Advanced Initiation of Synchronous Mitoses in *Physarum polycephalum* Following UV-irradiation

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Irradiation of multinuclear synchronous plasmodia of *Physarum polycephalum* with UV-light (2000 to 7000 ergs/mm², λ max 2537 Å) at any time during the first two thirds of the mitotic cycle (S-period and early G2-period) delays the onset of the next nuclear mitosis by 1 to 3 hours (10 to 30% of the normal cycle). The initial delay of the mitotic schedule is overcompensated by the advanced initiation of subsequent mitoses. This remarkable shortening of the nuclear division cycle very likely is related to the selective destruction of some irradiated nuclei during the first postirradiation S-period. Since the increase of total protein is little affected, the ratio of protein/DNA (cytoplasm/nuclei) is increased after the UV treatment. A model is discussed suggesting that the onset of nuclear mitosis is controlled by a cytoplasmic factor which accumulates during interphase at a rate proportional to the increase of the total plasmodial mass.

Multinuclear plasmodia of the myxomycete *Physarum polycephalum* exhibit synchronous nuclear mitoses¹ every 8 to 10 hours when cultivated axenically at 26 °C.² Previous experiments have shown that irradiation with UV light (2000 to 5000 ergs/mm², λ max 253.7 nm) during the first 2/3 of the mitotic cycle delays the onset of the next nuclear division by 2 to 4 hours; the second mitosis, however, appears almost at the normal time, thus after a period considerably shorter than the length of a normal interphase.³ ⁴ Similar observations were reported recently by Devi et al.⁵ The present report is concerned with the possible causes of this remarkable shortening of postirradiation division cycles. Our results indicate that during the shortened interphase after the first postirradiation mitosis overall DNA synthesis is reduced considerably because of the degradation of some irradiated nuclei; the surviving nuclei completely double their DNA content during the S-period. The selective reduction of the number of viable nuclei causes a decrease of the nucleo-cytoplasmic ratio which is assumed to be responsible for the advanced initiation of subsequent mitoses in irradiated plasmodia.

**Methods**

Disc shaped macroplasmodia of *Physarum polycephalum* with a diameter of 3 to 6 cm were cultivated in Petri dishes on a sterile liquid medium at 26 °C.² Mitotic stages of the nuclei were observed in ethanol fixed smear preparations with a phase contrast microscope. During mitosis more than 95% of the nuclei in a single plasmodium divide within 15 minutes. At various intervals between the second and the third synchronous mitosis whole plasmodia or dissected pieces (~ 2 cm²) were irradiated with UV light (Mineralight UVS-11, Ultraviolet Products Inc., San Gabriel, Cal.; λ max 253.7 nm 120 ergs/mm²/sec at a distance of 10 cm). The rate of DNA synthesis was measured by pulse labeling of excised plasmodial segments (~ 2 cm²) on medium containing 5 μCi/ml ³H-thymidine (specific activity 3 Ci/mmole, Schwarz Bioresearch Inc., Mount Vernon, N. Y.). Total DNA and protein were measured in the acid insoluble fraction (0.25 N

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trichloroacetic acid) by colorimetric techniques as described earlier. The relative DNA content of individual nuclei was determined in Feulgen stained smear preparations of small plasmodial pieces (≈ 1 mm²) with a Zeiss-UMSP-cytophotometer. For this purpose the smear preparations were fixed in 96% ethanol, treated with 5 N HCl at room temperature for 20 min and subjected to the Feulgen reaction. The smears were dehydrated with ascending concentrations of ethanol, xylene, and mounted with Canada balsam.

Results

Fig. 1 shows the cycle-dependent variation of the effects on the first and second synchronous mitosis following irradiation with a single dose of UV light (2600 ergs/mm²). The first mitosis (M) is delayed by about 20% (2 to 3 hours) of the normal generation time if the treatment occurs at any time during a period including the preceding mitosis (M - 1), S-phase and the first half of the G₂-period. The effect on this mitosis drops linearly during the last third of the interphase, approaching almost zero at the time of prophase (M). On the other hand the retarding effect on the second postirradiation mitosis (M + 1) sharply increases during the latter part of the interphase preceding (M). Two conclusions can be drawn from these results: a) the retarding effect of low doses of UV light on the initiation of the next mitosis is not mediated necessarily through an inhibition of nuclear DNA replication since the period of maximum UV sensitivity extends well into

Fig. 2. Timing of consecutive mitoses after UV irradiation. Two replicate macroplasmodia were cut into six equal segments each (~2 cm²). Eight pieces were irradiated with various doses of UV light in midinterphase and the appearance of up to the fourth postirradiation mitosis was observed. Fresh medium was added to all subcultures simultaneously after the irradiation treatment and every 12 hours thereafter. Four control pieces were treated identically except for the irradiation. The abscissa indicates the difference between the mitotic times of the irradiated plasmodia and their corresponding controls. 1, 2, 3, 4: number of postirradiation mitosis.

