
Can Cellular Responses to Continuous-Wave and Pulsed UV Radiation Differ?

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1. Introduction

Interest in the action of UV radiation (UVR) on mammalian cells stemmed initially from the need to interpret the effects of this radiation on humans. This interest was a focus at Finsen's Light Institute in Copenhagen, where some of the first basic studies on the effects of UVR on animal cells were carried out at the beginning of the century (Giese, 1964). During recent decades, investigation into UVR-induced effects on cells has been one of the most active and extensively studied areas in photobiology. For review see, e.g., Rauth (1970), Painter (1970), Peak and Peak (1989), and Moan and Peak (1989).

For most biological effects of UVR, such as lethality, mutation, and transformation, DNA is considered to be the main target. For example, action spectra for lethality and mutagenicity, which are similar for both prokaryotes and eukaryotes, follow the spectra for DNA absorption and pyrimidine dimer induction (Rothman and Setlow, 1979; Coohill, 1986; see also Fig. 16a).

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With the advent of lasers, new UV-R sources emitting pulsed radiation with various pulse durations and peak intensities have become available. It would be of interest to compare cellular responses to classical continuous-wave (CW) and pulsed UV radiation for two reasons. First, during evolution, cells have adapted to CW (solar) radiation, and it is not clear *a priori* that their responses to CW radiation and pulsed UVR at the same wavelengths are exactly similar. Second, in the case of very short and intense laser pulses, it is possible to excite singlet and triplet electronic states (two-quantum excitation) of the photoacceptor molecule (Fig. L b,c). It is known from experiments with DNA bases and viruses (Letokhov, 1983; Nikogosyan and Le-tokhov, 1983; Nikogosyan, 1990) that two-quantum excitation produces photoproducts different from those produced by single-photon UVR excitation.

The aim of our experiments (Karu *et al.*, 1981, 1982, 1983a,b; 1984a,b; 1988; Karu, 1986; Karu and Kalendo, 1987) was to find a way to damage quiescent (resting) cells. The high resistance of quiescent tumor cells to various damaging agents (e.g., ionizing radiation, chemotherapeutic drugs) is one of the factors decreasing the efficiency of tumor therapy (Baserga, 1971; Valeriote and van Putten, 1975; Zubrod, 1978; Epifanova *et al.*, 1983). The information available indicates that cultures of tumor cells in the plateau phase of growth, as identified by the content of cellular subpopulations, can serve as models

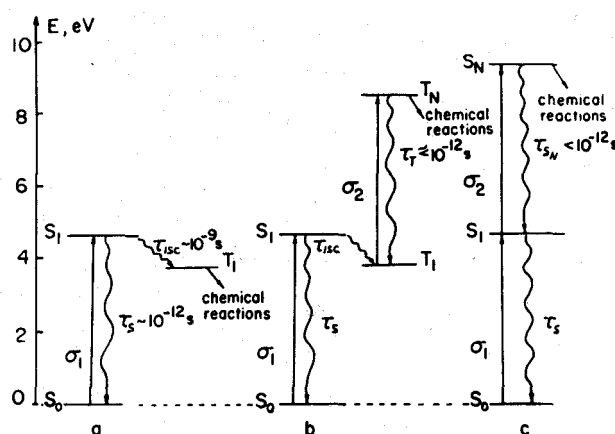


Figure 1. Excitation schemes of the electronic states of nucleic acid components: (a) one-quantum excitation of a singlet state with subsequent conversion to a triplet state with relaxation time τ_{isc} , (b) two-quantum excitation via intermediate triplet (T) states, and (c) two-quantum excitation via intermediate singlet (S) states. τ marks relaxation times of respective excited states, and σ_1 and σ_2 are cross sections of absorption of first and second photons.

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for solid tumors (Baserga, 1971; Hahn and Little, 1972; Hahn, 1975; Grdina *et al.*, 1977).

To damage cells, two laser UVR sources and one conventional UVR source were used (see details in Fig. 2). The wavelengths of all three UVR sources are very close and fit to the maximum of the first absorption band of DNA (see Fig. 16a for the absorption spectrum). Powerful picosecond pulses (PPP) at 266 nm can cause two-quantum excitation of DNA (Fig. 1 b,c), and high-repetition-rate pulses (HRRP) at 271 nm, as well as CW radiation at 270 nm, excite DNA by the single-quantum mechanism following classical laws of photobiology (Fig. 1a). The action of PPP on DNA bases, viruses, and

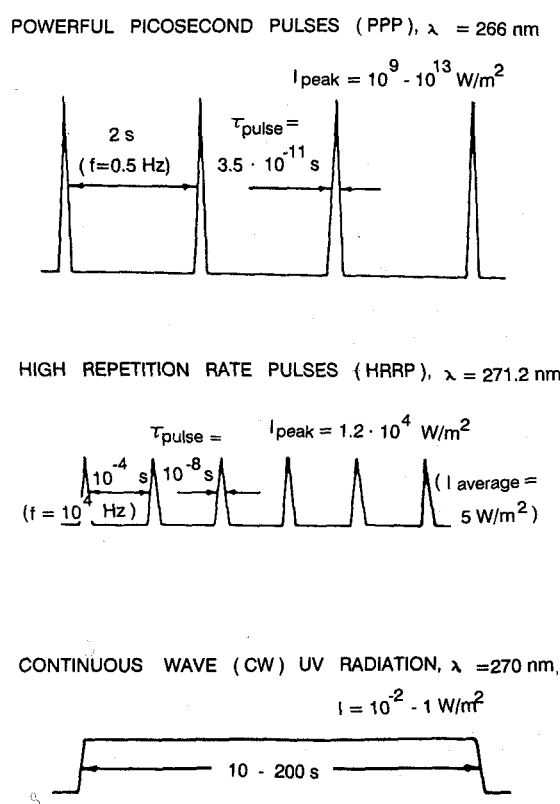


Figure 2. Parameters of UV pulses used in present work. The 4th harmonic of a Nd^{3+} :YAG laser emitting single powerful picosecond pulses (PPP) at 266 nm, and the 2nd harmonic of a Cu-vapor laser emitting high-repetition-rate nanosecond pulses (HRRP) at 271.2 nm with high peak and low average intensity (I) were the laser UVR sources used. A mercury lamp with a monochromator, $\lambda = 270$ nm, was the conventional UVR source used. (I_{peak} = peak intensity; τ_{pulse} = pulse duration; I_{average} = average intensity.)

bacteria (but not on mammalian cells) has been extensively studied (Nikogosyan and Letokhov, 1983; Nikogosyan, 1990; Schulte-Frohlinde *et al.*, 1990; Cadet and Vigny, 1990). Laser sources emitting HRRP are new to photo-biologic studies.

In this chapter, I will describe two ways to damage plateau-phase HeLa cells (as compared to exponentially growing cells) by pulsed laser UVR. First, it will be shown that certain pulse rates and fluence ranges of HRRP enhance colony-forming ability and induce replicative synthesis of DNA in a subpopulation of plateau-phase cells. Second, two intense picosecond pulses with a 4- to 6-s interval between them enhance the transport processes of cellular membranes without causing any changes in the viability of cells or in the rate of DNA synthesis.

It is well known that exposure to solar radiation and UVR under laboratory conditions can induce skin tumors. Recent experimental data suggest that an inappropriate expression of protooncogenes due to point mutation or gene amplification, deletion, or rearrangement may be involved in UV carcinogenesis (Ananthaswamy and Pierceall, 1990). The experimental data on UV-induced replicative synthesis in plateau-phase HeLa cells presented here may be of interest to those concerned with mechanisms of UV carcinogenesis, despite the fact that a HeLa culture is in itself transformed.

The increase in the permeability of cellular membranes can probably be used to introduce allogenic material or chemotherapeutic agents into quiescent cells.

2. Induction of Replicative DNA Synthesis in Plateau-Phase Cells

2.1. Basic Information about Cells and Experimental Technique

HeLa cell cultures grown as monolayers using nutrient medium 199 supplemented with 10% bovine serum and antibiotics were irradiated 3 days (exponentially growing, or log-phase, cells) or 10 days (plateau-phase cells) after plating. The cells were grown without changing the nutrient medium (unfed culture) and without CO₂ supply. The HeLa culture spread well and did not form multilayers (see Karu *et al.*, 1990, for details). The labeling index | of the log-phase population was $19.1\% \pm 3.0\%$, and that of the plateau-phase culture was $5.0\% \pm 0.8\%$. The mitotic indices of log-phase and plateau-phase populations were $1.1\% \pm 0.1\%$ and 0.1% , respectively. 3

Cultivation of cells, radiometric techniques of pulse labeling or continuous I labeling, and autoradiography are described in detail elsewhere (Karu *et al.*, |

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1983b, 1984a,c, 1987, 1990). These methods are rather standard and follow the description given by Hauschka (1973).

There are, however, two methodological points in our experiments which differed from usual experimental technique. First, cells were cultivated and irradiated in special chambers with quartz windows allowing irradiation of the monolayer without removal of the nutrient medium (Fig. 3). Under these conditions, changes in the partial pressure of oxygen and pH jumps in the nutrient medium could be avoided. Second, in contrast to the classical Puck technique for evaluation of clonogenicity (i.e., individual cells, either in suspension or attached to substrate, exposed to radiation and then allowed to form colonies), we irradiated confluent monolayers and then plated the cells into a fresh nutrient medium to form colonies.

