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Blue Light Inhibits Mitosis in Tissue Culture Cells

L. A. Gorgidze,[1] S. A. Oshemkova,[1] and I. A. Vorobjev[1,2]

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Irradiation of the mitotic (prophase and prometaphase) tissue culture PK (pig kidney embryo) cells using mercury arc lamp and band-pass filters postponed or inhibited anaphase onset. The biological responses observed after irradiation were: (i) normal cell division, (ii) delay in metaphase and then normal anaphase and incomplete cytokinesis, (iii) exit into interphase without separation of chromosomes, (iv) complete mitotic blockage. Cell sensitivity to the light at wavelengths from 423 and 488 nm was nearly the same; to the near UV light (wavelength 360 nm) it was 5-10 times more; to the green light (wavelength > 500 nm) it was at least 10 times less. To elucidate the possible mechanism of the action of blue light we measured cell adsorption and examined cell autofluorescence. Autofluorescence of cytoplasmic granules was exited at wavelengths of 450-490 nm, but not at > 500 nm. In mitotic cells fluorescent granules accumulated around the spindle. We suppose blue light irradiation induces formation of the free radicals and/or peroxide, and thus perturb the checkpoint system responsible for anaphase onset.

KEY WORDS: Blue light; mitosis; tissue culture cells; cell autofluoroescence.

INTRODUCTION

It is well known that animal cells are sensitive to the visible light. To minimize the influence of light irradiation all microscopic systems where living cells are observed and examined are supplied with shutters and filters (usually green band-pass filter is used). The action of visible light seems to be wavelength dependent. Strong irradiation with blue light damage cultured cells [1], and even kill them [2]. The lethal dose depends on the wavelength used and rapidly increases for longer wavelengths [2]. The more specific effects of blue light on living cells might be expected, yet they have not been described. Addressing this question we examined behavior of mitotic cells after irradiation with light of different wavelengths from the near UV (350 nm) to the green part of spectrum (515nm). Mitotic cells were chosen because mitosis is the period of the cell cycle when a cell is sensitive to the different external triggers.

'Laboratory of Cell Motility, A. N. Belozersky Institute of Physico-Chemical Biology, Moscow State University, Vorobievy Gory, 119899 Moscow, Russia. [2]To whom correspondence should be addressed.

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MATERIALS AND METHODS

Cell Culture

PK (pig kidney embryo) cells were grown in 199 tissue culture medium supplemented with 10% bovine serum and antibiotics (penicillin + streptomycin). For irradiation and observation cells were subcultured into the Rose chambers.

Microscopy and Irradiation Instruments

Cells were irradiated on the photomicroscope Opton-3 (Zeiss) equipped with epifluorescent condenser III-RS and high pressure mercury lamp HBO-50 as a light source. For irradiation we used band-pass interference filters (Carl Zeiss, German Democratic Republic). The transmission of the filters used was measured on spectrophotometer Cary-219 and is presented in the Table 1.

The irradiation was performed using oil immersion objective lens Neofluar 63/1.25. Power of the incident light was attenuated to the level of 2.5 mW with neutral density filters. The irradiated area was 100 μm in diameter. The dose of incident light in 1 s was ≈ 30 J/cm^2. The irradiation time varied from 2 s to 5 min. Constant temperature (37°C) on the microscope stage was sustained with air curtain incubator.

Autofluorescence of living cells was determined using cooled CCD camera with 16 bit controller (Princeton Instruments) on Zeiss ICM-405 inverted microscope using wide band pass excitation filters with transmission at 450-490 nm and 500-550 nm (Omega). For emission long pass filters 520 nm and 560 nm were used. Exposure time was 5-10 s, the final image was obtained using IPlab software and photographed on slidemaker.

Absorption of PK cells was determined using spectrophotometer SF-18 (USSR) in 10 mm cuvette supplemented with integrating spherical mirror. For measurements cells grown to the monolayer were detached with EDTA solution and resuspended in PBS to a final concentration of 4 x 10^5 cells/ml.

