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Studies on Synchronized Cells: Radiation-Induced Division Delay in the Flagellate *Astasia longa*[1]

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INTRODUCTION

Loss of viability, most often expressed as a failure of irradiated cells to divide indefinitely, has been the major criterion in evaluating radiation damage at the cellular level. In terms of the cell cycle, the ultimate aim is to find radiation-sensitive phases in the cell cycle, especially if they are intimately associated with cell division (*1–8*).

A variety of approaches have been devised to single out the response of those individual cells that were irradiated at a given stage in the cell cycle. Cells have been labeled with radioactive precursors of nucleic acids, irradiated, and followed for several generations (*1–3*). Puck and Steffen (*9*) have developed a system of mathematical analysis based on the kinetics of cellular proliferation that follows the introduction of a metabolic or radiological block of cell division. This approach permits the localization of radiation lesions in the cell cycle of cells whose pattern of division fits the mathematically derived formulations. Terasima and Tolmach (*10*) have developed a technique for isolating mitotic HeLa cells from exponentially growing cultures. These cells then divide synchronously for a limited number of generations. They can then be used to study radiation sensitivity throughout the cell cycle. Such a method, essentially representing a technique for synchronization of cell division, has been subsequently modified so that large populations of HeLa cells can be isolated and employed in biochemical studies (*11*). The technique has also been extended to other cell types (*12*, *13*). Other methods of synchronization of mammalian cells have included the use of nucleic acid precursors or analogs (*14*, *15*) and tem-

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perature shifts (*16*). The temperature-shift method of synchronization, which is of rather limited success with mammalian cells (*16*), has been used to greater advantage with protozoan cells such as the ciliate *Tetrahymena* (*17*) and the flagellate *Astasia longa* (*18*).

The present investigation employs synchronized cultures of *Astasia longa* grown in continuous culture (*19*). The chief advantage is that the synchrony is highly repetitive, a feature that is absent in mammalian cell systems. In addition, the synchronized growth characteristics of *Astasia* are well known (*19–22*). Thus, much of the information necessary to evaluate the radiation damage is already at hand. Further information has been gained on the response of protozoan cells to irradiation, in terms other than cell death (*23*). The present report constitutes an initial series of investigations, portions of which have been reported elsewhere (*24*).

MATERIALS AND METHODS

Cells were grown and maintained on a chemically defined salt medium as previously described (*20*). Synchrony was induced in cultures varying in volume from 125 ml to 20 liters by exposure to a repetitive 24-hour temperature cycle (17.5 hours at 14.5°C and 6.5 hours at 28.5°C) (*19*). Exponentially dividing cells were grown in the dark at 28.5°C. At desired intervals, aliquots of the log phase cells or synchronized cells (at different portions of the cell cycle) were transferred to a cylindrical water-jacketed vessel (3006, Bellco Glass, Inc.), maintained at the desired temperature, and exposed to X-rays. In some experiments, chloramphenicol was added to a final concentration of 200 μg/ml to minimize bacterial contamination. We find that cell division in *Astasia longa* is unaffected by this antibiotic to concentrations as high as 1000 μg/ml. In any case, the response to irradiation of the cells was the same whether or not chloramphenicol was present. Cell counts were made on suitably diluted samples with an electronic cell counter (Coulter Co., Hialeah, Florida) previously calibrated for this organism.

Cells were irradiated with a 250-kvp (30-ma) X-ray source (Maxitron 250, General Electric Co.) with a 3 mm Al filter. Exposure rates were below 1000 R/min, with 200 R/min constituting the average rate. Exposures were determined with an ionization chamber located in the middle of a model of the cylindrical vessel filled to the 100-ml level with Lucite (3.5 cm in thickness). During exposure the cell suspensions were continuously stirred with magnetic stirrers at about 100 rpm. The irradiated and control cells were at all times maintained at the temperature of the parent culture.

RESULTS AND DISCUSSION

Ionizing radiation induces a measurable division delay in exponentially dividing *Astasia longa* with exposures of approximately 500 R, as shown in Fig. 1. At the lowest level of exposure (105 R), the cells are unaffected and continue to divide at the control rate (doubling time 11.5 hours). With exposures in the range of 495 to