2.2. Specific Responses of Plateau-Phase Cells to Various Sources of UVR

Monolayers of plateau-phase HeLa cells were exposed to high-repetition-rate pulses (HRRP) at 271 nm, continuous-wave (CW) UVR at 270 nm, or powerful picosecond pulses (PPP) at 266 nm at various fluences. After 3 h the cells were removed from the culture chamber in maximal mild conditions (Versene instead of trypsin, temperature at 37°C), and plated in scintillation vials. Two experiments were performed. In the first, 100 cells were plated into every flask—control (nonirradiated) and test—and incubated for 14 days. Then the colonies were fixed with methylene blue, their numbers determined and their diameters measured under a high-power dissecting microscope. The results of this experiment, which reflects the colony-forming ability of plated cells, are presented in Figs. 4a and 5. In the second experiment, 100 cells were again plated into every flask, but the number of cells in three separate flasks was determined every day. The result of this experiment (growth curves) is presented in Fig. 4b.

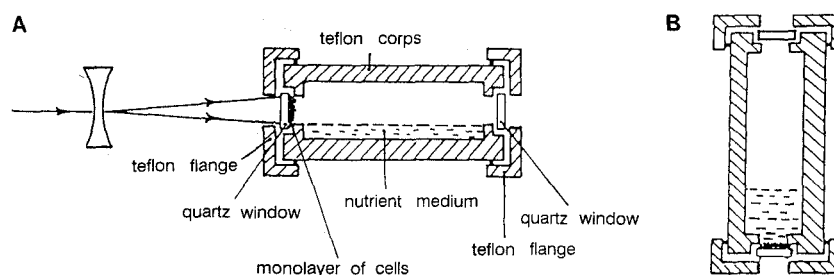


Figure 3. Cell culture chamber for UV irradiation of monolayers: (A) position during irradiation ; (B) position during cultivation.

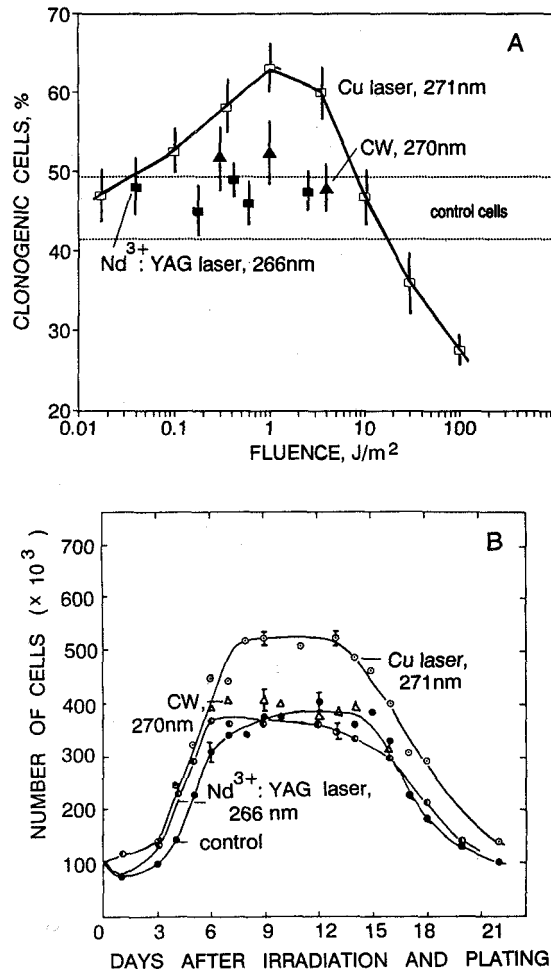


Figure 4. Colony formation and multiplication of HeLa cells exposed in the plateau phase of growth to various UVR sources and plated 3 h after irradiation into fresh medium. (Δ) = CW; (o) = Nd:YAG; (\bullet) = control; (\odot) = Cu laser. (A) number of colonies formed as a function of fluence after 14-day incubation period; (B) growth curves of cells given a fluence of 2 J/m^2 ; cells per vial counted daily.

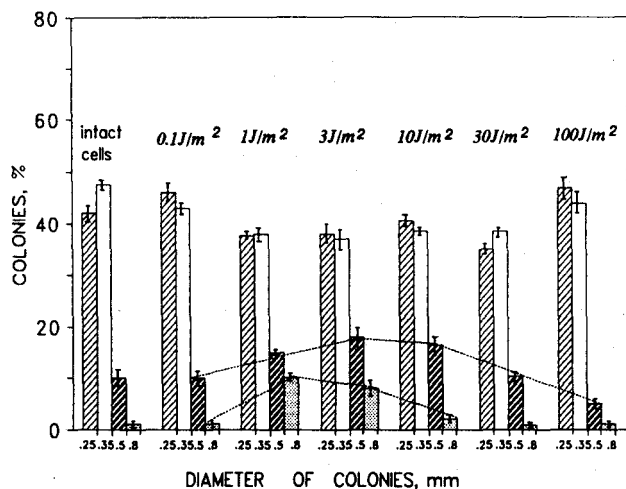


Figure 5. Distribution of colonies by diameter (d) after 271.2-nm HRRP at various fluences. Dashed lines mark the dependence of the percentage of large ($0.5 < d < 0.8$ mm) and intermediate-size ($0.35 < d < 0.5$ mm) colonies on the fluence.

The percentage of clonogenic cells in the nonirradiated culture was $45.2\% \pm 0.4\%$. As can be seen in Fig. 4a, irradiation with HRRP at fluences from 0.1 to $3 J/m^2$ caused an increase in the number of colonies. At higher fluences (from 10 to $100 J/m^2$), the number of colonies decreased as compared to the unexposed control. Under the same experimental conditions, PPP and low-intensity CW UVR in the fluence range 0.1 - $3 J/m^2$ did not cause statistically significant changes from the control level.

A comparison of the growth curves indicates that PPP and CW UVR did not have much effect on cell growth (Fig. 4b). Irradiation with HRRP at $2 J/m^2$ caused an increase in cell proliferation during the exponential phase of growth and the formation of a plateau with a higher number of cells than that of the control (Fig. 4b).

Investigation of colony size distributions after irradiation of plateau-phase cells with various fluences of HRRP (Fig. 5) indicated that in the cultures exposed to stimulating fluences (0.1 - $3 J/m^2$, as seen in Figure 4a), the number of intermediate-size ($0.35 < d < 0.5$ mm) and large ($0.5 < d < 0.8$ mm) colonies increased, and the number of abortive ($d < 0.25$ mm) and small ($0.25 < d < 0.35$ mm) colonies decreased. At fluences above $3 J/m^2$, however, the percentage of large and medium-size colonies decreased, whereas the action of light on the abortive and small colonies had no effect.

The data presented in Figs. 4 and 5 suggest that at certain low fluences (0.1-3 J/m²) of HRRP, irradiation not only results in an increase in the number of colonies but also induces increased proliferation of a subpopulation of the irradiated cells.

2.3. Specific Responses of Plateau-Phase Cells to High-Repetition-Rate UVR

Measurement of [³H]thymidine (a DNA precursor) incorporation into DNA is frequently done to estimate the effects of DNA-damaging agents. There are two different ways to perform such measurements.

In one method, the radioactive precursor is added to cell culture for a certain time period, e.g., 20 min, after which time the amount of radioactive DNA is determined, allowing changes in DNA synthesis rate to be evaluated (radiometric pulse-labeling). In the second method, the pulse-labeled cells are fixed and developed with photographic emulsion (autoradiography). This technique makes it possible to count the number of DNA-synthesizing cells, and by determining the number of silver grains above each nucleus, to evaluate the rate of DNA synthesis in individual cells.

Figure 6 presents the fluence-dependent changes in DNA synthesis rate measured by radiometric pulse-labeling in exponentially growing and plateau-phase HeLa cells 2.5 h after exposure to HRRP, PPP, or CW low-intensity UVR. As seen in Fig. 6a, the log-phase HeLa cells responded to HRRP and CW UVR in a similar way: DNA synthesis was inhibited by increasing the fluence. In the case of PPP, some stimulation was observed in the fluence range 10⁻¹-10 J/m², and inhibition of DNA synthesis occurred at fluences higher than 10 J/m².

The responses of the plateau-phase cells (Fig. 6b) were different from those of log-phase cells. PPP did not affect DNA synthesis in the fluence range used. HRRP and CW low-intensity UVR had pronounced but opposite effects on DNA synthesis rate: CW UVR inhibited DNA synthesis in a fluence-dependent manner; HRRP stimulated it (optimal fluences near 0.5-1 J/m²), and no inhibition of DNA synthesis was observed by increasing the fluence of HRRP.

