Laser Microsurgery in Cell and Developmental Biology

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The laser microbeam permits selective alteration of part of a subcellular organelle in a single living cell. This alteration can be in a specific class of molecules confined to an area of less than 0.25 micrometer. This capability has been developed over the last 15 years and is now generally available for studies in cell and developmental biology.

Summary. New applications of laser microbeam irradiation to cell and developmental biology include a new instrument with a tunable wavelength (217- to 800-nanometer) laser microbeam and a wide range of energies and exposure durations (down to 25×10^{-12} second). Laser microbeams can be used for microirradiation of selected nucleolar genetic regions and for laser microdissection of mitotic and cytoplasmic organelles. They are also used to disrupt the developing neurosensory appendages of the cricket and the imaginal discs of *Drosophila*.

Just over 12 years ago, the blue-green argon ion laser microbeam was introduced as a potential tool for subcellular microsurgery (*1*, *2*). There had been limited success earlier with the red ruby laser (*3*) and the classical ultraviolet microbeam (*4*). The work with the blue-green argon laser led to development of a tunable wavelength flash-lamp-pumped dye laser microbeam (*5*) and later to a dye laser that was pumped by the green wavelength of a low-power neodymium-YAG (yttrium-aluminum-garnet) laser (*6*). The recent establishment of a National Institutes of Health Biotechnology "user" resource has permitted the development of a dye laser microbeam completely tunable from 217 to 800 nanometers, by employing the second (532 nm), third (355 nm), and fourth (265 nm) harmonic wavelengths of a high-power 10-nanosecond pulsed neodymium-YAG laser (Fig. 1). In addition, a separate high-power 25-picosecond neodymium-YAG laser has been integrated into the system to permit exposure of living cells to ultrashort pulses of light. This dual laser system is interfaced with an inverted Zeiss Axiomat microscope and an image array processing computer, which permits the exposure of groups of cells, single cells, or individual organelles within single cells to a variety of wavelengths at various power densities, with time exposures as short as 25 psec. In addition, the use of the sophisticated Zeiss Axiomat microscope and the image processing computer permits a state-of-the-art optical and photometric examination of the biological material.

Since this user system is now available to the scientific community (*7*), we will review some of the earlier key experiments and several recent unpublished experiments that demonstrate the use and versatility of the system in cell and developmental biology.

Principles of Selective Damage

Laser light is intense, coherent, monochromatic electromagnetic radiation. The damage produced by a focused laser beam may be due to classical absorption by natural or applied chromophores and the subsequent generation of heat (*8*), or it may be caused by a photochemical process such as the production of monoadducts or of diadduct cross-linking in the case of laser light–stimulated binding of psoralen to nucleic acids (*9*). A third possibility is the generation of damage by an uncommon physical effect that occurs when ultrahigh photon densities are achieved in very short periods (a few nanoseconds or picoseconds). The resulting nonlinear optical effects, such as multiphoton absorption, dielectric breakdown, and pressure phenomena, occur when the classic law of reciprocity does not hold; these effects may be responsible for some of the disruption observed in biological material (*10*). Whichever of the above damage-producing mechanisms is operating, whether "classical" or "uncommon," the damage often can be confined to a specific cellular or subcellular target in a consistent and controllable way. In addition, once the biophysical mechanism of laser interaction with the molecules is ascertained, the investigator has a method for precise disruption of a specific class of molecules within a strictly delimited region of the living cell. The size of this region may be considerably smaller than the size of the focused laser beam because of the distribution of the target molecules in the target zone. However, the size of the focused laser spot also is of paramount importance, because it defines the maximum volume of biological material that will be available for direct interaction with the laser photons. Though the diameter of the focused laser spot is a direct function of the wavelength, the magnification of the focusing objective, and the numerical aperture of the objective, the actual diameter of the "effective" lesion area may be considerably less than the theoretical limit of the focused laser beam, which is half the wavelength. This is because a high-quality laser beam can be generted in the transverse electromagnetic (TEM_{00}) mode, which results in a beam with a Gaussian energy profile across it. The profile is carried over to the focused spot, producing a "hot spot" of energy in the center. It has been demonstrated consistently (*11*) that by careful attenuation of the raw laser beam, the damage-producing portion in

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the focused spot can be confined to the central hot spot (that is, the only region within the focused spot that is above the threshold for damage production). As a result, lesions can be routinely produced that are less than 0.25 μm in diameter and frequently 0.1 μm in diameter (see cover).