RESULTS Morphological

Observations

All the cells reported in this study were exposed to the whole-cell irradiation. For morphological observations cells were selected at late prophase or at prometaphase. The lamp was turned on for a given time period, and then turned off in order

Table 1. Transmission of Excitation Filters[a]

Filter #	1	2	3	4	5	6	7	8
λ_{max} nm	360	401	423	436	458	467	488	507
$\Delta\lambda_{1/2}$ nm	14	9	11.5	20	8	8.5	7	9.5

[a]Filter transmission was 30-50% in the peak and decreased exponentially for shorter and longer wavelengths. Transmission for wavelengths $\lambda_{max} \pm 3\Delta\lambda_{1/2}$ was less than 0.1% of the maximal one. No other transmission was detected for wavelengths in the range of 200-800 nm.

to observe subsequent effects. All the cells were then monitored at a minimal light level. The morphological and behavioral results of cells following irradiation fell into four categories: (i) normal completion of division, (ii) delay in metaphase and then normal anaphase and incomplete cytokinesis, (iii) exit into interphase without separation of chromosomes, (iv) irreversible mitotic blockage.

Often after irradiation mitotic cells contracted and rounded up. Rounded cells either divided into two poorly flattened cells, or stay in mitotic stage for > 3 hr and then detached from the coverslip.

To define mitotic delay we used a time criterion. Normal mitosis in PK cells continues for l-1.5hr [3]. Irradiated cell was defined as being delayed in mitosis when it remained in mitotic stage for at least 2 hr after prometaphase onset (dissolution of the nuclear envelope).

Some of the irradiated cells escaped into the interphase without chromosome separation. In this case chromosomes decondensed and large single nucleus formed 3-4 hr after irradiation. Formation of the interphase nucleus was supplemented with flattening of these cells. Other c-mitotic cells remained rounded, they detached from the coverslip or died in 5-8 hr. Cell death was detected by the beginning of Brownian motion of cytoplasma granules and by collapse of chromosomes. Dead cells were pushed out from the monolayer and detached from the coverslip.

Chromosomes in the irradiated mitotic cells became shorter and thicker (more condensed) than normal ones, and gathered at the central part of the cell (Figs. 1A-1E). Often two aggregates of granules appeared at the cell periphery. The granules never come to the area occupied with chromosomes.

Some of the cells irradiated at early and midprophase two hours and more after irradiation reverted to the interphase without separation of chromosomes. In reverting cells chromosomes gradually decondensed and later formed one large interphase nucleus. Cells which had irradiated at prometaphase never undertook reversion *(data not shown).*

PK Cells Response to the Blue-light Irradiation

The first question to be answered was whether irradiation of a whole cell with visible light inhibits mitotic progression. It was found out that irradiation of prophase and prometaphase cells with wavelengths 488 nm and shorter for 30 s and more induced different mitotic abnormalities. Thus using the wavelengths < 500 nm it was possible to determine minimal dose sufficient for mitotic arrest (see below).

The set of experiments was performed using the wavelength 467 nm (filter #6). A total of more than 250 mitotic cells were irradiated at different doses and monitored up to 8-10 hr afterwards. Sensitivity of individual cells varied in one and the same chamber. Some cells were arrested in c-mitosis after 15s irradiation, while others to achieve mitotic arrest required 2 min irradiation (Fig. 2).

To compare cell sensitivity at different wavelengths we introduced a special parameter—mean mitostatic dose. Mean mitostatic dose was determined as a minimal dose inducing prominent mitotic delay or mitotic arrest (description see below) for > 50% of cells irradiated with. For 467 nm light mean mitostatic dose was found