The initial delay of the mitotic schedule not only is made up but in fact is overcompensated by the advancement of subsequent mitoses. Thus the third and fourth mitosis already appear earlier than the corresponding mitoses in the untreated control plasmodia. This suggests that UV irradiation induces permanent changes which cause a negative phase shift or an overall time gain of the mitotic schedule. The permanent changes responsible for this phenomenon most likely involve the degradation of a number of nuclei after the first postirradiation
mitosis as indicated by the following experiments. DNA synthesis in normal plasmodia is initiated immediately following telophase and lasts for about 3 to 4 hours. Irradiation with UV light during the early G2-period delays the onset of the next S-period parallel to the delay of the first postirradiation mitosis (Fig. 3). The close correlation of the onset of DNA synthesis with mitosis in this organism therefore remains unaffected by the UV treatment. The rate of thymidine incorporation as well as the duration of the S-period is somewhat reduced compared to the control. Most strikingly the total increase of DNA during the S-period in the treated plasmodium is only about 40% of normal (white columns in Fig. 3). This result appears to disagree with earlier observations regarding the role of DNA synthesis on mitosis in this organism. According to these studies even a partial inhibition of DNA synthesis with the antimetabolite 5-fluoro-2'-deoxyuridine (FUDR) strongly inhibits the next mitosis suggesting that the onset of nuclear division requires the prior completion of DNA synthesis. One should expect therefore that the incomplete doubling of the DNA content after the first postirradiation mitosis (M*) would not permit the onset of the second mitosis. The experiment shows, however, that this mitosis does occur almost at the normal time. In fact, the interval between the first and the second postirradiation mitosis is even shorter than normal.

This apparent contradiction can be resolved on the basis of cytophotometric DNA determinations of individual nuclei as shown in Fig. 4. The histograms of Feulgen stained nuclei indicate that the average DNA content of individual nuclei in irradiated plasmodia is not significantly different from normal nuclei at the end of the S-period. The distribution of the nuclear DNA content in the irradiated plasmodium appears to be slightly more heterogeneous than in the control, perhaps due to a disturbance of the chromosome separation process in some of the nuclei.

The combined results from Fig. 3 and Fig. 4 indicate that most of the nuclei present at the end of the first postirradiation S-period contain a normal 2n DNA complement although the total amount of DNA in the irradiated plasmodium has increased only about 40% instead of 100% in the control. This suggests that approximately 30% of the irrada-
diated nuclei degenerate following the first post-irradiation mitosis (M*) whereas all surviving nuclei completely duplicate their DNA. Overall DNA synthesis in a multinuclear plasmodium therefore is differently affected by UV light and by the antimetabolite FUDR. The latter inhibits DNA replication uniformly in all nuclei whereas the former reduces the number of DNA synthesizing nuclei. This conclusion is further supported by the observation of many pycnotic nuclei in smears prepared shortly after the first postirradiation mitosis. By the end of the S-period these altered nuclei have disappeared almost completely. Since only a few pycnotic nuclei can be detected in irradiated plasmodia prior to the first (delayed) mitosis (M*) we assume that most of the irradiated nuclei are able to divide at least once, but a number of them de-generate shortly thereafter. Further, density labeling of newly synthesized DNA with 5-bromodeoxyuridine (BUDR) during the first S-period following irradiation and subsequent analysis of the DNA by CsCl density gradient centrifugation reveals only fully replicated DNA molecules with a buoyant density corresponding to hybrid DNA (Fig. 5). Any non-replicated (light) DNA from damaged nuclei therefore is either rapidly degraded during the S-period or is altered in a way which causes it to be lost during the isolation procedure.

![Fig. 5. CsCl density gradient analysis of BUDR labeled DNA from isolated nuclei of normal and UV irradiated plasmodia.](image)

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<th>DNA [µg]</th>
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<tr>
<td>C</td>
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<td>0 (M III)</td>
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<td>7 (G2)</td>
<td>196</td>
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Table I. Total DNA and protein content of normal and irradiated plasmodia. A series of 12 replicate macroplasmodia was prepared. 2.2 hours prior to the expected third synchronous mitosis, four cultures were irradiated with UV light (7000 ergs/mm²) which caused a 2.5 hours delay of this mitosis. Entire plasmodia were harvested at the time of the control mitosis (M III) as well as 7 hours later (G2-period) and analyzed for DNA and protein. C: Controls; UV: Irradiated plasmodia; figures in parentheses indicate % of control values.

While the increase of the total DNA and the number of nuclei are considerably reduced following UV irradiation, the synthesis of total protein is much less affected. Table I indicates that by the end of the first postradiation S-period the DNA content is reduced by 30% whereas total protein is only about 10% less than normal. The change of the protein/DNA ratio during the mitotic cycle in a normal and a UV treated plasmodium is shown in Fig. 6. The initial decrease of this ratio after telophase in both cultures reflects the rapid accumulation of DNA during the S-period. In the irradiated culture the ratio drops less and subsequently increases more than in the untreated plasmodium. By the time of the second postradiation mitosis the protein/DNA ratio considerably exceeds the control value. This results from the reduced synthesis of total DNA rather than from an increased protein production (Table I). Thus UV treatment selectively removes a certain number of nuclei without concomitantly reducing the overall increase of the cytoplasm. The increased ratio of the cytoplasm per nucleus ultimately may be responsible for the...
shortening of subsequent division cycles, as discussed below.