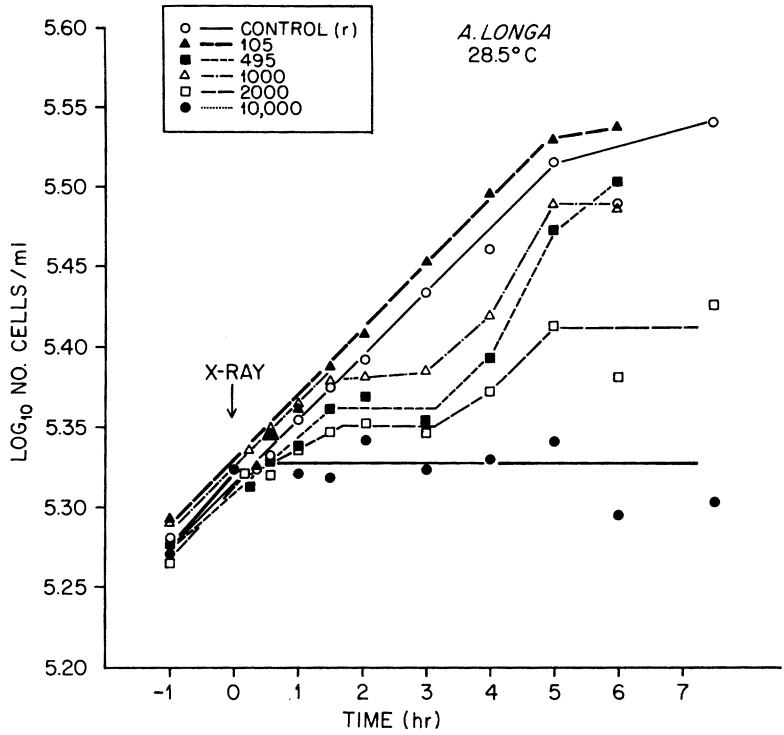

FIG. 1. Effects of X-rays on the division pattern of exponentially dividing *Astasia longa*. Individual cultures were grown at 28.5°C and exposed as indicated. Time of exposure is shown by the arrow.

1000 R, cell division is delayed for approximately 1.5 hours. The delay appears 1.5 hours after exposure to the X-rays, indicating either differential sensitivity throughout the cell cycle, or a delayed effect on the process of cytokinesis. In any event, at this range of exposures, the cells resume cell division at essentially the control rate after the delay. With 2000 R the postexposure growth curve is altered. There is an immediate reduction in the rate of cell division, followed by the characteristic cessation of cytokinesis; but the cells then resume division at a slower rate than the control cells or those receiving lower exposures. If the cells are exposed to 10,000 R, the division delay exceeds the period of observation, although the next day the cells appear to be viable when observed by phase microscopy. Since the division delay induced by X-rays at doses below 1000 R represented a constant period—that is, 13% of the doubling time at this temperature—synchronized cells were next irradiated to determine if the delay was due to the existence of a differentially sensitive portion of the synchronized cell cycle.

In this experiment (Fig. 2), the cells were exposed to X-rays at different times of

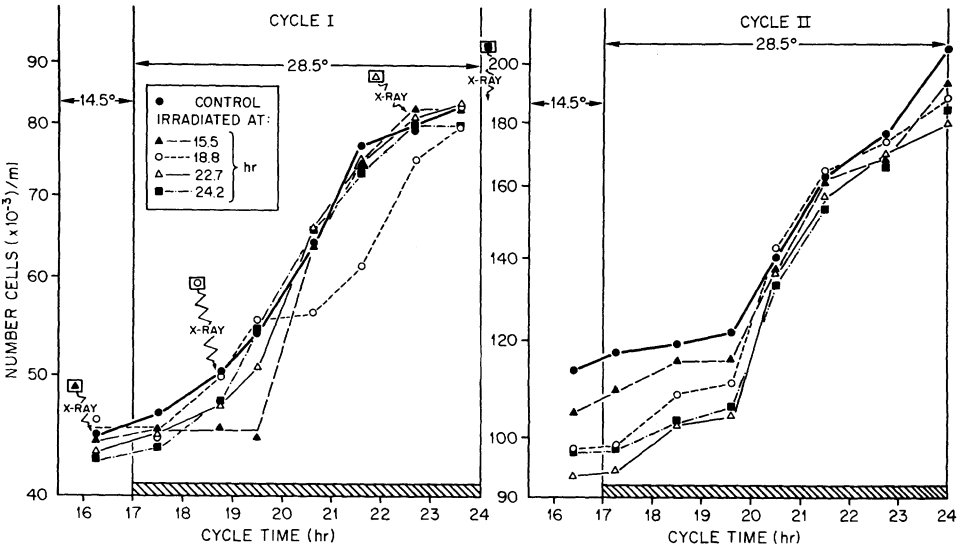

FIG. 2. Results of exposure of synchronously dividing *Astasia longa* to X-rays at various times in the temperature cycle. Portions of two consecutive temperature cycles are shown. In this experiment each 24-hour cycle consists of 17 hours at 14.5°C and 7 hours at 28.5°C. Approximate time of exposure of each culture to X-rays (400 R) is shown by arrows. The exact times are given in the insert.