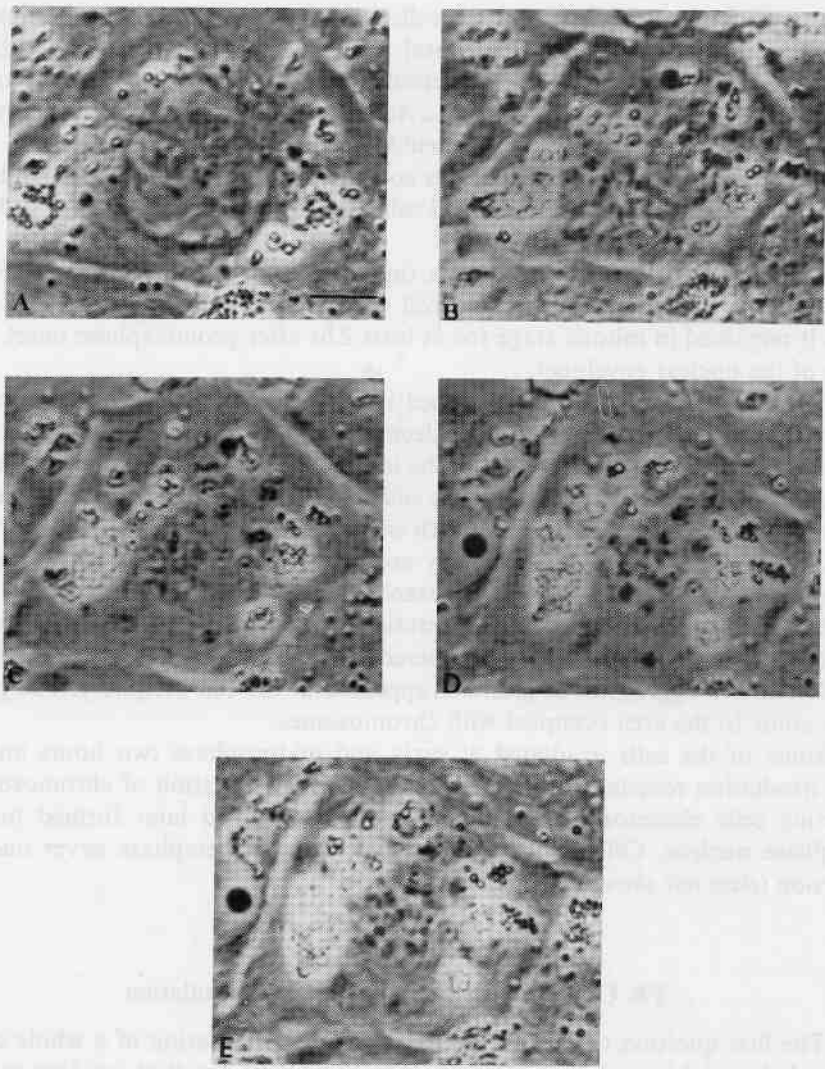

Fig. 1. Mitotic delay induced by the irradiation with blue light (λ_{max} = 467 nm, duration 2 min). (a) Immediately before irradiation; (b) 45 min after irradiation; (c) 1 hr 35 min after irradiation; (d) 2 hr 40 min after irradiation; (e) 4 hr after irradiation (bar, 20 μ*m).*

as 15s. This dose appeared to be the same in all the experiments with one and the same filter.

Action of Light of Different Wavelengths

The same approach was used for other wavelengths. The lowest mean mitostatic dose was found at 360 nm. 60 cells were irradiated at this wavelength. 2 s irradiation induced mitotic delay for two cells out of 10. Mean mitostatic dose (90 J/cm^2) was

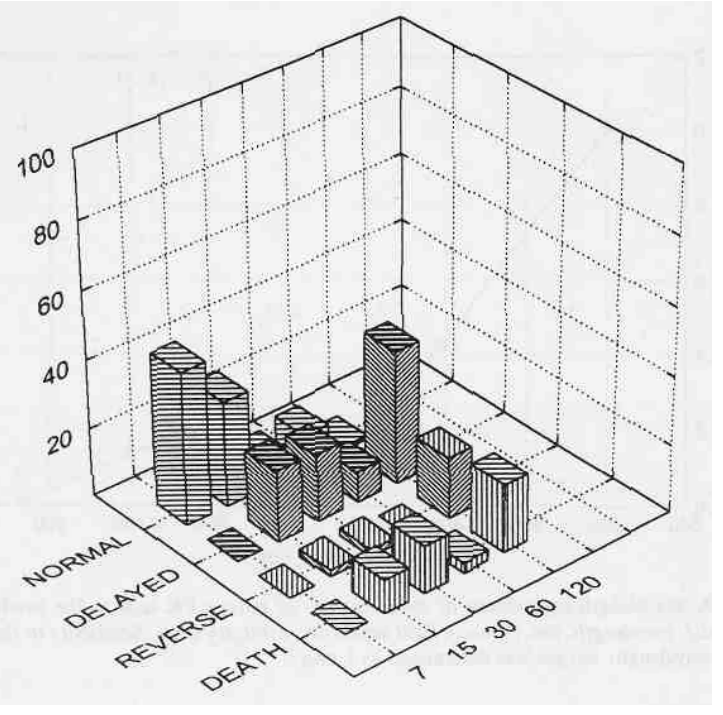

Fig. 2. Different responses of the mitotic cell on the irradiation with 467 nm light. (*X*-axis), time of irradiation; (*Y*-axis), different responses; (*Z*-axis), percentage of cells.