Discussion

The immediate effect of UV-irradiation on the nuclear division cycle in *Physarum polycephalum* is a delay of the onset of the first mitosis. The shape of the sensitivity curve suggests that this effect does not depend primarily on the inhibition of nuclear DNA synthesis, since the plateau of the curve extends well into the G\_2-period. This is true despite the fact that the incorporation of radioactive thymidine into DNA is significantly reduced if the UV-treatment occurs during the S-period\textsuperscript{12}. Nevertheless, the primary event responsible for the antimitotic effect of UV-light very likely involves an interaction with the chromosomal DNA or nucleoprotein since the effect is augmented by the prior incorporation of BUDR into the DNA during the S-period\textsuperscript{3}. A similar sensitizing role of BUDR on the UV-induced inhibition of septum formation in bacteria has been observed by Walker and Pardee\textsuperscript{15}. They conclude that the cytokinesis target must be nearly the size of the entire bacterial chromosome. Nucleic acid containing constituents as the UV-sensitive targets are also suggested for various other systems\textsuperscript{16-18}. The UV sensitive event may well be a DNA dependent process i.e. the production of a particular group of messenger RNAs required for the initiation of mitosis\textsuperscript{3} like in sea urchins\textsuperscript{19}. The declining part of the sensitivity curve during late interphase apparently reflects the degree of completion of this UV-sensitive event.

A somewhat similar pattern of the UV-sensitivity curve with a plateau and a declining linear transition part was observed in sea urchin eggs by Rustad\textsuperscript{20} and in *Tetrahymena* by Nachtwey\textsuperscript{21}. In these systems, however, the transition part was followed by a period of complete insensitivity prior to cell division which is absent in the case of *Physarum*. The constant sensitivity during the early part of the cycle (plateau) suggests that the radiation damage is stored until the UV sensitive process commences at the beginning of the transition period. In sea urchin eggs and in *Tetrahymena*, the transition period coincides with the separation and migration of the centrioles (kinetosomes) which leads the authors to speculate that an interference with the replication or separation of these organelles may be the cause of the UV induced division delay\textsuperscript{21, 22}. It is conceivable that DNA is associated with these organelles or that their function depends on DNA located elsewhere in the cell. No centrioles have yet been found in *Physarum polycephalum* which forms intranuclear mitotic spindles.

UV-irradiation during early stages of mitosis in *Physarum* did not prevent or delay further progress of this mitosis in our experiments. Devi et al.\textsuperscript{5}, however, were able to demonstrate a reversion of mitotic nuclei back to interphase upon irradiation with a much higher UV dose (14,500 ergs/mm\textsuperscript{2}) during prophase suggesting a second radiosensitive event at this early stage of mitosis.

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A peculiar aspect of the altered mitotic schedule in UV-treated plasmodia is the considerable shortening of the nuclear division cycle following the first (delayed) mitosis. The initial delay of the first mitosis was nearly compensated by the advanced initiation of the second mitosis and further mitoses occurred even earlier than the corresponding control divisions. This acceleration of the mitotic rhythm very likely is related to the selective destruction of some nuclei during the first postirradiation S-period. Since the increase of the total plasmodial mass (protein) is little affected under these conditions the ratio of the amount of cytoplasm per nucleus is increased compared to unirradiated controls. As a working hypothesis we assume that the artificial increase of this ratio ultimately causes the advanced initiation of subsequent mitosis.

In analogy to models suggested for the control of chromosome replication and cell division in bacteria we propose that nuclear division in Physoarum is triggered by a cytoplasmic factor (presumably a protein) which accumulates during the interphase at a rate proportional to the increase of the total plasmodial mass. The initiator molecules may interact with specific sites of the nuclei in a stoichiometric way. Onset of mitosis occurs as soon as all nuclear sites are covered with the initiator. During mitosis the number of nuclei doubles and a new set of binding sites becomes available which has to be covered with newly formed initiator molecules before the next division can occur.

According to this model, the effects of UV-irradiation on the mitotic schedule could be interpreted as follows. The first mitosis is delayed because the DNA dependent production of initiator molecules is transiently disturbed. Later on the primary irradiation damage is repaired and the synthesis of the cytoplasmic initiator resumes at a normal rate. After the first postirradiation S-period the UV-treated plasmodium contains fewer nuclei per volume of cytoplasm than the control. Therefore less time is required to cover all nuclear binding sites with initiator molecules and the onset of nuclear division is advanced. If this concept is true one should expect to find advancing effects on mitosis after nuclear damage only in multinuclear systems since the destruction of the nucleus of uninucleate organisms inevitably kills the cell. On the other hand the opposite effect of delaying mitosis by decreasing the ratio of the cytoplasm per nucleus was already demonstrated 40 years ago by HARTMANN. In this classical experiment which was later repeated by Prescott, growing cells of Amoeba proteus were prevented from entering mitosis by repeated partial amputations of the cytoplasm.

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