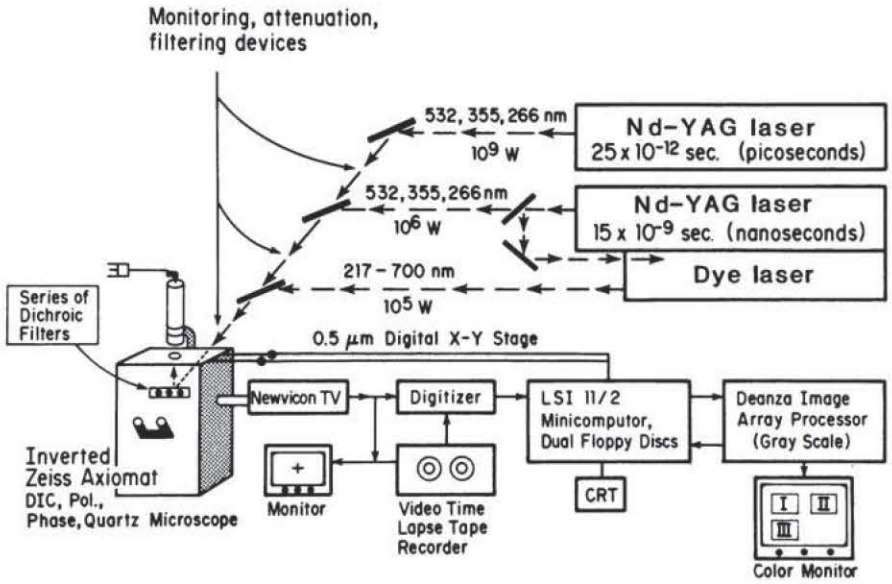


Fig. 1. Diagram of laser microbeam system. The three basic components of the system are the lasers (Quantel YAG 400 and 481/TDL III), the microscope (Zeiss inverted Axiomat equipped for phase contrast, bright field, polarization, and differential interference contrast), and the television computer system (DeAnza IP 5000 image array processor, Sierra LST-1 television camera, and GYYR DA 5300 MKIII videotape system). An LSI-11 minicomputer is used to drive the image array processor. In addition, the image processor–LSI combination is interfaced to the X-Y digital microscope stage in order to provide cell tracking capabilities.

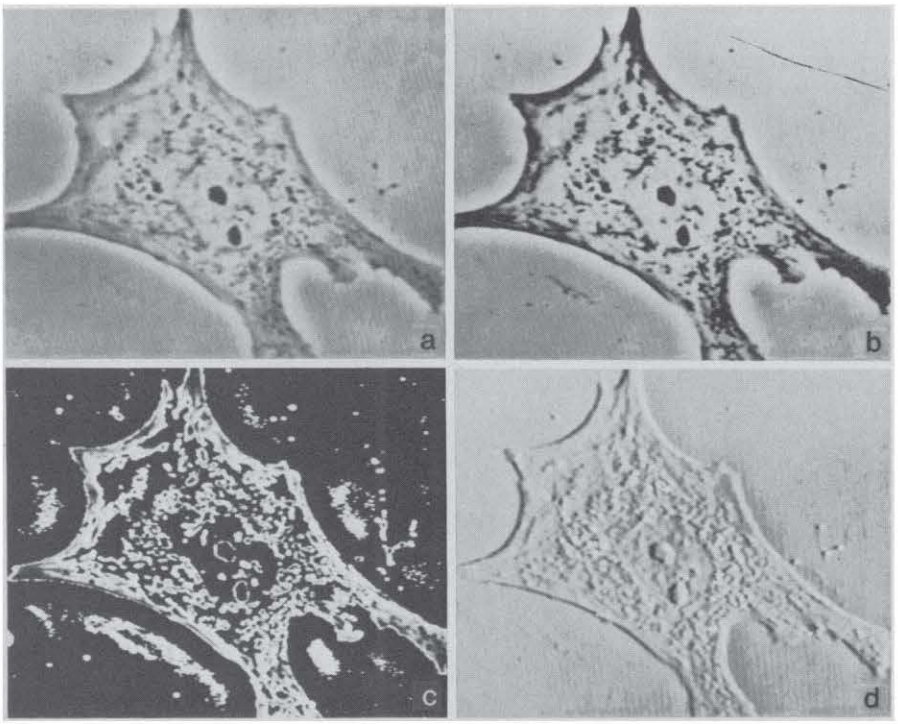


Fig. 2. (a) Live phase-contrast image of PTK_2 kangaroo kidney cell; image photographed directly from television monitor; (b) same cell after computer contrast enhancement by an intensity transformation that resulted in the reassignment of gray values and display of the image in real time; the result is an increase in contrast that enhances specific cellular structures; (c) same cell displayed after contrast enhancement of image boundaries; (d) real-time image of same cell after boundary enhancement by subtracting a slightly offset image from an original unshifted image. This image is virtually identical to a differential interference contrast image. It is generated by the computer by using a standard phase-contrast image with lower light level illumination.

Video Computer Microscopy

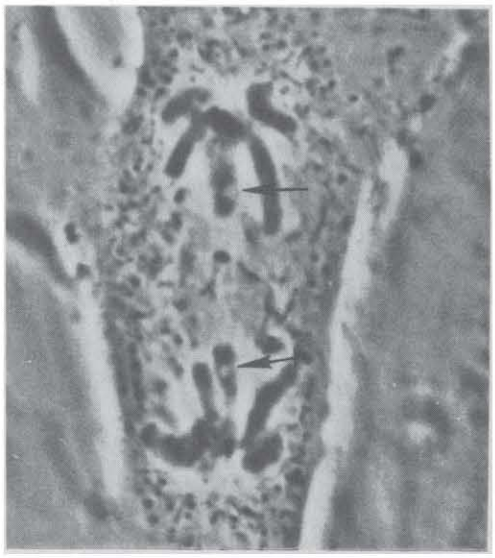
Since the laser beam can be focused to produce a damage spot less than the diffraction limits of the light microscope, one of the limiting factors becomes the quality of the optical image itself. It is difficult to focus the laser beam onto a target that is not readily visible.