achieved in 3 s. Irradiation at 401 nm was less effective—mean mitostatic dose was achieved in 7 s. The light at wavelengths 423 and 467 nm gave similar effect—mean mitostatic dose was achieved in 15 s. The mitostatic dose for 436 nm, 458 nm and 488 nm was achieved in 30 s. As mentioned above, we were not able to determine mitostatic dose for the light at wavelength 507 nm—almost all cells divided normally after 5 min irradiation. It means mitostatic dose for 507 nm exceeds 9000 J/cm^2. Mean mitostatic dose versus wavelength is plotted on Fig. 3.

To elucidate the possible mechanism of the blue light action we measured cell absorption and determined spatial distribution of cell autofluorescence.

Absorption and Autofluorescence of PK Cells

Absorption of PK cells in the visible range of spectrum was very low. It slowly decayed with an increase of the wavelength from 400 nm to 510 nm (Fig. 4). At 490 nm absorption was half of that at 400 nm. From 488 nm to 507 nm absorption decreases only 12%.

The autofluorescence of PK cells was extremely low, and detectable only when using for excitation broad band-pass filters ($\Delta\lambda_{1/2} > 15$nm). The autofluorescence was detected only with filters having $\lambda_{max} < 500$ nm. Autofluorescence was coming from cytoplasmic granules poorly visible under phase contrast. The distribution of

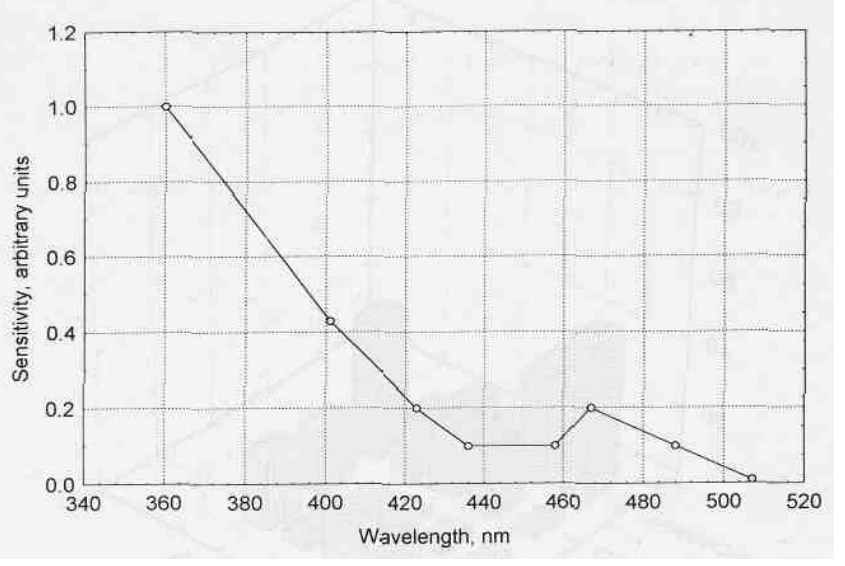

Fig. 3. Wavelength dependence of the sensitivity of mitotic PK cells to the irradiation. (X -axis), wavelength, nm; Y-axis), light sensitivity, arbitrary units. Sensitivity to the light with wavelength 360 nm was determined as 1 unit.