The stimulation of DNA synthesis by HRRP was specific for plateau-phase cells (Fig. 7). In this series of experiments, 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 of the cells. 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 illustrated by the schemes on the right of Fig. 7. As seen in Fig. 7, all plateau-phase cultures (10th, 11th, and 13th days of cultivation) responded to irradiation with increasing [³H]thymidine incor-

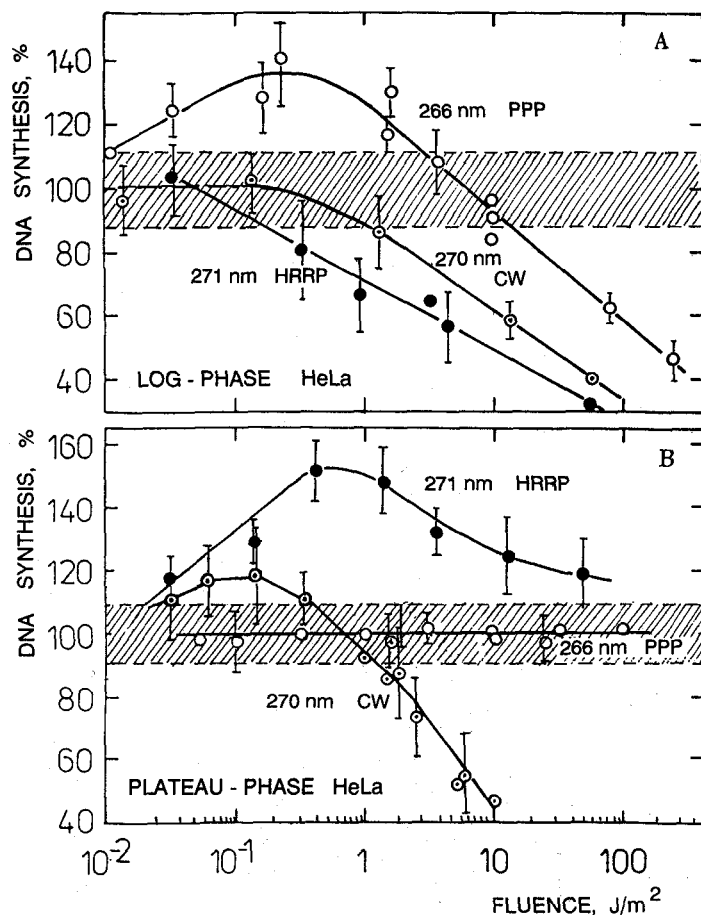
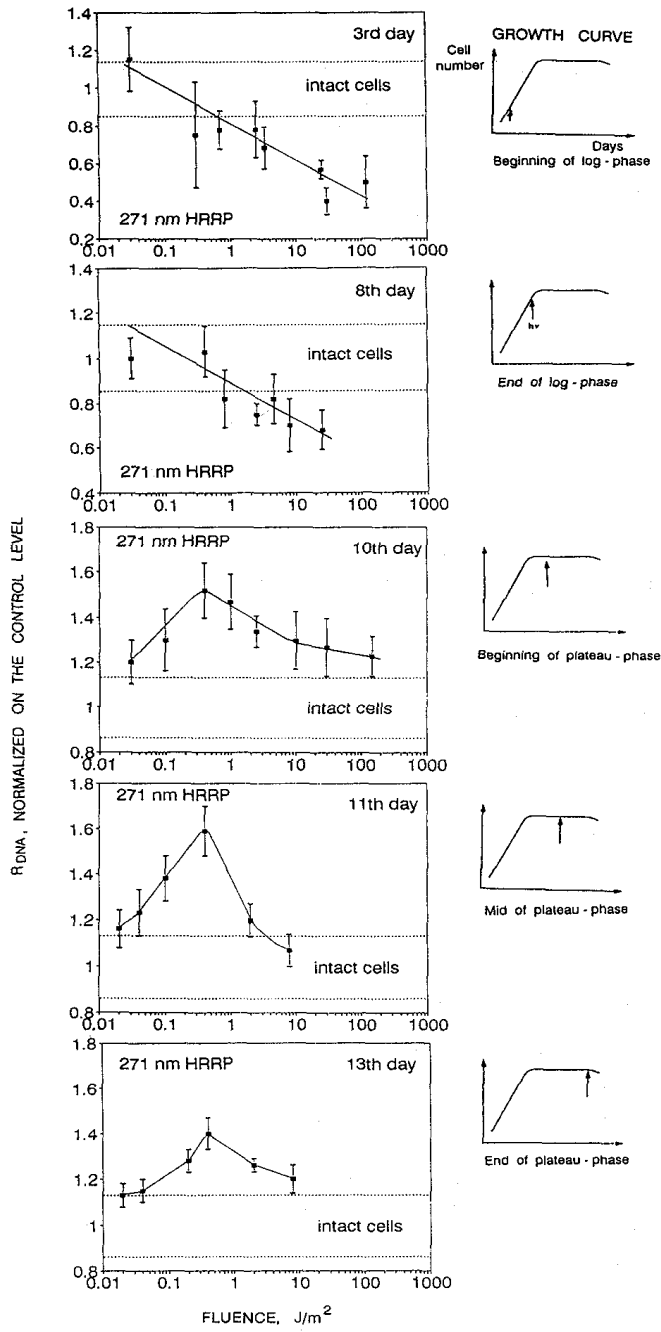


Figure 6. Incorporation of [³H]thymidine during 20-min pulse labelings measured 2.5 h (starting point of the pulse label) after the exposure of (A) log-phase or (B) plateau-phase HeLa monolayers to HRRP at 271 nm, PPP at 266 nm, or CW UVR at 270 nm. Control cells are denoted by the shaded area.

poration. No inhibition of DNA synthesis was observed as the fluence was increased.

The stimulation of DNA synthesis also depended on the pulse repetition rate of the radiation (Fig. 8). 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. In this experiment we used a Nd³⁺;YAG laser emitting at 266 nm, but the parameters of the pulses (their peaks, average intensities,



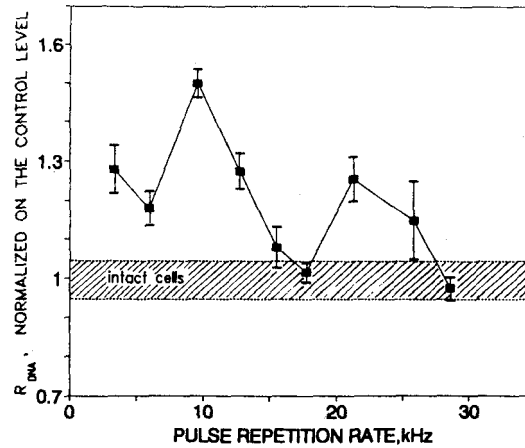


Figure 8. Incorporation of [^3H]thymidine into DNA during 20-min pulse labelings measured 2.5 h (starting point of the pulse label) after the exposure of plateau-phase HeLa cells to HRRP at a fluence of 5 J/m^2 as a function of pulse repetition rate.

and duration) were very close to the respective parameters of the 2nd harmonic of the Cu-vapor laser.

This result indicates that the action of HRRP on plateau-phase HeLa cells is connected with DNA damage specific and characteristic of this type of radiation.

The [^3H]thymidine experiments described above were performed 2.5 h after irradiation of the cells. The kinetics of [^3H]thymidine incorporation measured by pulse-labeling at various time points after irradiation varied with the type of radiation used (Fig. 9a,c). After irradiation with CW UVR (Fig. 9c), DNA synthesis was inhibited during the first hours and then returned to the control level. After irradiation with HRRP, DNA synthesis remained at the control level in the first 2 h of postirradiation and then increased rapidly (Fig. 9a).

We also measured [^{14}C]uridine incorporation into RNA after both types of irradiation. With CW UVR, no statistically significant changes were found in the first 5 h as compared to the nonirradiated cells (Karu *et al.*, 1983b).

Figure 7. Incorporation of [^3H]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. Ordinate is defined as the ratio of experimental and control.

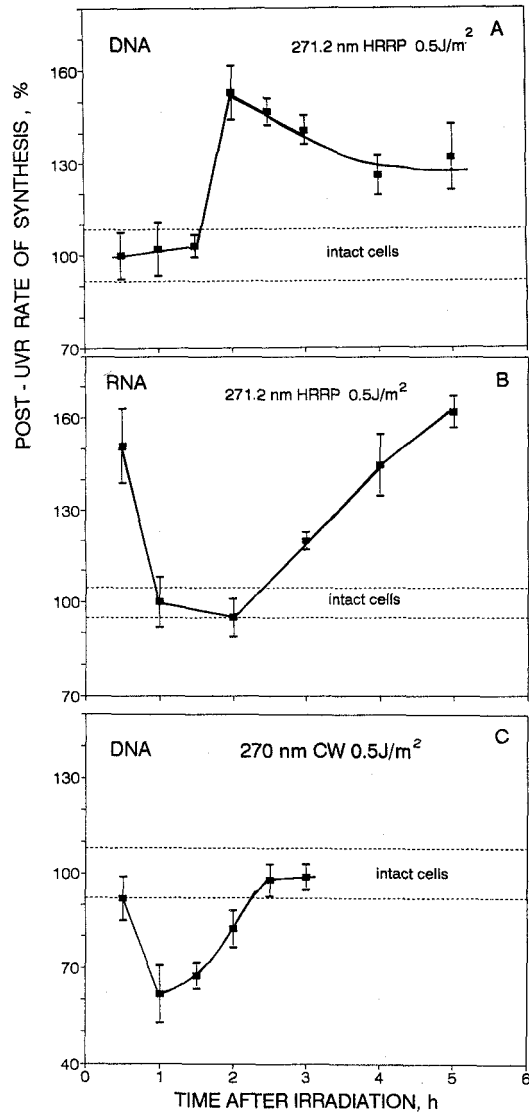


Figure 9. Incorporation of (A,C) [³H]thymidine or (B) [¹⁴C]uridine during 20-min pulse labelings measured at different time points (time in the abscissa marks the beginning of the pulse labeling) after the exposure of plateau-phase HeLa cells to (a,b) HRRP or (c) CW UVR.