We have been able to improve upon the optical image of the highest quality light microscope (the Zeiss Axiomat) by using a low light level television camera (Newvicon tube) whose signal is digitized and enhanced by a fast (real time) image-processing computer. Recent advances in computer technology have led to the development of small, relatively inexpensive image array processors that are capable of performing sophisticatd image-processing routines on video images in real time. Real-time processing allows sophisticated routines, such as contrast enhancement, edge detection, background subtraction, multiple image averaging, and pseudocolor enhancement; all of these can be performed on the microscope image during the time of the actual experiment. Figure 2 and the cover photograph are examples of computer-enhanced images. The processor can also be used for more analytical tasks such as calculation of object areas, boundary lengths, and intracellar distances. The integration of the computer into the laser microbeam system has greatly extended the capabilities of this system and the scope of the experiments to be described.

Chromosome Microsurgery

In 1969, a low-power pulsed argon ion laser was focused on chromosomes of living mitotic salamander cells that had been photosensitized with the vital dye acridine orange. The result was the production of a 0.5-μm lesion in the irradiated region of the chromosome (Fig. 3). Subsequent studies on salamander and rat kangaroo cells (PTK_1 and PTK_2) demonstrated that the laser microbeam could be used to selectively inactivate a specific genetic site, the nucleolar genes (*1*, *12*). Three different laser microbeam systems were used in these studies: the low-power argon laser, with acridine orange sensitization; a high-power argon laser, without dye photosensitization (most likely a multiphoton process mechanism); and the fourth harmonic (265 nm) of a neodymium-YAG laser. Not only can the nucleolar genes be selectively deleted, causing a loss of nucleoli in the subsequent cell genera-

tions, but a corresponding lack of one light-staining Giemsa band in the nucleolar organizer region of the chromosome can be demonstrated in cells cloned from the single irradiated cell (*13*) (Fig. 4). Experiments that include in situ hybridization with ^{3}H-labeled RNA and selective silver staining for the nucleolar organizer have demonstrated the loss of one group of ribosomal genes in the clonal population of cells (*14*) (Figs. 4 to 8). The use of the laser to destroy selected chromosome regions with the subsequent maintenance of this genetic loss is clearly feasible. In addition, it is now relatively easy to manipulate the ribosomal genes in vitro in order to study their regulation and function—a problem of considerable interest in light of the classic genetic studies on the bobbed mutant in *Drosophila* (*15*) and gene amplification



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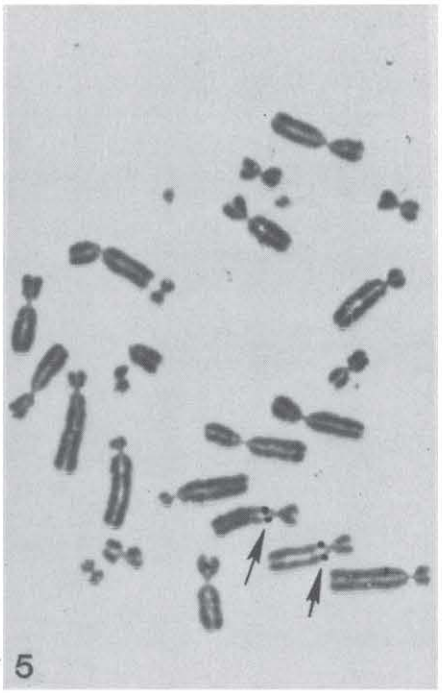

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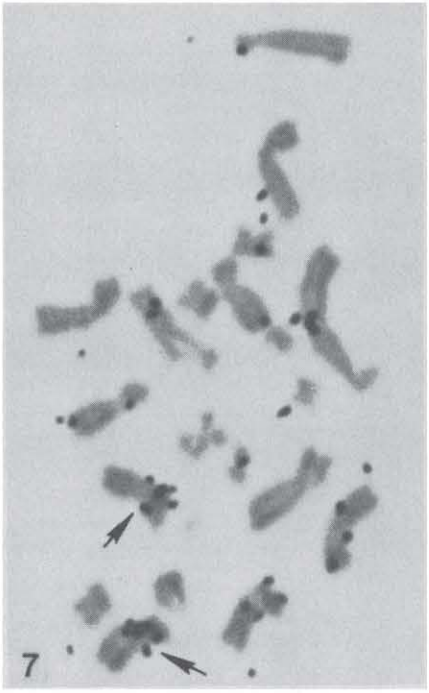

