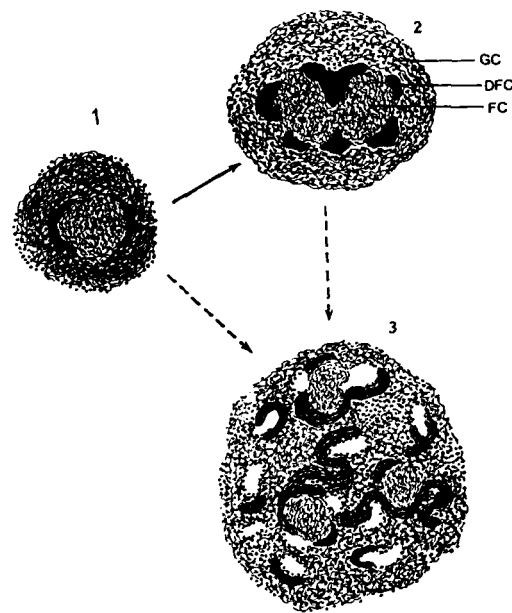


Fig. 8. A scheme of main modifications of a nucleolus activated by He-Ne laser radiation or PHA-treatment [38, 40]: (1), ring-shaped nucleolus of the intact lymphocytes; (2) changed nucleolus with a complex fibrillar center (FC); (3) strand-like nucleolus with several PC. Abbreviations: GC - granular component; DFC - dense fibrillar component; FC - fibrillar center.

experiments probably can be related to the enhancement of the pre-RNA processing and the preribosome production. Finally, the appearance of vacuoles can be related with the activation of transport of the r-RNP synthesized by transformed nucleolus as it was suggested earlier for PHA-activated rat lymphocytes [40].

The results of our experiments demonstrate the similarity in the nucleolar modifications of human lymphocyte nucleoli under the action of two different factors - PHA and laser radiation. On the whole these changes can be interpreted as an activation of rRNA metabolism, including its synthesis, processing and transport. These results are in agreement with the increase in total RNA synthesis rate in the human lymphocytes during the first hours after both types of treatment [36, 37]. The results of the present work show that the intensification of rRNA synthesis contributes to the increase of total RNA synthesis described in [36].

The irradiated with He-Ne laser lymphocytes revealed also tendencies in the changes of chromatin, which, on the whole, can be interpreted as functional activation of extranucleolar transcription: a decrease in the relative area of nucleoplasmic chromatin, an increase in the relative area of decondensation zones, an increase both in the number and twisting coefficients of nucleoplasmic chromatin clumps [39].

One of the intracellular systems involved in growth regulation is the c-myc proto-oncogene. It is known that PHA-treatment increases the c-myc mRNA level in human lymphocytes [46]. In our experiments the steady-state level of c-myc mRNA in human lymphocytes was measured 2 h after the irradiation. Under our experimental conditions, both the irradiation and PHA-treatment caused in the cells an accumulation of preliminary terminated proto oncogene c-myc RNA but not a variation in amount of full-length c-myc RNA [47].

3.2. DIFFERENCE IN LONG-TERM RESPONSES OF LYMPHOCYTES TO PHA AND IRRADIATION

Despite the similarities in early responses of cells to PHA and irradiation described in Section 2.1., the irradiated lymphocytes did not enter the S-phase [36]. This difference was explained by the absence of interleukin-2 receptor expression in irradiated lymphocytes [36].

At the same time, the irradiation elevated the DNA synthesis level in PHA treated cells (Table 2).

TABLE 2. Indices of ^{14}C -thymidine incorporation*: irradiation of lymphocytes with He-Ne laser (56 J/m²) and treatment with PHA in various concentrations [36].