the synchronized cycle. For example, irradiation within the last 2 hours of the cold period (for example, at 15.5 hours in the cycle time) delays the burst of division by approximately 1.5 hours. Subsequently, the cells resume division at an accelerated rate and complete their division with the control cells. This response is reminiscent of the stimulatory effects of X-rays on the DNA synthesis in onion root tip cells (*25*). However, exposure of the cells in the early portion of the warm period (18.8 hours cycle time) induces a smaller delay, which appears approximately 1 hour after irradiation. In this instance the cells resume their division at essentially the control rate. If the cells are exposed later in the warm period (for example, at 22.7 hours) or during the cold period (see Fig. 3), the effects on the following warm period are twofold: (1) cell division during the cold period (which amounts to approximately 10 % of the number of cell doublings in any one cycle) is completely blocked, and (2) although the treated and untreated cells begin to divide at the same time (usually at 19.5 hours in the cycle), only the control cells complete division by the end of the next cold period. This latter effect is comparable to the division delay seen in the exponentially dividing cells. From such data one can calculate the time required for the entire population to double in number after irradiation.

In a broad sense, the term "synchronized cell cycle" may be considered analogous to the *cell cycle* of cells grown at constant temperature. That is to say, the syn-

chronized cells have a mitotic phase during the warm period followed by an "interphase" during the cold period. Since the synchronizing treatment most likely alters the sequence and duration of events comprising the cell cycle, the analogy is not very strict. However, it has been shown repeatedly that under these temperature cyclings, where care is taken to use shifts within the physiological range, there is no unbalanced growth in synchronized *Astasia*, and the entire population doubles in number at each 24-hour period (*18*, *20*). Moreover, the synchrony persists for two division cycles when the cells are released from the synchrony-inducing temperature cycle (*19*). Thus, even if the "normal" temporal relationship between DNA synthesis and cytokinesis is altered, the effect of radiation on the events themselves may still be examined in the synchronized cells.

In order to derive a generalized response of synchronized cells to X-rays, the experiments of the type shown in Fig. 2 were repeated several times. The results from a series of four separate experiments which include the results shown in Fig. 2 are shown in Fig. 3. The cells were exposed to a dose of 400 R. In other experiments they were exposed to 200 R or 1000 R. However, a complete determination of the linearity of response versus dose throughout the entire range of doses and rates has

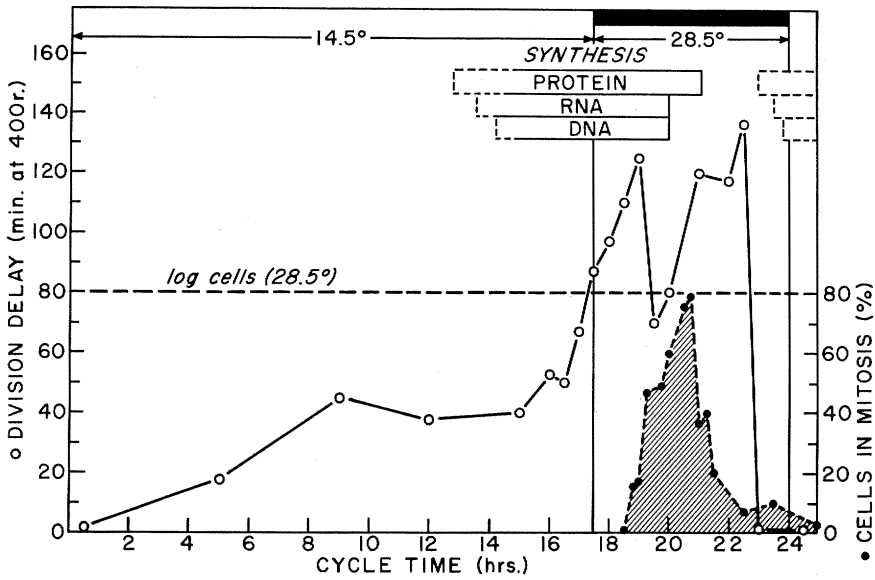

FIG. 3. X-ray-induced division delay as a function of the synchronizing temperature cycle in *Astasia longa* (O——O). Results are from four separate cultures of synchronized cells irradiated at 400 R; therefore, the division delay shown is limited to this dose. Shaded curve (·—·) shows incidence of mitoses in synchronized population (*20*). The horizontal line (-----) indicates the average division delay (80 minutes) for cells growing exponentially at 28.5°C. See text for details.

not been performed. The occasional fluctuations in the phase relationship between the onset of the burst of division and the beginning of the warm period make it difficult to detect division delay at low doses. At doses above 1000 R, the response is no longer a simple one, as shown in Fig. 1. We thus limit the presentation to experiments in which synchronized *Astasia* were exposed to doses at about the 400-R level as indicated above. For purposes of comparison, the frequency distribution of mitoses and the time of DNA, protein, and RNA synthesis are also shown in this fig. (*20*). The average division delay for exponentially growing *Astasia* (28.5°C) is indicated by the horizontal line.