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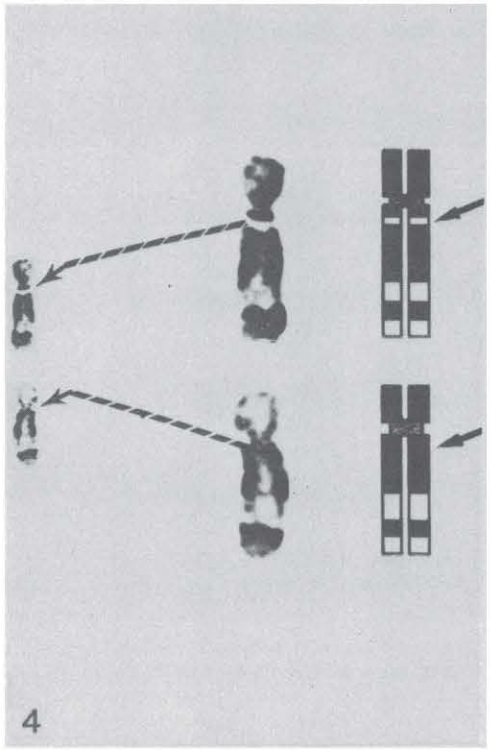

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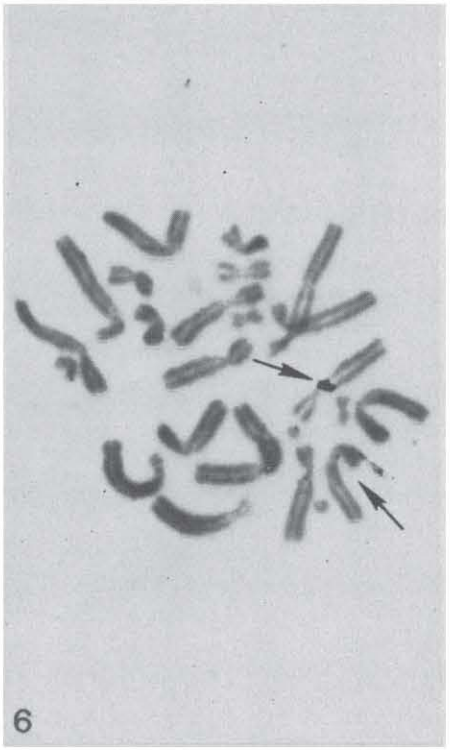

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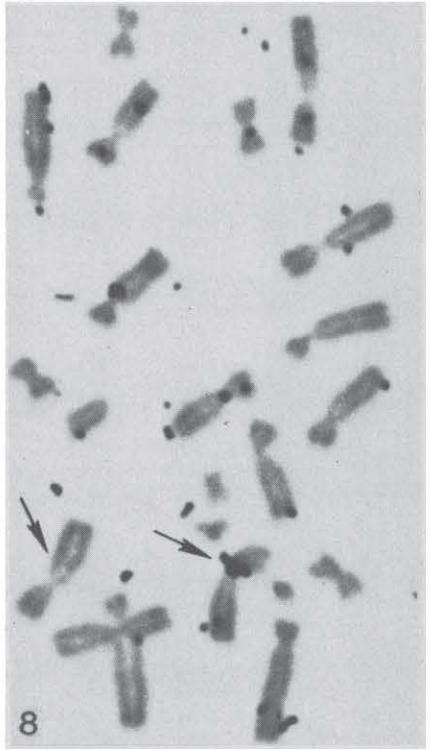

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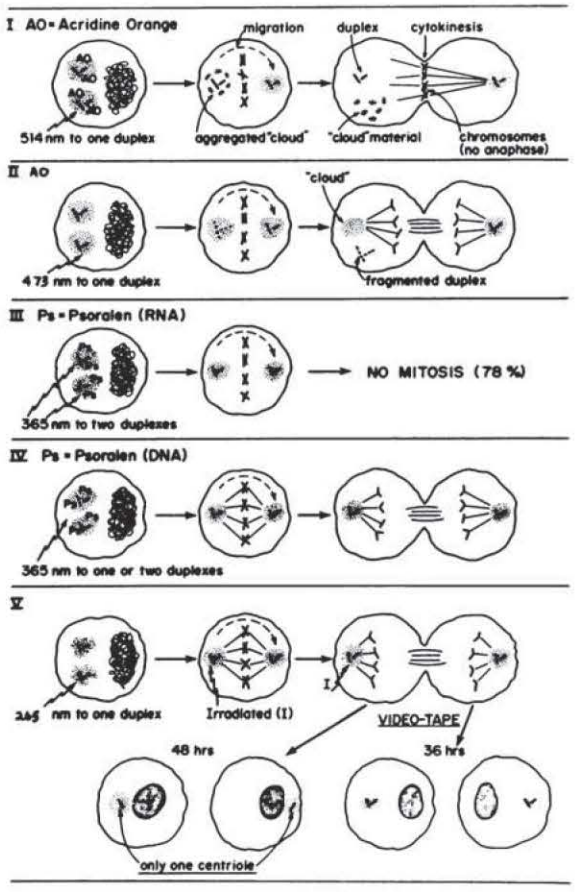
Fig. 3. Phase-contrast photomicrograph of anaphase PTK_2 chromosomes after placement of two lesions, 1 μm in diameter, on the chromosome arms (arrows). Lesions were produced by irradiation with the 514-nm beam of an argon laser with an energy density of 1000 microjoules per square micrometer without dye sensitization. Fig. 4. Giemsa-trypsin–banded chromosomes from PTK_2 clone in which originating cell had one nucleolar organizer secondary constriction irradiated with a 265-nm beam of a YAG laser. Note the deletion of one light-staining chromosome region (arrows). Fig. 5. Silver-stained chromosomes from control nonirradiated PTK_2 cells. Note two clearly stained nucleolar organizer regions. Arrows point to two homologs of a pair. Fig. 6. Silver-stained chromosomes from irradiated clone. Note one heavily stained nucleolar organizer. Fig. 7. In situ hybridization of ^{3}H-labeled ribosomal RNA to control nonirradiated PTK_2 cell. Note two chromosomes with selective hybridization to the nucleolar organizer. Fig. 8. In situ hybridization to cell cloned from the irradiated cell. Note only one chromosome with hybridization to the nucleolar organizer.