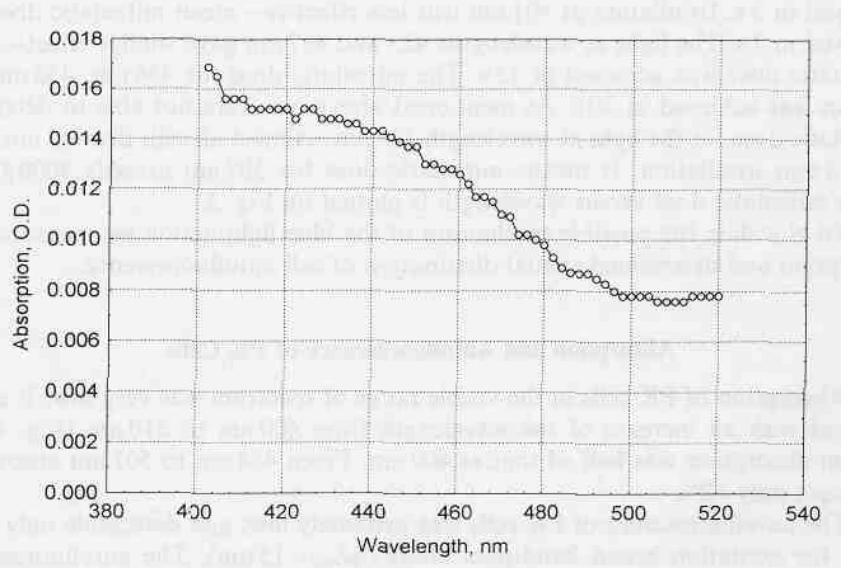

Fig. 4. Wavelength dependence of the absorption of PK cells. (X-axis), wavelength, nm; (Y-axis), absorption (O.D.).

fluorescent granules in cytoplasm was different in interphase and mitosis. In the interphase cells tiny autofluorescent granules were scattered throughout the cytoplasm or encircled cell nucleus (Fig. 5). In the mitotic cells autofluorescent granules were more prominent. They surrounded mitotic spindle and lacked in the peripheral cytoplasm. In the prometaphase cell autofluorescent granules (or small clusters of granules) encircled spindle area with chromosomes (Fig. 6, single arrow). In the metaphase cell granules were associated in two caps located close to the spindle poles (Fig. 6, arrowheads). Spindle area was free from these granules. Pattern of the autofluorescent granules in interphase and mitotic cells was dissimilar from mitochondria, as revealed by Rhodamine 123 staining *(data not shown)*.

DISCUSSION

This study examines the damage potential of the near UV and visible light. Mitotic PK cells show remarkable sensitivity to the UVC, violet and blue light, and are much more resistant to longer wavelengths. The results obtained are of relevance for fluorescent studies of living cells. Even without sensitization mitotic cells have limited ability to complete normal division after irradiation from a standard mercury lamp using typical band-pass filters. The maximal irradiation time not inhibiting cell division is 15-30 s when using blue light and only 2 s for the near UV light. This gives strong limitations to such studies. Special precautions should be undertaken in approaches using short wavelengths irradiation of living cells. Otherwise nonspecific effects are likely to be observed.

Previously similar limitations were reported for the optical trapping microbeam from titanium-sapphire laser applied onto mitotic chromosomes. Long enough irradiation inhibited chromosome separation and induced mitotic arrest [4]. Nonspecific effect of microirradiation on mitotic cells was found in some other cases [3, 5]. In our experiments whole cell irradiation have much stronger effect when compared to the microirradiation with visible or near infrared light. The applied dosage sufficient for mitotic arrest was in our experiments approximately 30 mJ per cell (for the blue light wavelengths), while in the centrosome irradiation and optical trapping experiments it exceeded 1 J [4, 5]. In all irradiation experiments mentioned above mitotic arrest was observed after the beam was turn off. This is different from the action of mitotic drugs like nocodazole and taxol, whose effect is reversible. Cells arrested by application of nocodazole, taxol and other anti-tubulins undergo normal mitosis after drugs are washed out [6, 7]. Taken together these observations give strong evidence for the existence of a light sensitive metaphase checkpoint.