Donor	0.5 µg/ml PHA			1.0 µg/ml PHA			2.0 µg/ml PHA		
	PHA + PHA	He-Ne	%**	PHA	He-Ne + PHA	%	PHA + PHA	He-Ne + PHA	%
I	40.8±3.4	48.4±3.5	118.6	85.4±11.0	129.4± 3.9	151.5	102.4± 6.7	143.5±28.0	140
II	-	-	-	82.9± 4.5	142.2± 7.8	171.6	84.0± 3.0	84.9± 8.2	101.1
III	-	-	-	59.1±8.5	60.2±11.7	101.9	88.5± 5.4	120 ±5.1	136.5
IV	-	-	-	22.8± 3.0	39.8±4.6	174.0	47.5± 7.8	73.6±11.3	154.9
V	-	-	-	-	-	-	100.5±12.3	123.5± 7.0	122.9
VI	-	-	-	-	-	-	118.1±15.5	121.0±15.3	102.4

* Index of ^{14}C -thymidine incorporation = $\text{CPM}_{\text{experiment}} : \text{CPM}_{\text{intact control}}$

** % of efficacy of irradiation = $\text{index}_{\text{He-Ne+PHA}} : \text{index}_{\text{PHA}}$

The mechanism of the irradiation-induced boosting effect (Table 2) can be probably explained with the fact that a part of metabolic processes connected with activation (short-term responses) occurs under action of light as well (Figure 7). In this way, the effective dose of PHA per cell decreases and more cells can be activated by PHA. The role of He-Ne laser irradiation here could be a transcriptional activation of lymphocytes (and may be other Go-cells) making these cells more sensitive to growth factors and antigens.

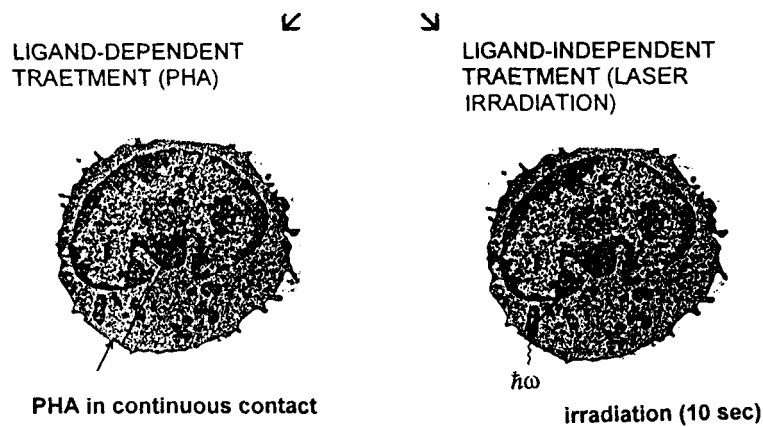
3.3. COMPARISON OF LYMPHOCYTE ACTIVATION BY PHA AND He-Ne LASER RADIATION

Specific binding between a surface receptor and a ligand (PHA in our experiments) is required for lymphocyte activation and replication (a ligand-dependent treatment). Interactions between lymphocyte surface and PHA initiate a signal which is transduced from the cellular membrane to the nucleus and subsequently induces proliferation [34, 35].

The activation of lymphocytes by laser radiation is a ligand-independent treatment [48,49]. According to the laws of photobiology, in this case, as a first step the light quanta should be absorbed by some molecules-photoacceptors which are considered to be components of the respiratory chain [48,49]. The principal difference between two types of activation is that PHA is constantly present in cell suspension, whereas the laser irradiation lasts only the first 10 s.

Figure 9 presents the summary of results of comparative experiments (PHA-treatment and He-Ne laser irradiation) performed with human lymphocytes. As seen in Fig.9, the early responses of cells to both types of treatment are similar, but the long-term responses are entirely different.

LYMPHOCYTE ACTIVATION



SIMILARITY IN SHORT-TERM RESPONSES (first minutes and hours after treatment)

- | | |
|---|---|
| + Changes in chromatin template activity
(synthesis of total RNA, decondensation of chromatin) | + |
| + Ca^{2+} influx | + |
| + Expression of rRNA genes (rRNA - 80% of total RNA) | + |
| + Accumulation of preliminary terminated proto oncogene
c-myc RNA | + |

DIFFERENCE IN LONG-TERM RESPONSES (48-72 h after treatment)

- | | |
|--|---|
| + Blasttransformation | - |
| + DNA synthesis and mitosis | - |
| + Expression of genes of interleukin-2
and interleukin-2 receptor | - |

Fig. 9. Comparison of results [36-39,47] of ligand-dependent and ligand-independent types of lymphocyte activation

Figure 9 presents the summary of results of comparative experiments (PHA-treatment and He-Ne laser irradiation) performed with human lymphocytes. As seen in Fig.9, the early responses of cells to both types of treatment are similar, but the long-term responses are entirely different.