in amphibian oocytes (*16*). Our preliminary experiments with silver staining and in situ hybridization suggest the possibility of ribosomal gene "magnification" in vitro after the laser deletion of one group of ribosomal genes (Figs. 4 to 8). This suggestion is based on the finding that the one remaining nucleolar organizer region (after deletion of one) appears to stain twice as deeply as those in cells with the normal two nucleolar organizers. Similarly, there appears to be roughly twice the normal amount of in situ hybridization to the one nucleolar organizer region in the cells cloned from the irradiated cell. Final conclusions on this point, however, must await quantitative ribosomal DNA determinations in vitro.

Whereas the studies discussed above dealt with selective removal of portions of chromosomes, other studies have been done in which entire chromosomes have been removed from mitotic cells (*17*). This can be accomplished by irradiation of the centromere region at metaphase of mitosis. When a centromere with its microtubule attachment site (the kinetochore) is destroyed, the chromatid no longer remains attached to the mitotic spindle. Frequently the chromatid remains behind at the metaphase plate and is caught within the constriction ring at cytokinesis. The chromosome may be incorporated into the cytoplasm of one of the daughter cells. The genetic result is the frequent production of one daughter cell that is missing an entire chromosome and one daughter cell that has an extra chromosome enclosed within a micronucleus. These daughter cells have been observed through the subsequent mitosis (*18*), and the irradiated chromosome duplicates itself without a functional kinetochore. At the next mitosis, the duplicated irradiated chromosome cannot attach to the spindle, and once again a micronucleus is formed. The capability of directed whole chromosome removal permits a class of cytogenetic studies in which investigators can selectively delete chromosomes and thus have a method to complement the already well-developed methods of somatic cell fusion. In addition, the ability to damage a restricted region of a chromosome (such as the centromere) and to observe the cell