When the plateau-phase cells were irradiated with HRRP, [^{14}C]undine incorporation increased 30 min after irradiation, then returned to the control level, and later increased again (Fig. 9b). Similar kinetic changes in RNA synthesis rate have been described for cells overcoming the $G_0 \rightarrow S$ transition (Epifanova, 1977). The data presented in Fig. 9b are clearly insufficient to answer the question of whether or not the increased RNA synthesis found in our experiments (Fig. 9b) is connected with an activation of proliferation.

This series of experiments suggests that the increase of [^3H]thymidine incorporation after irradiation with HRRP is a specific response of plateau-phase HeLa cells under our experimental conditions.

2.4. Does High-Repetition-Rate UVR Induce Replicative or Reparative DNA Synthesis?

One way to distinguish between replicative and reparative synthesis of DNA is by counting 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 10 presents the labeling index 2.5 h 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

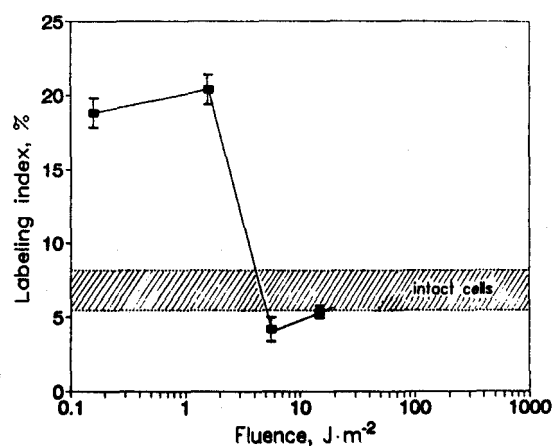


Figure 10. Labeling index (percentage of cells incorporating [^3H]thymidine) 2.5 h after the exposure of plateau-phase HeLa cells to 271-nm HRRP at various fluences (radioautographic measurements).

times at fluences of 0.16 or 1.6 J/m². After increasing the fluence (5.6 and 16 J/m²), the number of DNA-synthesizing cells decreased and dropped to near the control level. The stimulative fluences here (0.16 and 1.6 J/m²) coincide with those determined by radiometric measurements of [³H]thymidine incorporation (Figs. 6b and 7).

Figure 11 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/m², 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/m²), 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. 12a that the percentage of labeled cells increased in 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.

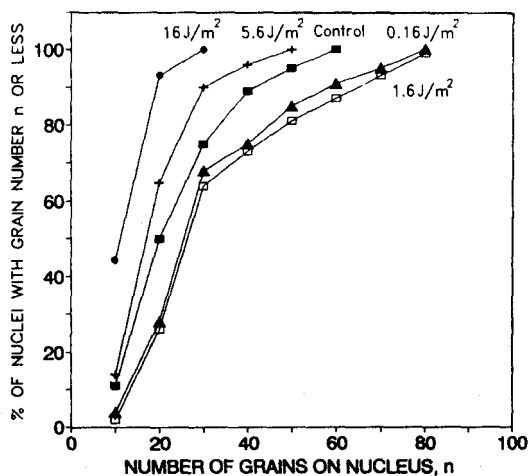


Figure 11. Silver grain counts in control and 271.2-nm HRRP-irradiated plateau-phase HeLa cells after 20-min pulse labeling with [³H]thymidine (radioautographic measurements). The respective labeling indices are shown in Fig. 10.

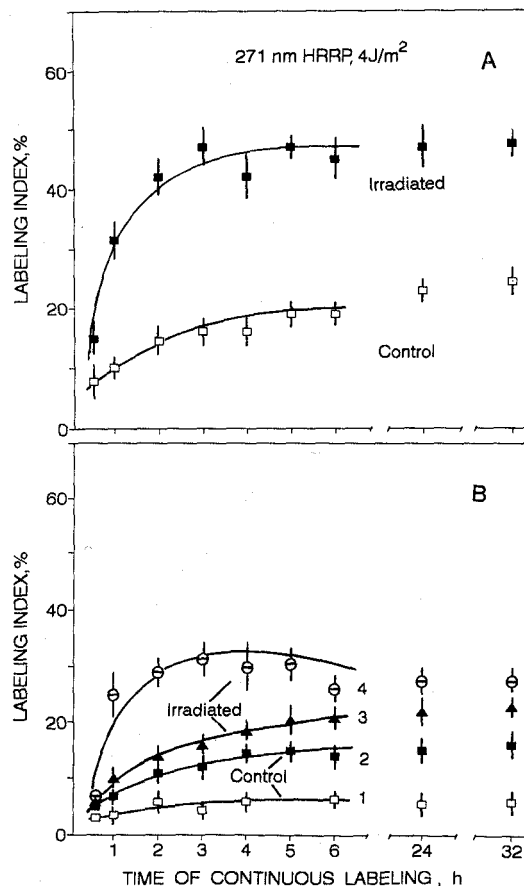


Figure 12. Labeling index (percentage of DNA-synthesizing cells) after the exposure of plateau-phase HeLa cells to 271-nm HRRP at 4 J/m^2 , measured by the autoradiographic continuous-labeling technique: (A) percentage of labeled cells in irradiated and control cultures, and (B) percentage of cells with 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.

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 (Paribok and Semyonova, 1970).

As seen in Figure 12b, 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 (0.1-3 J/m² (Figs. 6b, 7, 9a) and at certain pulse rates (near 10 and 21 kHz, Fig. 8) is mainly due to reparative DNA synthesis (Fig. 12b). A lesser [³H]thymidine uptake is accomplished via replicative DNA synthesis (Figs. 11 and 12b). Under our experimental conditions, HRRP is able to induce both types of DNA synthesis, replicative as well as reparative.

2.5. Is the Induction of Replicative Synthesis by DNA-Damaging Agents a Common or a Unique Phenomenon?

A stream of literature data suggest that UVR suppresses replicative synthesis of DNA and induces reparative synthesis of DNA in cellular cultures of mammalian origin (for review see Painter, 1970; Rauth, 1970; Moan and Peak, 1989). 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 (Little, 1969; Hahn, 1975; Chan and Little, 1979; Wechelbaum *et al.*, 1980; Kantor *et al.*, 1980; Kantor, 1986; Gill and Coohill, 1987) 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 (Kantor, 1986).

We found that irradiation with HRRP at 271 nm induced replicative DNA synthesis in a fraction (subpopulation) of the plateau-phase HeLa cultures (Figs. 5, 12). The cells irradiated at low fluences not only retained the capacity for further growth but their proliferative capacity was stimulated (Figs. 4, 5).

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 (2-20 J/m²) or with carcinogenic agents *N*-methyl-*N*-nitrosourea and *N*-acetoxy-2-acetylaminofluorene (Cohn *et al.*, 1984). Another carcinogenic agent, *N*-methyl-*N*-nitrosoquandine, stimulated DNA replication in a post-confluent culture of Syrian hamster embryo cells (Mironescu *et al.*, 1980). Gamma irradiation induced replicative synthesis of DNA in plateau-phase HeLa cultures (Synsyns and Saenko, 1986).

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 these experiments (Mironescu *et al.*, 1980; Cohn *et al.*, 1984; Synsyn and Saenko, 1986) is that all DNA-damaging agents, each of them producing 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.* (1991) 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 (Ronai *et al.*, 1990). UVR is known to activate viral DNA sequences in transformed cells and to induce deletion of growth suppression genes (Ananthaswamy and Pierceall, 1990).

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 stimulate growth of the complete virus in human cells (Valerie *et al.*, 1988; Zmudzka and Beer, 1990). UVR-sensitive sites in RNA transcripts of hepatitis delta virus and potato spindle fiber viroid have been found (Branch *et al.*, 1989). 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 (Alpatov and Nastjukova, 1933, Carlson *et al.*, 1961; Walicka and Beer, 1973; Samoilova, 1979; Abbaszade, 1986; Belenkina *et al.*, 1990). Most of this work has been done with microorganisms, except that of Walicka and Beer (1973). 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 to 53.5 J/m², and cultured for up to 70 days after irradiation. UV-exposure stimulated growth in about 70% of the cultures; growth started immediately after post-irradiation 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 5178-S strain: maximal shortening of the mean doubling time for the L 5178Y-R strain was 43%, whereas it was 20% for L 5178-S cells (Walicka and Beer, 1973). The most important result of

this investigation was that growth stimulation also occurred at later stages of postexposure development, demonstrating UVR-induced heritable changes.