The results obtained make it possible to estimate action spectrum of the UV/blue light on mitotic cells (Fig. 3). This spectrum is different from the absorption spectra of the PK cells. Cell absorption decreases between 488 nm and 507 nm only 12%, while mean mitostatic dose increases at these wavelengths interval more than tenfold. Thus absorption mechanism of cell damage by irradiation is unlikely. Better is correlation between the action spectrum and the cell autofluorescence. Action spectrum on the mitotic cells observed in this study is almost identical with the excitation spectra of flavins and flavoproteins described elsewhere [8,9] the range of wavelengths between 400 nm and 500 nm. Thus photochemical mechanism of cell

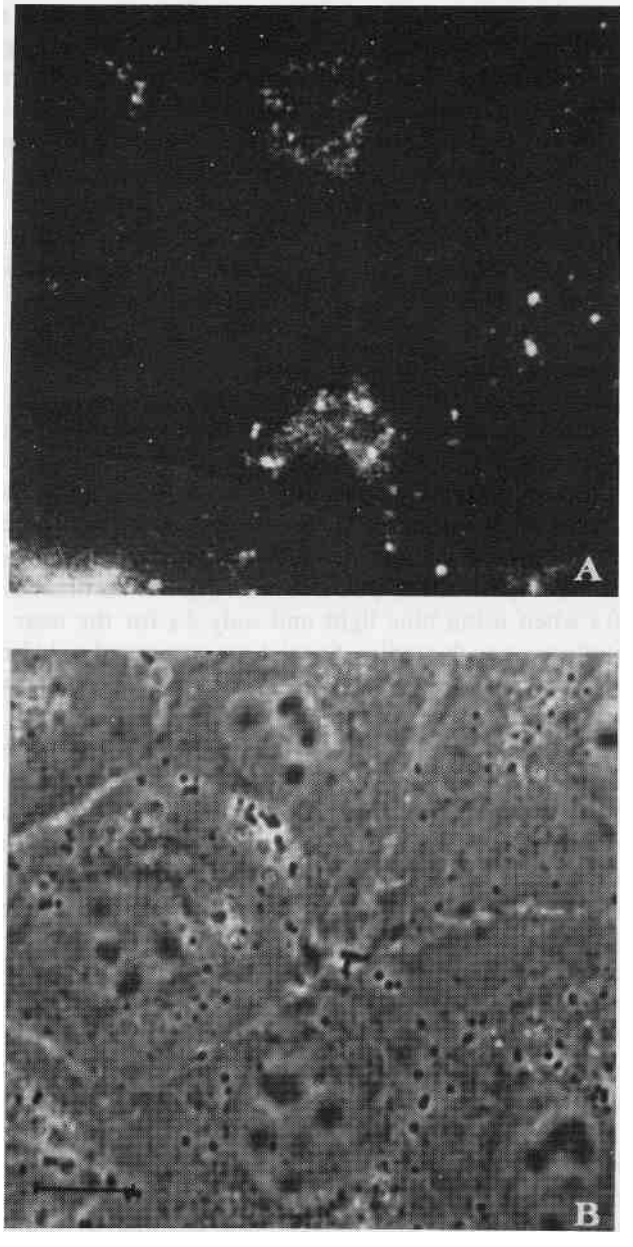

Fig. 5. Autofluorescence of the interphase PK cell (excitation wavelength 480 nm). (a) Fluorescence, (b) Phase contrast (bar, 10 μm).