It is clear that the sensitivity of synchronized *Astasia* to X-rays is variable. It gradually increases during the cold period and reaches a maximum level 1 hour after the warm period begins. It then decreases to the level seen in exponentially dividing cells at a time corresponding to the early portions of mitosis in the synchronized population of *Astasia*. There is a second peak of radiation sensitivity occurring in the second half of the warm period. There appears to be a correlation between the first period of increasing radiation sensitivity and the period when the preponderance of DNA, RNA, and protein is being synthesized. Previous studies (*19*, *20*) have shown that *Astasia* begins to synthesize these cellular components in the latter portions of the cold period. This period essentially matches the period of increasing radiation sensitivity. It is also during the first half of the warm period that the cells show increased oxygen consumption (*21*, *31*) and a requirement for CO_2 (*26*). After the twentieth hour the division burst occurs, and, reflecting a lower rate of synthesis than of division, the cellular level of these constituents drops. The second period of X-ray sensitivity occurs at a time when the cells are completing division and again renew their biosynthetic activities, although to a much lesser extent than before (*20*). It should also be noted that the cold period is not a nonmetabolic interlude (*22*). It is during the cold period that *Astasia* synthesizes its carbohydrate and lipid reserves (as well as the constituents mentioned above), showing an increase in dry weight from 1100 to 1400 μg per 10^6 cells (*19*).

In other cell systems, variation in sensitivity in terms of cell survival as a function of the cell cycle has been well demonstrated (*2*, *3*, *10*, *12*, *15*, *25*, *27*, *28*). Terasima and Tolmach found two periods of increased sensitivity in HeLa S3 cells when colony-forming ability is the index of radiation damage. It was found, in contrast to the present investigation, that the cells showed decreasing sensitivity as they entered into the DNA synthetic period. However, Terasima and Tolmach (*10*) indicated that HeLa cells also show the smallest division delay early in interphase. Such a delay, or prolongation of interphase, increases progressively as the cells are irradiated later in the interphase (comparable to the cold period in synchronized *Astasia*). It may thus be possible that in *Astasia* the X-rays also cause a similar differential prolongation of some of the subdivisions of interphase. In another synchronized mammalian system, cultured Chinese hamster cells are found to be most

resistant to X-rays, also in terms of the colony-forming ability, in the latter part of the DNA synthetic period, and least resistant during G_1 and G_2 (*13*).

In *Astasia longa* it is premature to ascribe the division delay to an impairment of a specific cellular constituent. The level of sensitivity varies from cycle to cycle in a fashion that can be correlated with certain phases of cellular activity such as mitosis and cytokinesis. Yet the shifts in the phase relationship between the burst of division and the temperature cycle do affect the magnitude of the observable division delay and introduce a level of statistical uncertainty characterized by a rather large standard error (average, 27.5 minutes during warm period). Such fluctuations, in turn, also obscure the periodicity of nucleic acid synthesis (*22*). However, the decrease in sensitivity during the time when most of the synchronized cells are in mitosis is in agreement with the results found in the alga *Oedogonium cardiacum* (*27*). In some strains of synchronized *Escherichia coli*, it is more difficult to associate radiation sensitivity with the division process *per se* (*28*, *29*). Such results possibly reflect the different nature of the replicative process in bacteria or its dissociation from other phases of the cell cycle through synchronization. The experimental links between radiation damage and the disruption of DNA synthesis have been reviewed by Lajtha (*30*).

In conclusion, the flagellate *Astasia* responds to X-rays in a manner similar to that of other cells. X-rays appear to delay those cells that are metabolically, rather than mitotically, active. Since we have examined a reparable insult—division delay rather than cell death—there is a difference in the degree of response of *Astasia* to radiation, in comparison to mammalian cells, which are more sensitive (*9*). The synchronized cells, because they offer a system that resembles the single cell, do indicate an opportunity and need for studying radiation damage against a well-defined biochemical and cellular sequence of events characteristic of repetitively dividing cells.

SUMMARY

The protozoan flagellate *Astasia longa*, dividing both exponentially and synchronously, has been exposed to X-radiation. Exponentially dividing cells are insensitive to doses of 105 R, but show a 1.5-hour division delay when exposed to 495 to 1000 R, after which they resume a normal rate of division. At larger doses, 2000 and 10,000 R, there is an immediate cessation of cytokinesis for an extended period, after which the cells resume cytokinesis at a rate slower than that of the control. Synchronized cells were exposed to 400 to 1000 R at various times during the cycle, and the delay in the time of cell burst was noted. Sensitivity to radiation is variable over the cell cycle. It gradually increases during the latter portions of the cold period, reaching a maximum level 1 hour after the warm period begins. It decreases during the time of mitosis and then increases again during the rest of the warm

period. This same sensitive period corresponds to the periods when synthesis of DNA, RNA, and protein is taking place.

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