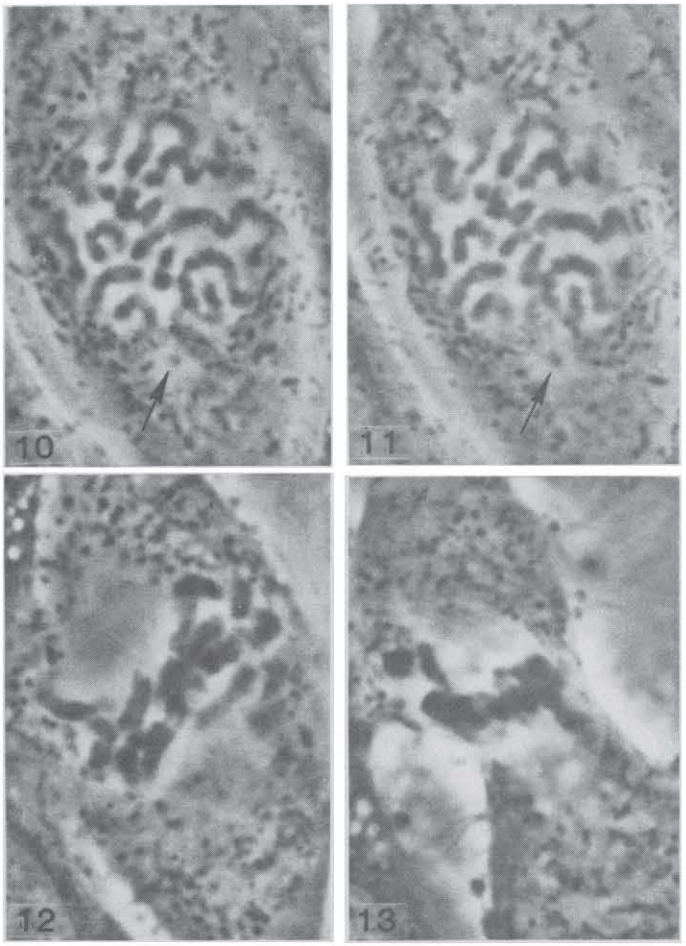


Fig. 9. Summary of experiments in which the centriolar region was irradiated under conditions to produce selective disruption to specific components. (*I*) Acridine orange sensitized the pericentriolar cloud to 488 and 514 nm. (*II*) Irradiation with 473 nm selectively disrupted the centriole proper and left the pericentriolar cloud unaffected; acridine orange was also used as a sensitizer. (*III*) Psoralen (4′-aminomethyl-4,5′,8-trimethylpsoralen) selectively sensitized the pericentriolar cloud by binding to RNA. (*IV*) Psoralen specific for DNA (4′-hydroxymethyl-4, 5′, 8-trimethylpsoralen, 4′-methoxymethyl-4,5′,8-trimethylpsoralen, and 4,5′,8-trimethylpsoralen) had no effect on the immediate process of cell division. (*V*) Irradiation with 265-nm laser light was effective in preventing centriole replication but did not inhibit the immediate cell division process. Furthermore, the fact that the irradiated cell went through a subsequent division without duplicating centrioles demonstrates that centriole replication is not needed for mitosis to occur. This result also implicates the nucleic acid in the process of centriole duplication. Fig. 10. Prophase PTK_2 cell treated with acridine orange to sensitize the centriolar region to the argon ion laser beam of 514 nm. The arrow indicates the centriolar region (dark spot) in the perinuclear clear zone. This is before irradiation. Fig. 11. Immediately after irradiation of the centriolar region. Note the slight increase in the extent of darkening (arrow). Fig. 12. The irradiated cell about 15 minutes after irradiation. Note that the chromosomes have continued to condense and align in a metaphase-like configuration. Fig. 13. At 30 minutes after irradiation, the cell undergoes cytokinesis without any anaphase movement of chromosomes. Ultrastructural examination of this and similarly irradiated cells demonstrated that the pericentriolar material had been selectively damaged.

through its cell cycle to a subsequent mitosis permits studies on chromosome damage and repair from a new perspective.

Mitotic Organelles

Centriolar Zone. Extensive work has been devoted to the use of the laser to selectively disrupt three mitotic structures (centrioles, kinetochores, and microtubules) in order to elucidate their organization and function in the process of cell division. These studies may be the most demanding in terms of understanding and applying the principles of selective damage discussed in the first section of this article. Figure 9 summarizes the centriole experiments.

Centrioles are just within the resolution of the light microsocope. In the PTK_2 cell line, the centriolar duplex is frequently visible in prophase as a phase dark dot 0.25 μm in diameter within a perinuclear clear zone (*19*). Ultrastructurally, the centriolar complex is composed of the centriole proper and a surrounding cloud of material called the pericentriolar cloud. Treatment of prophase cells with nontoxic levels of acridine orange selectively sensitized the pericentriolar cloud to the green beam of either the argon or YAG laser (*20*). Irradiation of the centriolar complex after acridine orange treatment resulted in selective disruption of the cloud without apparently affecting the centriole. The cells progressed toward metaphase, but no anaphase movement of chromosomes occurred, even though the cells went through cytokinesis (Figs. 10 to 13). Since acridine orange binds selectively to nucleic acid, this study supports the earlier finding (*21*) that some nucleic acid is located in the pellicle of *Paramecium*. In our studies, the high degree of sensitivity of the pericentriolar cloud implicated this region as a major site of nucleic acid localization. In addition, the lack of microtubule organization after disruption of the cloud suggests that this region is a microtubular organizing center in vivo, a fact confirmed by Gould and Borisy (*22*) using isolated pericentriolar material. In later laser microbeam studies of the centriolar region, a psoralen compound that is photochemically bound to DNA did not inhibit mitosis after exposure of the centriolar region to the appropriate cross-linking wavelength (365 nm) of laser light (*23*). However, another psoralen compound that, upon exposure to long-wavelength ultraviolet, binds to both DNA and RNA effectively inhibited mitosis after laser microirradiation of the centriolar region. These results suggest that an RNA with secondary structure in the pericentriolar region has a major role in the organization and function of the mitotic spindle. Recent ribonuclease digestion studies support this finding (*24*).

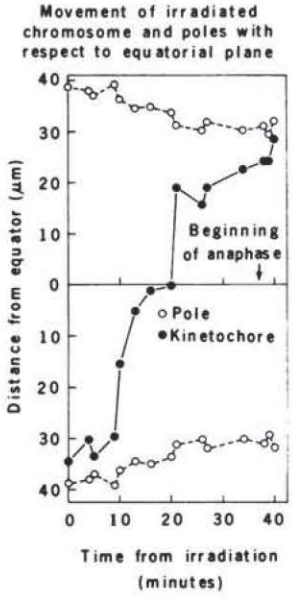
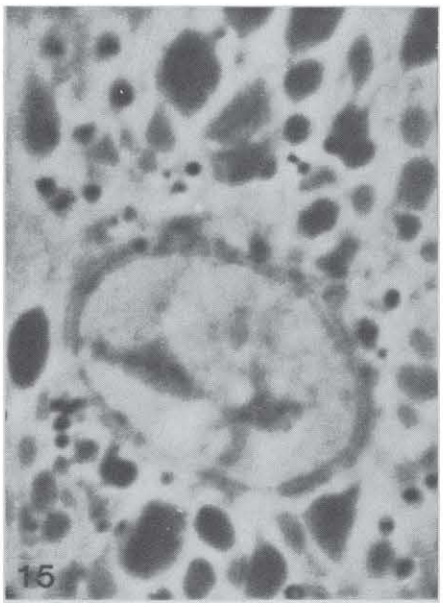


