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Free electron laser irradiation at 200 μm affects DNA synthesis in living cells

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ABSTRACT **We describe the effect of a 200-μm wavelength free electron laser beam on the ability of asynchronized and synchronized mammalian tissue culture cells to incorporate tritiated thymidine. Compared to controls (unexposed cells), a significant proportion of exposed cells exhibited a reduction in isotope incorporation. The results suggest that this wavelength may affect DNA synthesis.**

Recent studies on DNA lattice dynamics have utilized the apparent existence of vibrational and resonant modes of the DNA molecule in the infrared spectral region between 100 and 600 μm (1–3). These investigations have been performed on either extracted DNA or polynucleotides. Other than a preliminary study reported in 1987 (4), no studies have been conducted in this spectral region on living biological systems, though there have been investigations in the microwave region (1-cm wavelength) on living yeast (5). Those studies demonstrated a direct effect on yeast growth with a strong frequency dependence with resonances as narrow as 8 MHz. Because the power used was in the milliwatt range, the authors concluded that the effect was not thermally mediated and was of an unexplained biological nature.

We report here a series of studies in which a standard Chinese hamster ovary (CHO) cell line was exposed to the 200-μm irradiation of a free electron laser (FEL). Experiments were conducted on asynchronous and synchronous cell cultures. Three different statistical tests were used to analyze the data, and all demonstrated a statistically significant (P = 0.0001–0.01) inhibition of DNA synthesis.

MATERIALS AND METHODS

The FEL used in these studies was the Van De Graff generator-based machine at the University of California, Santa Barbara. The optical pulse from the FEL had a beam waist of ≈1 cm. One percent of the beam was sampled with a Mylar beam splitter and measured with a pyroelectric detector.

This reference detector monitored fluctuations in the power of the beam. The portion of the beam passing through the beam splitter was focused with a 90° off-axis paraboloidal mirror onto the sample. The reference detector was calibrated by measuring the energy in the beam at the sample with a Laser Precision RJP-735 energy meter. With an optical pulse length of 2.0 μsec (full width at half maximum) and a typical energy per pulse of 2.6 mJ, the power per pulse was 1.3 kW. The profile of the focused beam was measured with a Spirocon pyroelectric array detector placed at the position of the sample. The array detector consisted of a 16-by-32 array of pyroelectric elements with a separation of 0.8 mm. The profile of the beam was observed to be nearly Gaussian, with a waist of 2.7 mm (Fig. 1).

In all of the experiments, each cell culture was exposed to 100 pulses of the FEL operating at 0.5–0.33 Hz.

The cells used were Chinese hamster (*Cricetulus griseus*) ovary cells obtained from the American Type Culture Collection (CCL no. 61).

The cells were maintained in GIBCO's minimum essential medium (MEM) with 10% (vol/vol) fetal bovine serum and were regularly subcultured with 0.25% trypsin. In preparation for the experiment, the cells were grown in Corning 150-cm^2 tissue culture flasks. Approximately 11 hr before transporting the cells, half were placed in 6 μl of Colcemid per ml of medium for 3 hr. The mitotic phase cells were removed by agitation and were centrifuged for 5 min at 200 × g. The synchronized cells were resuspended in fresh MEM with 10% fetal bovine serum and injected into standard Rose tissue culture chambers.

For the next 8 hr the cells were allowed to attach to the fused silica windows in the Rose chamber. It had been previously shown that the fused silica windows transmit 60% of the FEL wavelength at 200 μm (4). After 8 hr the cells were finishing G_1 of the cell cycle and beginning the DNA synthesis (S) phase. At this point the cells were chilled to 4°C on ice for 3 hr during their transport to the laser in Santa Barbara. Previous studies had demonstrated that the cells could be held in S phase for up to 6 hr by this method. When returned to 37°C, the cells continued through the cell cycle. The total cell cycle for CHO cells is 12.5 hr, and the length of S phase is 4 hr. Once at the FEL laser facility, the cells were placed at room temperature 2–5 min prior to irradiation. The nonsynchronized CHO cells were subjected to identical temperature changes and culture conditions as the synchronized cells.

For irradiation, each chamber was placed into a holder that was prealigned so that the FEL beam was directed onto the fused silica surface with the cells growing as a monolayer on the opposite side of the glass. Because of the tight adherence of the cell membrane to the glass surface, there was no water interface between the cells and the glass surface. In this configuration, the beam passed directly through the window and exposed the cells without passing through any intervening fluid. A 3-mm-thick stainless steel shield with a 6-mm aperture was placed over the glass surface so that the FEL beam exposed only those cells under the aperture window. All the other cells growing on the glass served as control cells and, except for the FEL exposure, were treated exactly the same as the exposed cells.