Taken together this research suggests that the induction of DNA replication by low doses of damaging agents is not a unique 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 higher doses of the agents (Mironescu *et al.*, 1980; Karu *et al.*, 1982, 1983a; Cohn *et al.*, 1984, Saenko and Synsyns, 1986; Figs. 6, 7, 10). Similar bell-shaped fluence dependencies were also established for HIV-1 promoter induction in HeLa cells by UVR (Zmudzka and Beer, 1990). As established by Cohn *et al.* (1984), 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, Cohn *et al.*, 1984; 0.5 J/m², Karu *et al.*, 1983a; Figs. 6b, 7) and magnitudes of effects (1.6, Cohn *et al.*, 1984; 1.4-1.6, Karu, 1982, 1983a; Fig. 7) 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.* (1984) established this fraction to be 6% in plateau-phase cultures and 8% in serum-arrested cultures. We did not perform detailed measurements, but an analysis of auto-radiographic data and clone size distributions allows us to estimate this fraction to be 10-15%. Cohn *et al.* (1984) 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 was a specific cellular response to HRRP; the CW UVR administered at practically the same wavelength, the same fluence, and during the same postirradiation period had no effect (Fig. 6b) or was inhibitive (Fig. 9c). In these experiments, the measurements were made during the first 5 h of postirradiation. With HRRP, the first point with increased [³H]thymidine incorporation was 2 h after exposure (Fig. 9a), 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 (Cohn *et al.*, 1984). 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 (Synsyns and Saenko, 1986). 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. 9b), 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.

Upon reaching the plateau-phase, populations of normal cells accumulate in the early G₁ phase of the cell cycle, while most cells of transformed lines, HeLa included, accumulate in a late G₁ phase (Hittelman and Potu, 1978). Similar differences in the morphology of the G₁ phase between cells from normal tissues and solid tumors are reported (Grdina *et al.*, 1977).

It has been shown in cell 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 (w/5 h). UV irradiation of the quiescent cells before fusion induced decondensation of chromatin in a fluence-dependent manner in the nuclei of these cells (Rao and Smith, 1981). Decondensation of chromatin must occur before reparative (Schor *et al.*, 1975) and replicative (Baserga, 1979) 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.

3. The Effect of Powerful Ultrashort Pulses on Transport Processes in the Membranes of Plateau-Phase Cells

3.1. Does Two-Quantum Excitation Occur in Living Cells?

As explained in the introduction, PPP can excite absorbing molecules by a two-quantum mechanism (see Figs. 1c,d). The two-quantum mechanism of excitation occurs in DNA bases in solutions, in viruses, and in bacteria (Nikogosyan and Letokhov, 1983; Letokhov, 1983; Nikogosyan, 1990). It would be of interest to estimate the probability of two-quantum excitation of DNA molecules when mammalian cells are irradiated by ultrashort pulses with $\lambda = 266$ nm. If the target is a DNA molecule, cells with a higher quantity of DNA have a greater chance of being damaged. In other words, as the organism becomes more complex, the number of DNA bases increases and the fluence needed to cause damage to at least one base in the DNA chain decreases. This relationship, which is shown in Fig. 13, is also

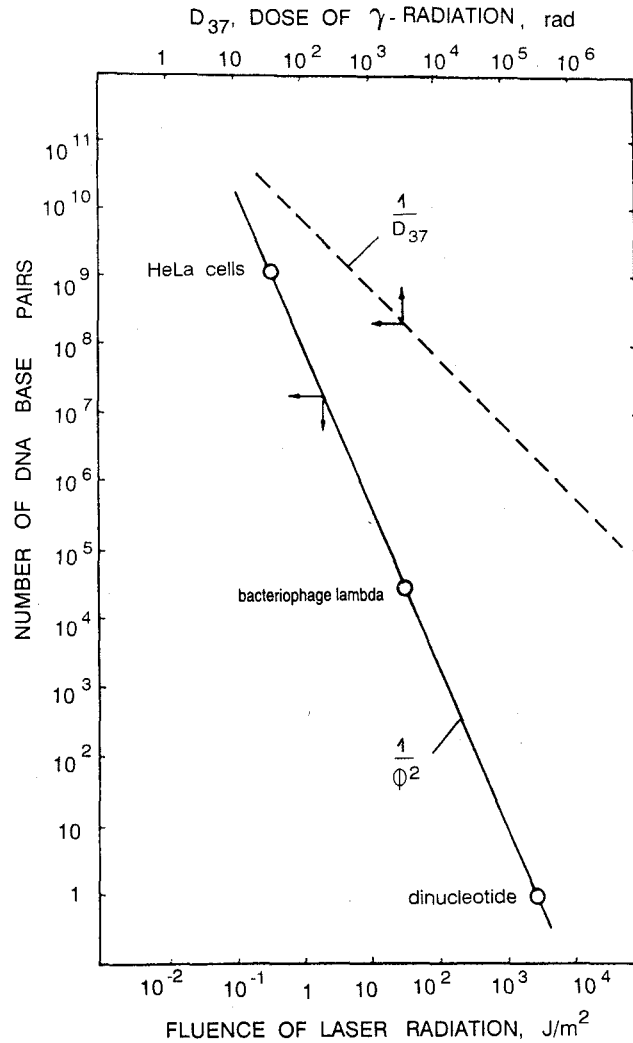


Figure 13. DNA-damaging fluence (ϕ) of picosecond UV pulses ($\lambda = 266$ nm) (solid line) as a function of the number of DNA base pairs in dinucleotides, viruses, and mammalian cells. The dashed line denotes the same type of dependence for γ -irradiation.

observed for γ -radiation (Kaplan and Moses, 1964). If the ordinate represents the quantity of DNA (number of base pairs, K_p) and the abscissa, the dose of radiation, D_{37} (a dose at which 63% of DNA molecules are damaged), one observes an inverse dependence, $K_p \sim 1/D_{37}$, between damage and dose (Fig. 13).

Let us estimate the minimal damaging fluence for HeLa cells irradiated with UV PPP at 266 nm. Figure 14a presents the changes in DNA synthesis rate after exposing exponentially growing HeLa cells to PPP at $\lambda = 266$ nm. $[^3\text{H}]$ thymidine incorporation was found to increase with fluences in the range 0.1-10 J/m^2 , with a maximum at 1 J/m^2 . Irradiation with PPP at 1,064 nm

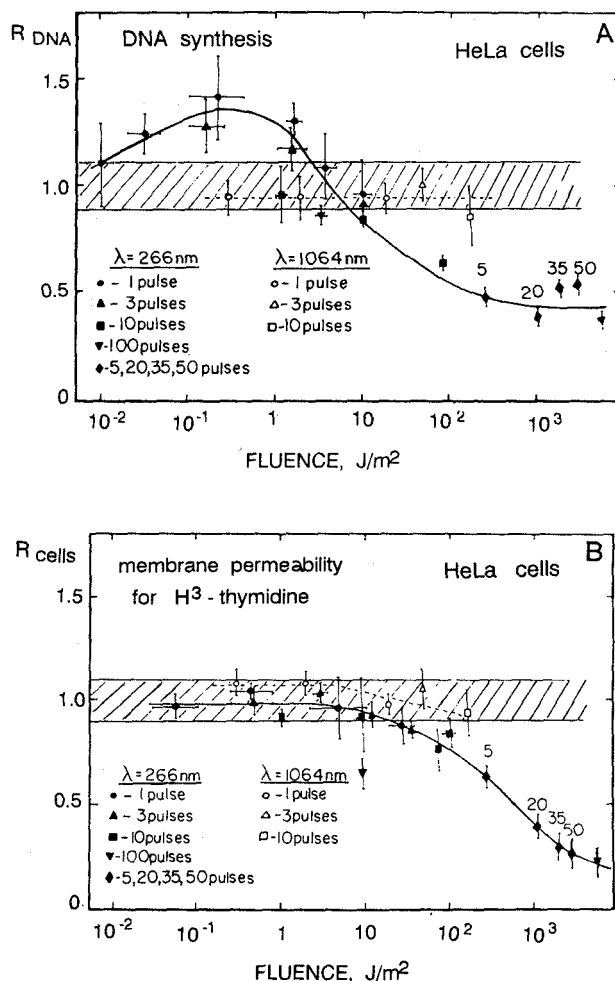


Figure 14. Radioactivity of (A) DNA and (B) whole cells measured by 20-min pulse labelings 2.5 h (starting point of the pulse label) after the exposure of exponentially growing HeLa cells to powerful picosecond UV ($\lambda = 266$ nm) and IR (infrared) ($\lambda = 1,064$ nm, dashed line) pulses. R is defined as the ratio of radioactivity values in irradiated and control samples.

produced no change in [³H]thymidine incorporation (Fig. 14a). Because this wavelength ($\lambda = 1064$ nm) is not absorbed by DNA, one can suggest that the effects at $\lambda = 266$ nm are connected with the absorption by DNA. Membrane permeability for [³H]thymidine did not differ from the control level with fluences varying from 0.1 to 10 J/m² (Fig. 14b). The trypan blue exclusive test showed that the cells were viable after irradiation.

There is every reason to believe that the stimulation of [³H]thymidine incorporation in the fluence range between 0.1 and 1 J/m² (Figure 14a) is a response to DNA damage. HeLa cells have 2.9×10^9 base pairs. The bacteriophage lambda contains 4.65×10^4 base pairs, and its damaging fluence (on the basis of survival curves) lies between 10 and 10² J/m² (Nikogosyan and Letokhov, 1983). Experiments with PPP on DNA fragments have shown that the lethal fluence for nucleotides is 30 J/m² (Nikogosyan and Letokhov, 1983). Since the damage yield at this fluence is about 1% (Letokhov, 1983), the fluence needed for guaranteed damage of a dinucleotide lies in the range 10³-10⁴ J/m².