Fig. 14. Graphic depiction of the movement of a double-chromatid chromosome after laser irradiation of one kinetochore. In the diagram, the black circle represents the centromere with two kinetochores, one on each side. The kinetochore closest to the bottom pole was irradiated at time 0. The entire double-chromatid chromosome with only one functional kinetochore subsequently went through the movements depicted by the black circle in the figure. The rate of movement was equivalent to the normal rate of anaphase movement even though the chromosome mass was twice that of a single chromatid.

A final series of laser microbeam studies on the centriolar region involved selective destruction of the centriole proper without damage to the pericentriolar cloud (*25*). In these studies, the blue second harmonic wavelength of the YAG laser was used with acridine orange. The biophysical mechanism of damage production was most likely an uncommon physical effect because of the short exposure time (nanoseconds) and high power. It was demonstrated that cells with destroyed centrioles but intact pericentriolar material were capable of proceeding through mitosis in a normal fashion. The role of the centriole in mitosis remains debatable. Selective alteration of the centriolar region and computerized tracking of cells should elucidate centriolar replication and its relation to control of mitosis.

Kinetochores. The other major mitotic structure that participates in the organization of microtubules is the kinetochore. Using a very finely focused green laser beam, we have destroyed this region of the chromosome and then investigated the dynamics of chromosome movement. When both kinetochores of a metaphase double-chromatid chromosome are destroyed, the chromosome drifts about in the cell and the chromatids separate slightly from each other at the exact time that the rest of the chromosomes initiate their anaphase movements. This observation illustrates that the initial separation of chromatids at anaphase is not mediated by a microtubule force (*18*).



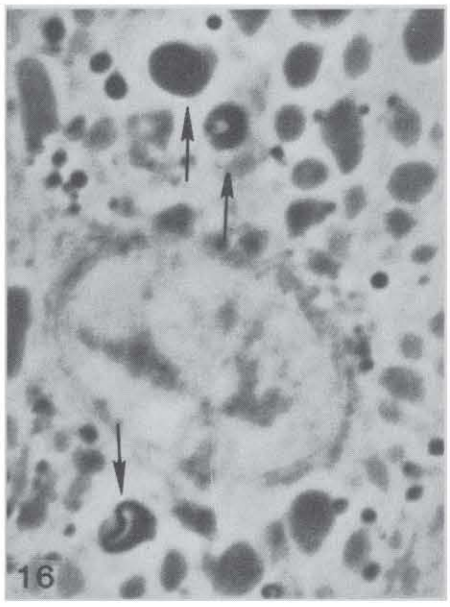
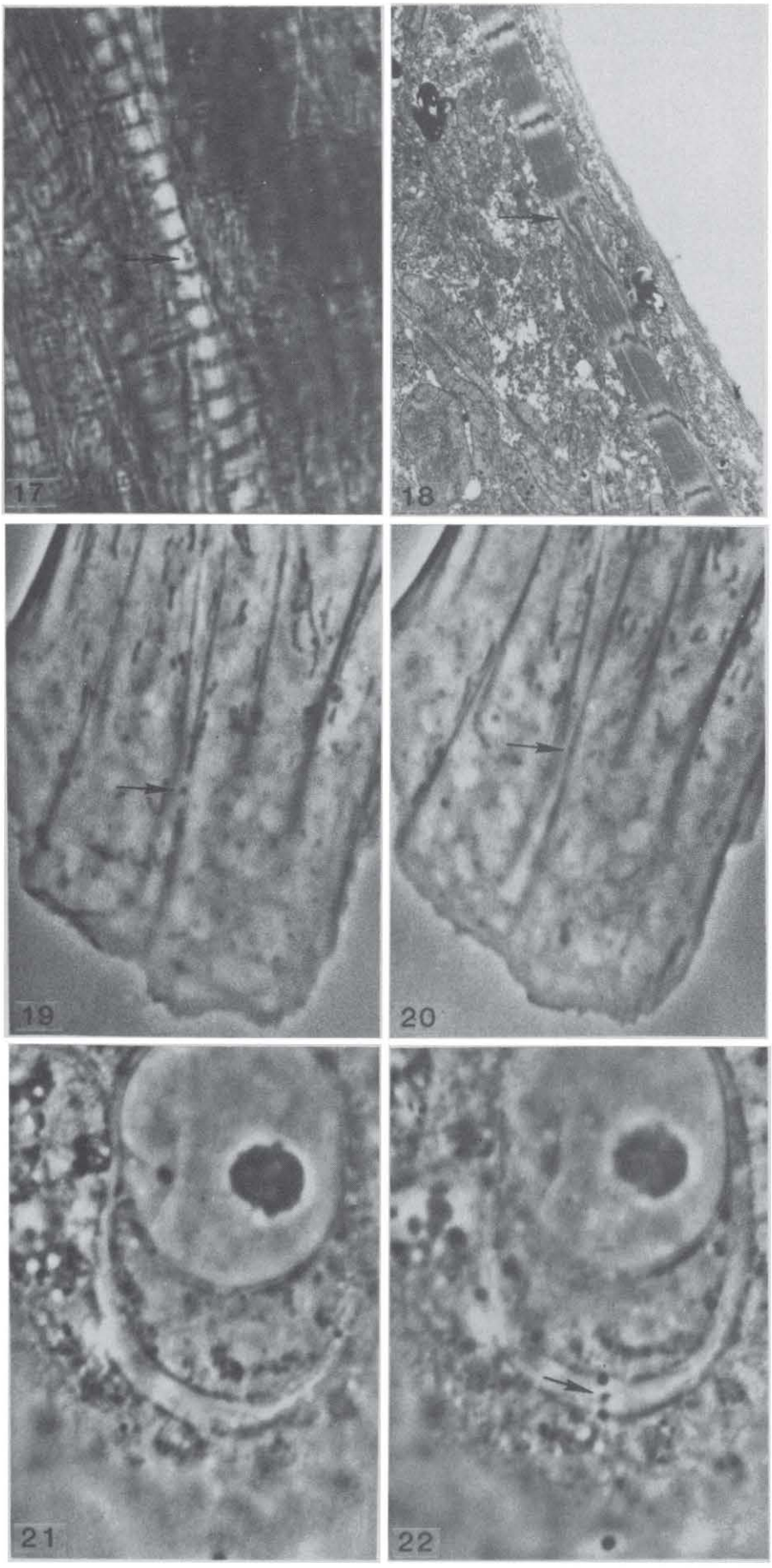


