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Microirradiation of Living Fungal Cells with a Laser¹

John L. Robertson and Charles L. Wilson

INTRODUCTION

The introduction of the laser into biology has provided a tool with which new questions can be asked, and possibly answered. It has proven to be a useful instrument for microsurgery because a very small area of the cell can be destroyed with little apparent damage to the remaining portions. There is evidence that laser radiation can be used as a mutagen in much the same manner as x-ray or other types of radiation. This paper discusses the methods involved in preparation of living fungal materials for irradiation with a laser beam and describes some responses of irradiated cells. To our knowledge, experiments of this nature have not been performed previously with the fungal cell.

MATERIALS AND METHODS

Material to be irradiated was prepared by inoculating agar-dipped slides with blocks of agar containing fungal mycelium. Inoculated slides were incubated at 24°C for 15 to 18 hours in a moist petri dish. Agar-dipped slides were prepared as follows: sterile slides, .96 to 1.06 mm in thickness, were dipped into hot Difco Oak Wilt agar in a sterile petri dish and allowed to become well covered. They were then held on end to drain off excess agar, leaving only a thin film on the slide. After inoculation and incubation of the fungus the excess agar was scraped off the slide leaving a small square of the agar with fungal hyphae. A cover slip, no. 0, was then placed on the slide and all edges of the coverslip were sealed with paraffin.

A Leitz phase-contrast microscope with Heine condenser was used in conjunction with a TRG bio-coupling model 153A laser system. Shots were fired through a Leitz 70x oil fluorite objective. The laser has a maximum power input of 230 joules and could be fired once every minute with a pulse duration of 150 μ sec. The laser was aimed by use of a set of crosshairs in the ocular of the microscope. Because of the large size of its cells, *Basidiobolus ranarum* Eidam was the fungus used in this study.

RESULTS

The results of microirradiation are affected by the activity of the cell at the time of irradiation. If the cell is dividing, a muta-

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genic effect may be obtained. When a shot is directed into the chromatin at telophase, a portion of the chromatin is destroyed with the resulting formation of a nucleus which is much reduced in size.

If a shot is directed into the spindle during division, no cell wall subsequently forms, so that a cell with two nuclei is produced.

When a nucleolus is irradiated at one end (Fig. 1) a darkened area appears at the focal point of the beam. After a period of about 2 minutes the nucleolus can be seen to constrict at the point of irradiation (Fig. 2). Approximately two hours after the initial shot, a portion of the nucleolus may separate from the main body (Fig. 3). The separated portion may fragment into small bodies which remain within the nuclear envelope. (Fig. 4).

The elongation of a cell can be arrested by irradiation of the tip. This, however, must be done with care because it is quite possible to burn a hole in the cell wall permitting the cytoplasm to escape through it. These holes generally range in size from 1 to 3 μ (Fig. 5).

DISCUSSION

The amount of the cell damaged, and the type of damage done by laser irradiation depends on several factors. The amount of energy input and the size of the aperture affects the size and intensity of the beam in the TRG system. Magnification also affects the size of the damaged area: the higher the magnification, the smaller the damaged area. If the microscope is out of focus, a much larger portion of the cell will be damaged. The depth at which the cell is embedded in the agar also determines how effective a shot will be; this is one reason for the importance of the thin film of agar on the slide. Thin preparations are absolutely essential in order to obtain good results with laser irradiation through the microscope. It also encourages the fungus to grow in one plane, thus allowing entire cells to be brought into focus at the same time.

The reaction of the nucleolus when irradiated indicated that there is something which bounds its contents. Although no membrane has been found around the nucleolus with ultrastructure studies, the evidence presented here appears to indicate that one may be present. Such a membrane might not have the generally accepted unit membrane structure and therefore, would not be detectable by the methods generally used in electron microscopy. A membrane that is highly elastic and capable of repairing itself following breakage is suggested by responses of the nucleolus to microirradiation.

With laser radiation there is a possibility of increasing or reducing ploidy by selectively destroying the spindle or part of the nuclear material.

Laser irradiation should provide a valuable tool for studies to better understand the fungal cell. The opportunity to selectively destroy parts of the cell should allow morphogenic and genetic studies to be made that were hitherto impossible.

DESCRIPTION OF FIGURES

Microirradiation of *Basidiobolus Ranarum* With Laser

- Fig. 1. 650x Nucleolus shortly after irradiation. Arrow at point of irradiation.
- Fig. 2. 650x. Constriction of same nucleolus (arrow) 2 minutes after irradiation.
- Fig. 3. 650x. The point of initial irradiation (R) at the far left and constriction of nucleolus (c).
- Fig. 4. 1500x. Fragments (F) of nucleolus within the nuclear envelope.
- Fig. 5. 1500x. Irradiated area at the tip.