Figure 13 shows the dependence of the damaging fluence of PPP on the number of base pairs. This dependence is quadratic, $K_p \sim 1/\phi_2$ and thus suggests a two-quantum damage mechanism in dinucleotides, bacteriophage lambda, and HeLa cells irradiated with PPP at 266 nm.

3.2. Irradiation with Two Ultrashort Pulses Increases the Permeability of Membranes of Plateau-Phase Cells

Nucleosides (precursors of DNA and RNA synthesis) in concentrations less than 2 μ M are taken up into mammalian cells by means of a passively mediated transport mechanism, also known as *facilitated diffusion* (Hopwood *et al.*, 1975). The nucleosides that have entered the cell are phosphorylated at once and thus lose their ability to diffuse out (Schlotissek, 1968). Since [³H]thymidine incorporation into the DNA of cells irradiated with PPP does not differ from the control level (Fig. 15, curve 4), this suggests that an increase in [³H]thymidine-labeled cells is due to an alteration of diffusion and/or transport properties of the cellular membrane.

When plateau-phase HeLa cells were irradiated with two UV (266 nm) PPP, no increase in [³H]thymidine uptake occurred when the interval between the two pulses was 1-2 s (Fig. 15, curves 1 and 2). Uptake increased as the interval between the two pulses increased, and a maximal increase occurred at a pulse interval of 4 to 6 s. Further increase in the pulse interval from 8 to 100 s caused the effect to be reduced to the control level. [³H]thymidine uptake did not depend on wavelength; the same effect occurred at 266, 532 (Fig. 15, curves 1 and 3) and 1,064 nm (Karu, 1986). Increase in the intensity of the pulses, however, decreased the effect (curves 1 and 2 in Fig. 15).

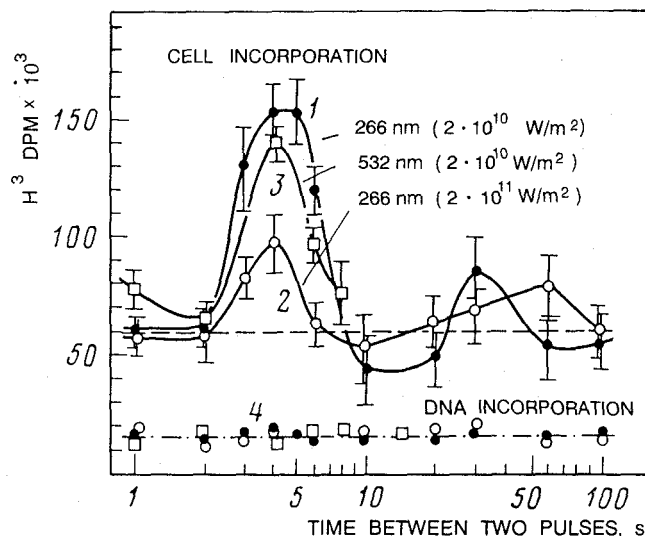


Figure 15. Changes in the permeability of cellular membranes to $[^3\text{H}]$ thymidine measured 1.5 h after exposure of plateau-phase HeLa monolayers to two PPP at 266 or 532 nm, as a function of the time between the two pulses. DPM = decays per minute.

These effects are specific for plateau-phase HeLa cells. The membrane permeability of cells in the exponential phase of growth did not vary from the control level (Karu *et al.* 1984a).

The results obtained from irradiating cells with two PPP (Fig. 15) enable us to assume that the first pulse is responsible for some change in the cell transport system for thymidine which may be realized within several seconds with another pulse but which itself cannot cause a change in membrane permeability (Karu *et al.*, 1984a). If the second pulse comes at the right moment, a change in the transport system occurs which accelerates the passage of nucleosides through the membrane. If the second pulse arrives too late, when the preparatory reactions caused by the first pulse have decayed, no change in cell membrane permeability is observed.

Using a powerful, single, nanosecond pulse (third harmonic of Nd^{3+} ;YAG laser, $\lambda = 335$ nm), it is possible to perforate the cell membrane so that some allogenic material penetrates into the cell (Tsukakoshi *et al.*, 1984). The threshold energy used was about 1 mJ, and the hole in the membrane closed after a second. It should be emphasized that the fluence and pulse energies used in our experiments were essentially less than those used in the work of Tsukakoshi *et al.* (1984).

It is hardly probable that the increased permeability of the cellular membrane found in our experiments is due to purely mechanical destruction of the membrane since (1) there is a complex nonlinear dependence of the effect on the time between the two pulses (Fig. 15, curves 1-3), (2) the effect is reduced at increased light intensities (Fig. 15, curves 1 and 2), and (3) the increased permeability lasts for hours (at least 2.5 h; Karu, 1986).

One possible explanation for increased permeability is that irradiation results in some conformational changes in the structure of the thymidine transport system. Even slight changes of this kind may be important because some components of the transport systems of nucleotides are allosteric proteins (Eilam and Cabantchik, 1977; Pardee and Palmer, 1973).

What mechanisms could explain our results? A photochemical mechanism seems unlikely because there is no dependence of permeability on wavelength (Fig. 15). It is possible that the modulated transport functions are connected with local heating. The permeability of membranes of ascite tumor cells for fluoresceine and trypan blue was shown to increase with increasing temperature (Strom *et al.*, 1973). In this experiment, tumor cells were incubated at different temperatures for hours. This kind of total heating is impossible in our experiments because pulse duration is only 3×10^{-11} s. The total increase of temperature will not exceed $10^{-3}-10^{-2}$ °C (Karu, 1986).

Due to the nonhomogeneity of the cellular membrane, irradiation with PPP can cause local heating. The possible local increase of temperature caused by one PPP was estimated to be ≈ 36 °C (Karu, 1986). This short-interval increase is sufficient for causing some conformational changes in the molecules of the cellular membrane transport system.

4. Concluding Remarks

Two characteristic responses of plateau-phase HeLa cells to pulsed UVR are described: an increase in cellular membrane permeability to nucleosides after irradiation of the cells with two ultrashort UV pulses, and the induction of replicative DNA synthesis in a cellular subpopulation after irradiation with high-repetition-rate UV pulses. The first effect is actually a specific response to pulsed UVR for a very simple reason: the parameters afforded by pulsed UVR are not possible with ordinary CW UVR sources. It would be more correct to say that this effect is laser specific.

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

It is a common misconception that UVR is photochemically much more active than visible light (Smith, 1980). It is also believed that UVR has mainly negative effects (inactivation of cellular reactions) and that laser radiation has special properties.

The experiments discussed in this chapter suggest that UVR has not only negative but positive effects: stimulation of colony-forming ability (Fig. 4a), enhancement of proliferation (Figs. 4b, 5), and modulation of transport processes in cellular membranes (Fig. 15). As to the photochemical activity of visible light, it is possible to stimulate DNA synthesis with this kind of radiation as well (Fig. 16b). 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 (Karu 1987, 1988, 1989). This scheme forms the basis for low-power laser therapy ("laser biostimulation"). Effecting DNA synthesis in this way is completely different from the mechanism discussed in this chapter, as one can see from a comparison of Figs. 16a and b.

Many people believe that properties of laser radiation such as coherence, high intensity, and monochromaticity make laser radiation unusual. An example is the so-called laser biostimulation, a purely photobiological phenomenon, the effects of which depend on the wavelength and fluence of the light used for irradiation (Fig. 16b), but not on its coherence (Karu, 1987). In this case, lasers just have the benefit of being a convenient, monochromatic light source.

Lasers can provide light with parameters that vary greatly from those of light from ordinary sources, e.g., the intense, ultrashort pulses used in the experiments discussed in this chapter (Section 3). But even here, where the primary excitation mechanism (two-quantum excitation, Fig. 1c,d) is entirely different from the classical one-quantum excitation (Fig. 1a), the laws of classical photobiology are valid. As an example, Fig. 17 illustrates the lethal action of PPP at 266 and 532 nm on plateau-phase and log-phase HeLa cells. It is known that visible light is ineffective in causing lethal and mutagenic effects. PPP at 532 nm kills both plateau-phase and log-phase cells, but with a very low efficiency not differing from the lethal action of CW green light (Karu *et al.*, 1988). This finding confirms the rule known in classical photobiology:

the damage inflicted by visible radiation is not responsible for the death of cells, or in other words, even intense laser pulses cannot be effective when the wavelength is wrong. Plateau-phase cells have a higher resistance than log-phase cells to PPP at 266 nm (Fig. 17). The same is true for plateau-phase and log-phase cells exposed to CW low-intensity 254-nm UVR, as discussed in Section 2.4. These examples only illustrate some misconceptions about light. Readers may find better examples in their surroundings.