When cultured mammalian cells are irradiated and the proliferation is considered, the cascade of reactions starts from absorption of light in mitochondria and only after that the signal is

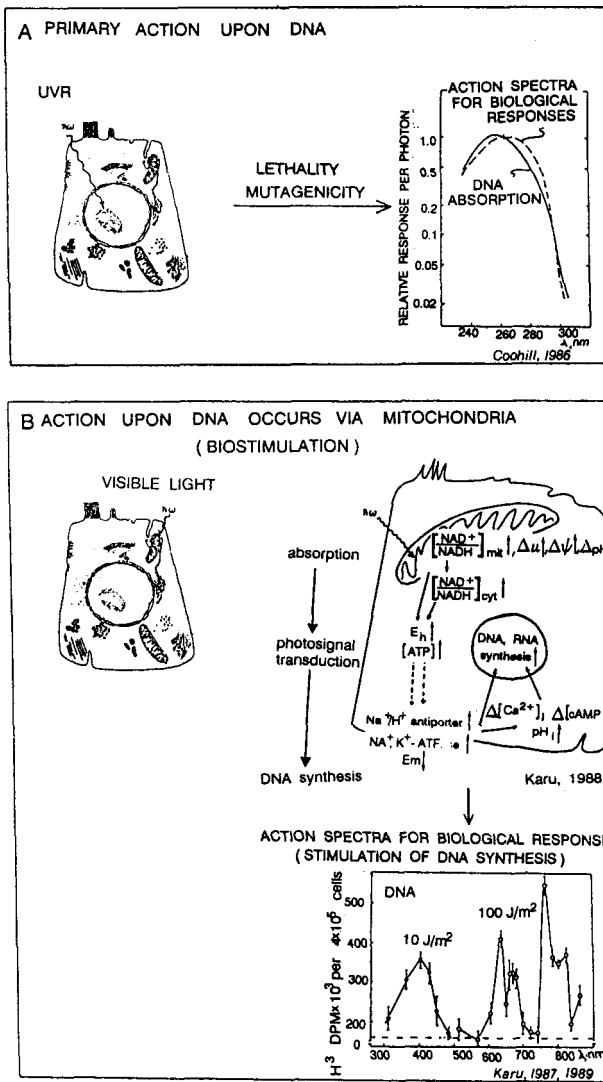


Fig. 10. Two different mechanisms for exerting effects on DNA synthesis: (a) primary action after absorption of UVR by DNA forms the basic for lethal and mutagenic effects as well as for structural effects considered in the Section 1; (b) a mechanism whereby monochromatic visible (laser) light is absorbed by mitochondrial pigments, and stimulation of DNA synthesis occurs as a final step after many dark reactions in a cell. This scheme forms a basis for low-power laser therapy [47,48].

transduced to the cellular membrane and then to the nucleus (Figure 10b). Whether this is the case in lymphocytes is not clear. An action spectrum reflecting the absorption spectrum of the primary photoacceptor(s), is absent.

On the other hand the changes in structure of chromatin after the irradiation (Fig.7) which do not have absorption bands at 632.8 nm clearly show the existence of a signal transduction chain from a photoacceptor to the nucleus. The question if these photoacceptors are located in mitochondria, needs further investigation. Electron microscopy studies show that there are changes in mitochondria in short time periods after He-Ne laser irradiation of human lymphocytes [38,50]. The mechanism of lymphocyte activation by He-Ne laser radiation is not yet investigated.

4. Concluding Remarks

The experiments discussed in Section 2 suggest that UVR has not only negative but also positive effects: stimulation of colony-forming ability and enhancement of proliferation. It is believed that when the cells are irradiated by UVR, the primary photoacceptor is the DNA (Figure 10a). As to the photochemical activity of visible (laser) light, it is possible to stimulate DNA synthesis with this kind of radiation as well (Fig.10b). In this case, DNA synthesis is the last step in a long chain of photosignal transduction and amplification reactions triggered by the absorption of visible light in mitochondria (Figure 10b). Probably, the results discussed in Section 2 are based on this type of cell-radiation interaction. Effecting DNA synthesis in this way is completely different from the mechanism discussed in Section 1, as one can see from a comparison of Figures 10a and b.

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