Within 2 min of completion of exposure to the FEL beam, the culture medium was removed from the culture chamber, and fresh medium containing [^{3}H]thymidine (specific activity of 5 μCi/ml; 1 Ci = 37 GBq) was injected into the chamber. The isotope-containing chamber was then placed in a 37°C incubator for 30 min, after which 2.5% glutaraldehyde and MEM without fetal bovine serum was injected. The fixed cultures were then subjected to standard light microscope

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Abbreviation: FEL, free electron laser.

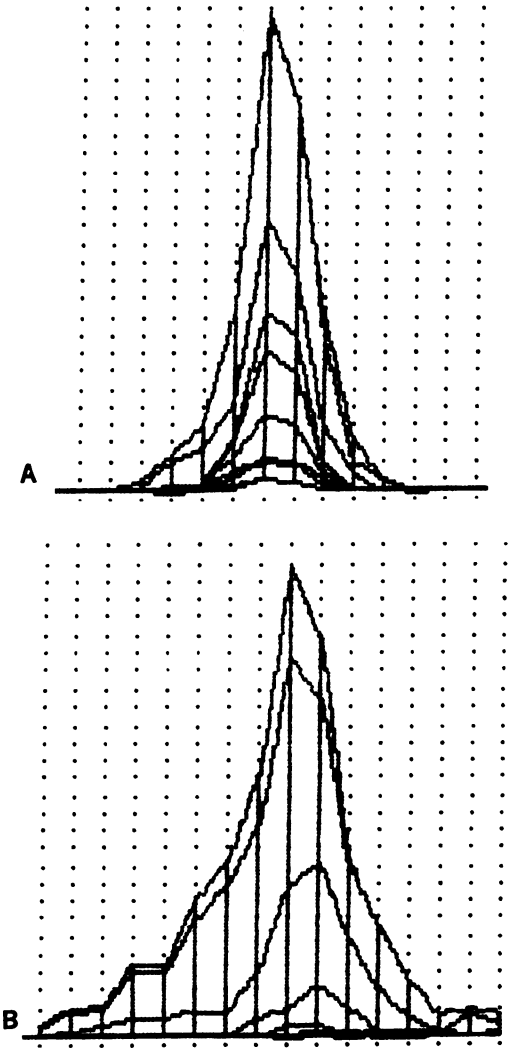

FIG. 1. Horizontal (*A*) and vertical (*B*) profiles of the FEL beam at 200 μm. Each curve represents a profile of one FEL pulse. The beam was progressively attenuated. The vertical axis represents the relative intensity. Spacing on the horizontal axis is 0.8 mm.

autoradiographic analysis using Ilford emulsion K-5. After being dipped in the emulsion, the fused silica windows were placed in a black box in a refrigerator for 1 week. The slides were developed in Kodak D-19 developer for 3 min, Kodak stop bath (8 ml per 500 ml of distilled H_2O) for 30 sec, and Kodak acid fixer for 5 min.

RESULTS AND DISCUSSION

A total of 40 culture chambers was exposed to the FEL. Twenty contained asynchronized cells and 20 contained synchronized cells such that the exposure to the FEL occurred during the S phase of the cell cycle.

Autoradiographic analysis of each chamber involved scoring 500 randomly selected cells from within the irradiation zone (the aperture) and 500 cells from outside the irradiation zone (shielded by the metal cover). To insure nonbias of the person performing the analysis, a second individual without knowledge of the experimental design scored a random number of chambers in order to verify the counts of the former individual. On the basis of the density of autoradiographic grains over the nucleus, a cell was scored as either "light" or "heavy." This scoring was rather easy to do because the cells generally either had a heavy or light concentration of isotope. Computer-enhanced video microscopy clearly demarcated the lightly labeled cells from the heavily labeled cells (Fig. 2). The mean number of cells exhibiting a light (reduced) amount of isotope was compared for both the synchronized and asynchronized cell populations (Fig. 3). Each group was compared to its own control (the cells outside the irradiation zone). Statistical tests used to

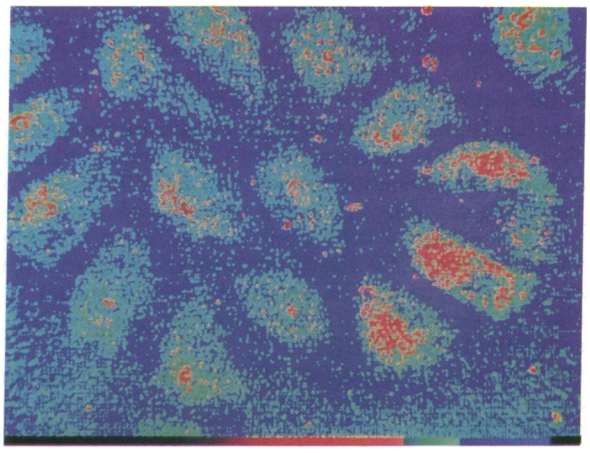

FIG. 2. Computer-enhanced video image of an autoradiogram of a field of cells exposed to the FEL beam. The red pixels represent silver grains over the cell nucleus and denote radioactivity associated with newly synthesized DNA. The three cells in the right of the field would be categorized as heavy and the remaining cells in the field would be categorized as light. (×2500.)