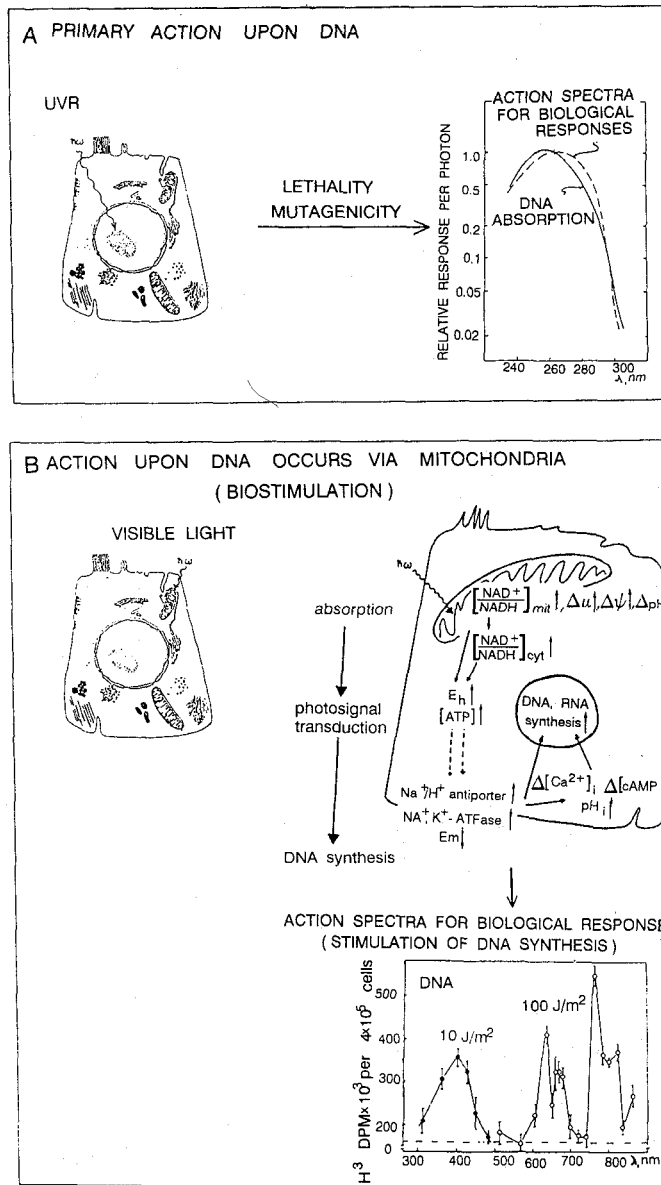


Figure 16. Two different mechanisms for exerting effects on DNA synthesis: (A) primary action after absorption of UVR by DNA forms the basis for lethal and mutagenic effects (from Coohill, 198), and (B) a mechanism whereby visible light is absorbed by mitochondrial pigments, and stimulation of DNA synthesis occurs as a final step after many dark reactions in a cell (photosignal transduction and amplification). This scheme forms a basis for low-power laser therapy (Karu, 1987, 1988,1989).

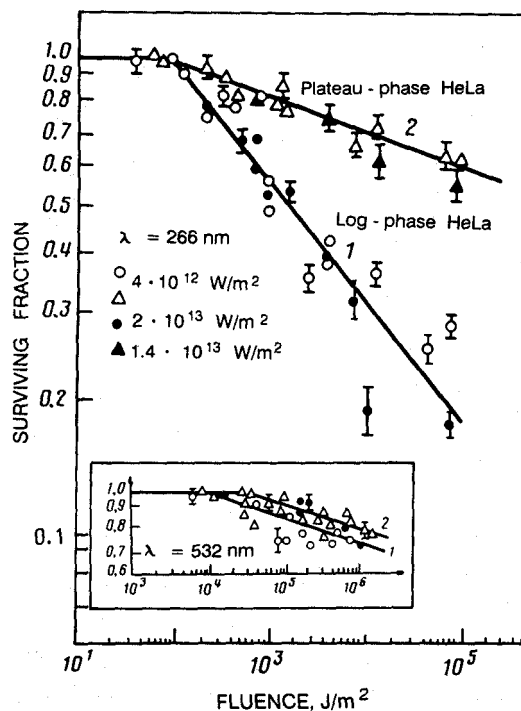


Figure 17. Lethal action of PPP at 266 nm on (1) log-phase and (2) plateau-phase HeLa cells measured by trypan blue exclusive test. The insert presents the same data for 532-nm PPP.

References

- Abbaszade, I. G., 1986, Action of low doses of UVR on *E. coli*, in: *Molecular Biophysics of Cells and Cellular Processes*, Azerbaijan State University Publ., Baku, pp. 17-23 (in Russian).
- Alpatov, W. W., and Nastjukova, O. K., 1933, The influence of different quantities of ultra-violet radiation on the division rate in *Paramecium*, *Protoplasma* 18:281-285.
- Ananthaswamy, H. N., and Pierceall, W. E., 1990, Molecular mechanisms of ultraviolet radiation carcinogenesis, *Photochem. Photobiol.* 52:1119-1136.
- Baserga, R. (ed.), 1971, *The Cell Cycle and Cancer*, Marcel Dekker, New York.
- Baserga, R., 1979, *Multiplication and Division of Mammalian Cells*, Marcel Dekker, New York and Basel.
- Belenkina, N. S., Strakhovskaya, M. G., and Fraikin, G. Ya., 1990, Activation effect of UV laser radiation on yeast growth, *Biofizika* 35:618-620.
- Branch, A. D., Benenfield, B. J., Barondy, B. M., Wells, F. V., Gerin, J. L., and Robertson, H. D., 1989, An ultraviolet-sensitive RNA structural element in a viroid-like domain of the hepatitis delta virus, *Science* 243:649-652.
- Cadet, J., and Vigny, P., 1990, The photochemistry of nucleic acids, in: *Bioorganic Photochemistry* (H. Morrison, ed.), Vol. 1, Wiley Interscience, New York, pp. 1-272.

- Carlson, J. G., Gaulden, M. E., and Jagger, J., 1961, Mitotic effects of monochromatic ultraviolet irradiation of the nucleolus, in: *Progress in Photobiology* (B. C. Christiansen and B. Buchman, eds.), Elsevier, Amsterdam, pp. 251-253.
- Chan, G. L., and Little, J. B., 1979, Responses of plateau-phase mouse embryo fibroblasts to UV light, *Int. J. Radial. Biol.* 35:101-110.
- Cohn, S. M., Krawicz, B. R., Dresler, S. L., and Lieberman, M. W., 1984, Induction of replicative DNA synthesis in quiescent human fibroblasts by DNA damaging agents, *Proc. Natl. Acad. Sci. U.S.A.* 81:4828-4832.
- Coohill, T. P., 1986, Virus-cell interaction as probes for vacuum-ultraviolet radiation damage and repair, *Photochem. Photobiol.* 44:359-363.
- Eilam, G., and Cabantchik, Z. I., 1977, Nucleoside transport in mammalian cell membranes: A specific inhibitory mechanism of high affinity probes, *J. Cell Physiol.* 92:185-202.
- Epifanova, O. N., 1977, Metabolism of proliferating and resting cells, *Cytologia* 21:1379-1396 (in Russian). Epifanova, O. I., Terskikh, V. V., and Polunovskii, V. A., 1983, *Resting Cells*. Nauka, Moscow (in Russian).
- Giese, A. C., 1964, Studies on ultraviolet radiation action upon animal cells, in: *Photophysiology* (A. C. Giese, ed.), Vol. 2, Academic Press, New York, pp. 203-245.
- Gill, R. F., and Coohill, T. P., 1987, A comparison of mammalian cell sensitivity to either 254 nm or artificial "solar" stimulated radiation, *Photochem. Photobiol.* 45:264-271.
- Grdina, D. W., Hittelman, R. A., White, R. N., and Meistrich, M. L., 1977, Relevance of density, size and DNA content of tumour cells to the lung colony assay, *Br. J. Cancer* 36:659-669.
- Hahn, G. M., 1975, Radiation and chemically induced potentially lethal lesions in non-cycling mammalian cells: Recovery analysis in terms of x-ray and UV-like synthesis. *Radial. Res.* 64:533-545. Hahn, G. M., and Little, J. B., 1972, Plateau-phase cultures of mammalian cells, *Current Topics in Radial. Res.* 8:39-83.
- Haushka, P. V., 1973, Analysis of nucleotide pools in animal cells, in: *Methods in Cell Biology* (D. M. Prescott, ed.). Academic Press, New York, pp. 361-462.
- Hittelman, W. N., and Potu, N. R., 1978, Mapping Gi phase by the structural morphology of the prematurely condensed chromosomes, *J. Cell. Physiol.* 95:333-342.
- Hopwood, L. E., Dewey, W. C., and Hejny, W., 1975, Transport of thymidine during cell cycle in mitotically synchronized CHO cell, *Exp. Cell Res.* 96:425-429.
- Kantor, G. J., 1986, Effects of UV, sunlight and x-ray radiation on quiescent human cells in culture, *Photochem. Photobiol.* 44:371-378.
- Kantor, G. J., and Ritter, C., 1983, Sunlight-induced killing of nondividing human cells in culture, *Photochem. Photobiol.* 37:533-538.
- Kantor, G. J., Sutherland, J. C., and Setlow, R. B., 1980, Action spectra for killing nondividing normal human and xeroderma pigmentosum cells. *Photochem. Photobiol.* 31:459-464.
- Kaplan, H. S., and Moses, L. E., 1964, Biological complexity and radiosensitivity, *Science* 145:21-25.
- Karu, T. I., 1986, Action of pulsed UV radiation on membrane permeability of nonproliferating tumor cells HeLa, *Radiobiologiya* 26:793-797 (in Russian).
- Karu, T. I., 1987, Photobiological fundamentals of low-power laser therapy, *IEEE J. Quantum Electron.* 23:1707-1717.
- Karu, T. I., 1988, Molecular mechanisms of the therapeutic effect of low-intensity laser radiation, *Lasers in the Life Sciences* 2:53-74. Karu, T. I., 1989, *Photobiology of Low-Power Laser Therapy*, Harwood Academic, London.