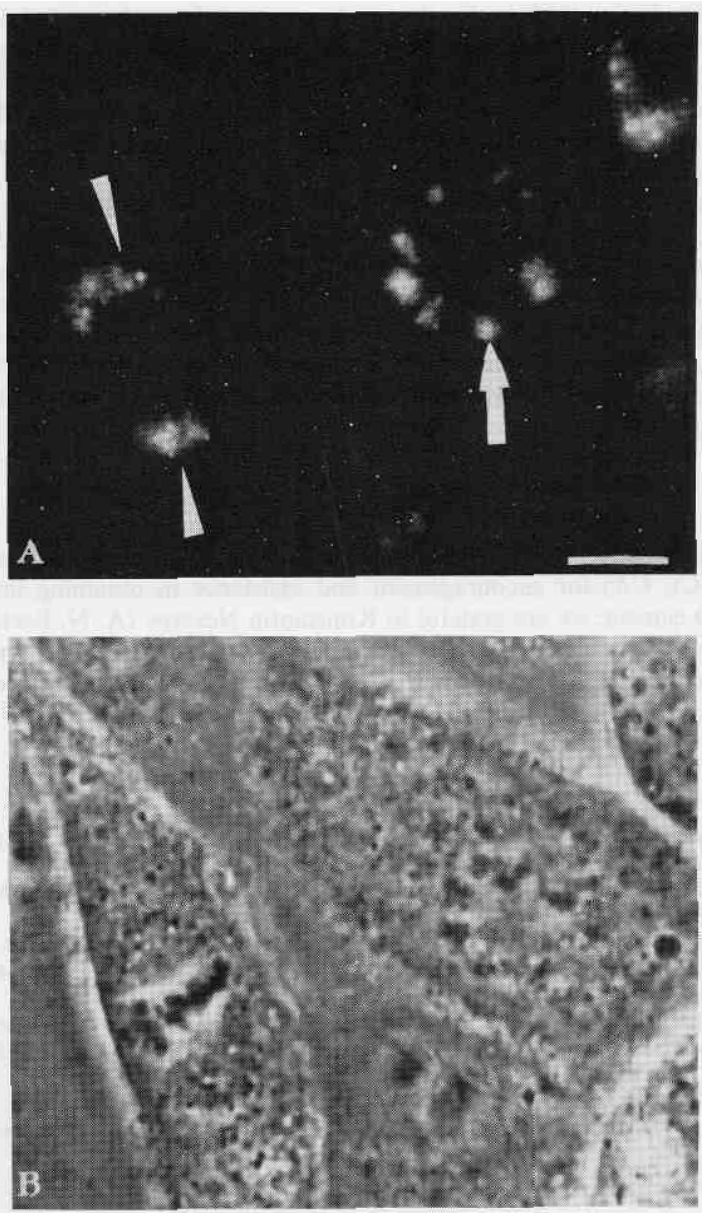

Fig. 6. Autofluorescence of the mitotic PK cells (excitation wavelength 480 nm). (a) Single arrow indicates autofluorescent granules in the pro-methaphase cell. Arrowheads indicate two caps of granules in the metaphase cell, (b) Phase contrast (bar, 10 μm).

damage by irradiation is likely. Further evidence comes from the spatial distribution of autofluorescence. Whether autofluorescent granules are the main intermediates in the blue light action, one would expect them to be close to the mitotic spindle. This is what we observed in the mitotic PK cells. During the interphase autofluorescent granules in PK cells were scattered throughout the cytoplasm, while in mitosis they were always located close to the mitotic spindle.

Summarizing the data discussed above as mechanism of the blue light action on mitotic cells seems to be as follows. Excitation of flavins generates free radicals, who are extremely toxic, yet short-lived [10]. Free radicals could be generated by irradiation anywhere in a cell, yet their yield is much higher where blue light absorbing flavins are concentrated. These are compartments from where autofluorescence is generated. When strong enough light is directed onto prophase/prometaphase cell it induces formation of the free radicals and/or peroxide who perturb the system responsible for the anaphase checkpoint.

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REFERENCES

1. Sharovskaya, Yu. Yu., Chailakhjan, L. M., and Margolis, L. B. (1988) *Exp. Cell Res.* 175:404-408.
2. Beck, K. and Bereiter-Hahn, J. (1984) *Eur. J. Cell Biol. Suppl.* 5:6.
3. Uzbekov, R. E., Votchal, M. S., and Vorobjev, I. A. (1995) *J. Photochem. Photobiol. (ser. B)* 29:3163-3170.
4. Vorobjev, I. A., Liang, H., Wright, W. H., and Berns, M. (1993) *Biophysical J.* 64:533-538.
5. Vorobjev, I. A., Drachev, V. A., and Chentsov, Yu. S. (1988) *Biopolymeri i Kletka* 4:313-320 *(in Russian).*
6. Alieva, I. B., Vaisberg, E. A., Nadezhdina, E. S., and Vorobjev, I. A. (1992) in: *The Centrosome* (Kalnins, V. C. ed.), Academic Press, New York, London, Sydney, Toronto, pp. 103-129.
7. Joshi, H. C. and Baas, P. W. (1993) *J. Cell Biol.* 121:1191-1196.
8. Aubin, J. E. (1978) *J. Histochem. Cytochem.* 27:36-43.
9. Benson, R. C., Meyer, R. A., Zaruba, M. E., and McKhann, G. M. (1979) *J. Histochem. Cytochem.* 27:44-48.
10. Cheeseman, K. H. and Slater, T. F. (1993) *British Medical Bulletin* 49:481-493.