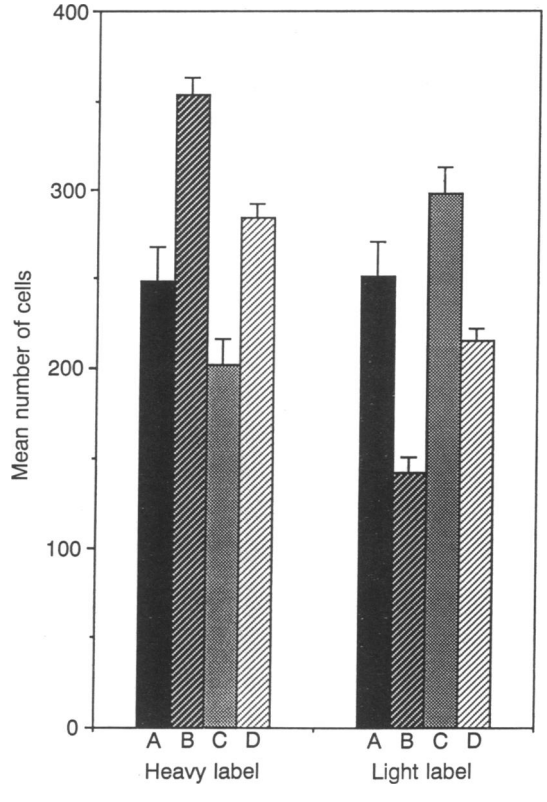

FIG. 3. Autoradiographic data on cells exposed to FEL beam. The first group represents mean number of cells exhibiting heavy radioactive label and the second group represents the mean number of cells exhibiting light radioactive label. Bars: A, synchronized experimental cells; B, synchronized control cells; C, asynchronized experimental cells; D, asynchronized control cells. All means are based on 500 cell counts in twenty chambers. Controls are based upon scoring 500 cells from outside the irradiation zone in the same chambers as the experimental. The error bar indicates the SEM.

compare the experimental with the controls were (*i*) Wilcoxon rank sum, (*ii*) Student's *t* test, and (*iii*) χ^2. All three tests demonstrated statistical differences of the experimental from the control with $P = 0.0001$–0.01.

It is clear from the data that there was significant inhibition of thymidine incorporation in both the synchronized and asynchronized populations exposed to the FEL when compared to the controls. As would be expected, there was a greater percentage of cells exhibiting inhibition in the synchronized group (22% above control) as compared to the asynchronized group (17% above the control value). This finding is consistent with the idea that the radiation is affecting the DNA directly.

There should be a greater effect on those cells in the S phase of the cell cycle during the time of actual laser exposure. Since a 30-min pulse of [^{3}H]thymidine was given, only those cells actively undergoing DNA synthesis would exhibit an effect as assayed by autoradiography. This also explains why >72% of the synchronized control cells exhibited heavy labeling (i.e., most were in S phase at the time of isotope exposure).

One additional possible explanation of the reduced labeling caused by the 200-μm FEL laser exposure is that the effect was on the cell membrane rather than the DNA directly. In this case, the entry of the isotope into the cell would have been affected. However, in other studies conducted at the same wavelength using an RNA precursor ([^{3}H]uridine), there was no difference in isotope incorporation between the control and experimental populations.

We believe that this study demonstrates clearly that the 200-μm wavelength affects the physiology of cells, and most likely the absorption site is the DNA molecule. On the basis of the studies with extracted DNA (1–3), it is possible that the photons are influencing the DNA molecule by means of the weak vibrational modes in this region of the spectrum. We believe this to be the case even though the absorption bands described in extracted DNA (1–3) are in the wavelength region of 90–140 μm as compared to the 200 μm reported here. Of course, the physical–chemical state of the DNA molecule and the numerous associated molecules are quite different in a living cell. It may not be unreasonable to assume that the absorption characteristics of the DNA will be different in the *in vivo* situation.

Because of the relatively low fluences and the thermal diffusion time between pulses, it is unlikely that the effect is thermally mediated. Furthermore, calculations made assuming water as the target molecule with absorption of 500 cm^{-1} at the wavelength used (cell thickness of 4 μm) indicated a possible maximum temperature rise of only 2.96°C.

Though the nature of the effect described in this report remains to be determined, the implications for both probing polymeric macromolecules in living cells and manipulating cell physiology, with light in this region of the spectrum, is intriguing. A tunable FEL in this region of the spectrum certainly would facilitate these studies.

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