- Karu, T. I., and Kalendo, G. S., 1987, Response of plateau-phase HeLa cells to low-intensity pulsed and continuous-wave UV light near 270 nm, Abstr. of 2nd Congress of ESP, Padova, 6-10.09.1987, Abstr. No C-98, p. 111.
- Karu, T. I., Kalendo, G. S., Letokhov, V. S., Matveets, Yu. A., Semchishen, V. A., 1981, Action of ultrashort UV laser pulses upon tumor cells HeLa, *Kvantovaya Electroni.* 8:2540-2545 (Engl. translation: *Sov. J. Quantum Electron.* 11:1550-1553, 1981).
- Karu, T. I., Kalendo, G. S., Letokhov, V. S., and Lobko, V. V., 1982, Response of proliferating and resting tumor HeLa cells to high repetition rate laser UV pulses, *Dokl. Akad. Nauk SSSR* 262:1498-1501 (Engl. translation: *Dokl. Biophys.* Febr. 1982, pp. 30-33).
- Karu, T. I., Kalendo, G. S., Letokhov, V. S., and Lobko, V. V., 1983a, Different responses of proliferating and resting tumour HeLa cells to pulsed high repetition rate low-intensity laser light at 271 nm, *Laser Chemistry* 1(3):153-161.
- Karu, T. I., Fedoseeva, G. E., Yudakhina, Ye. V., Kalendo, G. S., and Lobko, V. V., 1983b, Action of low-intensity high repetition rate UV laser pulses on nucleic acids synthesis rate in proliferating and resting cells, *Tsitologiya*, 25:1207-1212 (in Russian).
- Karu, T. I., Kalendo, G. S., and Letokhov, V. S., 1984a, Comparison of action of ultrashort powerful UV pulses on replicative and transcriptive function of DNA in proliferating and nonproliferating HeLa cells, *Radiobiologiya*, 24:17-20 (in Russian).
- Karu, T. I., Kalendo, G. S., Letokhov, V. S., and Lobko, V. V., 1984b, Action of pulsed UVR on proliferating and resting tumour cells HeLa, *Radiobiologiya* 24:273-276 (in Russian).
- Karu, T. I., Kalendo, G. S., Letokhov, V. S., and Lobko, V. V., 1984c, Biostimulation of HeLa cells by low intensity visible light. III. Stimulation of nucleic acid synthesis in plateau phase cells, *IlNuovo Cimento D* 3:319-325.
- Karu, T., Pyatibrat, L., and Kalendo, G., 1987, Biostimulation of HeLa cells by low-intensity visible light. V. Stimulation of cell proliferation *in vivo* by He-Ne laser radiation. // *Nuovo Cimento D* 9:1485-1494.
- Karu, T. I., Pyatibrat, L. V., Tiphlova, O. A., and Nikogosyan, D. N., 1988, Investigation into specificity of lethal and mutagenic action of picosecond laser pulses at 532 nm, *Radiobiologiya* 28:499-502 (in Russian).
- Karu, T., Kalendo, G., and Pyatibrat, L., 1990, On the role of cells attachment and spreading in biostimulation effects. *Lasers in the Life Sci.* 3:229-232.
- Letokhov, V. S., 1983, *Nonlinear Laser Chemistry*, Springer-Verlag, Berlin, Chap. 8. Little, J. B., 1969, Repair of sub-lethal and potentially lethal radiation damages in plateau phase cultures of human cells. *Nature* 224:804-806.
- Mironescu, G. D., Epstein, S. M., and DiPaolo, J. A., 1980, Relationship between morphological transformation and H³-thymidine incorporation stimulated by a chemical carcinogen in postconfluent cultures of hamster embryo cell. *Cancer Res.* 40:2411-2416.
- Moan, J., and Peak, M., 1989, Effect of UV radiation on cells, *Photochem. Photobiol.* 4:21-34.
- Nikogosyan, D. N., 1990, Two-quantum UV photochemistry of nucleic acids: Comparison with conventional low-intensity UV photochemistry and radiation chemistry. *Int. J. Radial. Biol.* 57:233-299.
- Nikogosyan, D. N., and Letokhov, V. S., 1983, Nonlinear laser photophysics, photochemistry and photobiology of nucleic acids, *Riv. Nuovo Cimento* 6:1-74.
- Painter, R. B., 1970, The action of ultraviolet light on mammalian cells, in: *Photophysiology* (A. C. Giese, ed.). Vol. 5, Academic Press, New York, pp. 169-189.
- Pardee, A. B., and Palmer, L. M., 1973, Regulation of transport systems by means of controlling metabolic rates. *Proc. Soc. Exp. Biol.* 27:133-144.
- Paribok, V. P., and Semyonova, F. G., 1970, Unscheduled synthesis of DNA and reparation of sublethal damages in HeLa J-63 cells, *Tsitologiya* 12:1423-1431 (in Russian).

- Peak, M. J., and Peak, J. G., 1989, Solar-ultraviolet-induced damage to DNA, *Photodermatology* 6:1-15.
- Peak, J. G., Woloschak, G. E., and Peak, M. J., 1991, Enhanced expression of protein kinase C gene caused by solar radiation, *Photochem. Photobiol.* 53:395-397.
- Rao, P. N., and Smith, M. L., 1981, Differential response of cycling and noncycling cells to inducers of DNA synthesis and mitosis, *J. Cell Biol.* 88:649-653.
- Rauth, A. M., 1970, Effects of ultraviolet light on mammalian cells in culture, in: *Current Topics in Radiation Research* (M. Ebert and A. Howard, eds.), Vol. 6, North Holland, Amsterdam and London, pp.197-247.
- Ronai, Z. A., Lambert, M. E., and Weinstein, I. B., 1990, Inducible cellular responses to ultraviolet light irradiation and other mediators of DNA damage in mammalian cells. *Cell Biol. Toxicol.*6:105-126.
- Rothman, R. H., and Setlow, R. B., 1979, An action spectrum for cell killing and pyrimidine dimer formation in Chinese hamster V-79 cells, *Photochem. Photobiol.* 29:57-61.
- Samoilova, K. A., 1979, Light action on cells: Morphological, cytogenetic, physiological and biochemical aspects, in: *Photobiology of Living Cells*, Nauka, Leningrad, pp. 167-185 (in Russian).
- Schlotissek, Ch., 1968, Studies on the uptake of nucleic acid precursors into cells in tissue culture, *Biochim. Biophys. Acta* 158:435-447.
- Schor, S. L., Johnson, R. T., and Waldren, C. A., 1975, Changes in the organization of chromosomes during the cell cycle: Response to ultraviolet light, *J. Cell Sci.* 17:539-565.
- Schulte-Frohlinde, D., Simic, M. G., and Gomer, H., 1990, Laser-induced strand break formation in DNA and polynucleotids, *Photochem. Photobiol.* 52:1137-1151.
- Smith, K. C., 1980, Common misconceptions about light, in: *Lasers in Photomedicine and Photobiology* (R. Pratesi and C. A. Sacchi, eds.). Springer-Verlag, Berlin, pp. 23-25.
- Strom, R., Santoro, A. S., Criefo, C., Bozzi, A., Mondovi, B., and Fanelli, A. R., 1973, The biochemical mechanism of selective heat sensitivity of cancer cells. IV. Inhibition of RNA synthesis, *Eur. J. Cancer* 9:103-112.
- Synsins, B. I., and Saenko, A. S., 1986, DNA synthesis in stationary-growing HeLa culture after γ -irradiation, *Radiobiologiya* 26:800-802.
- Tsukakoshi, M., Kurata, S., Nomiya, G., Ikawa, G., and Kasuya, T., 1984, A novel method of DNA transfection by laser microbeam cell surgery, *Appl. Phys. B* 35:135-140.
- Valerie, K., Delers, A., Bmck, C., Thiriart, C., Rosenberg, H., Debouck, C., and Rosenberg, M., 1988, Activation of human immunodeficiency virus type 1 by DNA damage in human cells. *Nature* 333:78-81.
- Valeriotte, F., and van Putten, L., 1975, Proliferation dependent cytotoxicity of anticancer agents: A review. *Cancer Res.* 35:2619-2630.
- Walicka, M., and Beer, J. Z., 1973, UV-light as a stimulating factor for growth of murine lymphoma L 5178Y cells *in vivo*. *Stud. Biophys.* 36/37:165-173.
- Wechelbaum, R. R., Nove, J., and Little, J. B., 1980, Radiation response of human tumor cells *in vitro*, in: *Radiation Biology in Cancer Research* (E. Meyn and H. R. Witherom, eds.), Raven Press, New York, pp. 345-351.
- Zmudska, B., and Beer, J. Z., 1990, Activation of human immunodeficiency virus by ultraviolet radiation, *Photochem. Photobiol.* 52:1153-1162.
- Zubrod, G. G., 1978, Selective toxicity of anticancer drugs: Presidential address. *Cancer Res.* 38: 4374-4377.