Fig. 15. Phase-contrast micrograph of myocardial cell with large mitochondria just before microirradiation with 514 nm of an argon laser. Fig. 16. The same cell after irradiation. The lesions have different degrees of severity (arrows indicate irradiated mitochondria).



In other studies, only one kinetochore was destroyed, and the chromosome with both chromatids and only one functional kinetochore was tracked (*26*) (Fig. 14). The results are quite dramatic and demonstrate that (i) two functional kinetochores are necessry for the alignment of a chromosome on the metaphase plate and for normal anaphase movement; (ii) bipolar tension on the kinetochore is necessary to stabilize the orientation of the chromosome on the metaphase plate; (iii) irradiation and inactivation of one kinetochore lead to nondisjunction of the irradiated chromosome; (iv) chromatids with irradiated kinetochores retain their ability to replicate but are unable to repair the damaged kinetochore region; and (v) within limits, the velocity with which a kinetochore moves is independent of the mass associated with it.

Microtubules. The microtubules were among the first mitotic structures successfully irradiated with the classical ultraviolet microbeam instruments (*27*). In our studies we have initiated microtubule studies with the laser microbeam on the highly visible dense band of microtubules in dividing fungal cells (*28*).

Of particular interest is the function of the dense microtubular band that extends between the two separating nuclei at the end of fungal mitosis (*29*). Earlier observations led to the hypothesis that this band of microtubules served to push the two nuclei apart. However, laser disruption of the bundle resulted in a threefold increase in rate of nuclear separation; 22.4 μm/min as opposed to 7.6 μm/min in control unirradiated cells (*28*). In addition, damage to the outside of the nucleus (distal to the bundle) resulted in a significant decrease (6.1 μm/min) in the rate of nuclear separation. These experiments indicate that the intranuclear band of microtubules is rate-limiting (slowing down the movement of the nuclei). Furthermore, it appears that the forces for nuclear separation may be coming from

Fig. 17. Polarization photomicrograph of myofibrillar region of contracting myocardial cell in vitro. A single A band (arrow) has been irradiated with the 532-nm beam of the YAG laser. Damage is localized within one A band. Fig. 18. Electron micrograph of myofibrillar network that has had one Z line (arrow) irradiated while the cell was in the living state. The damage to one Z line resulted in considerable disarray of the myofilaments in proximity to the irradiated Z line. Fig. 19. Phase-contrast micrograph of a single stress fiber of a rat endothelium that has been cut by one pulse of 532-nm light from the YAG laser microbeam. Fig. 20. Same cell 1 hour after irradiation. Note that there has been regeneration of the cut stress fiber. Fig. 21. Band of 100-Å filaments in a nonmuscle cell from a tertiary culture of neonatal rat heart. Fig. 22. Same cell with three laser lesions placed across the band of 100-Å filaments by the 532-nm beam of the YAG laser. This type of system has been used to study relative movement within the band.

the other side of the nuclei, where electron microscopy has revealed a substantial array of astral microtubules.

Cytoplasm

Mitochondria. The laser microbeam has been extensively applied to the subcellular disruption of single mitochondria (*30*). Much of this work has been conducted in contracting mammalian cardiac cells in culture and has had as its major aim elucidation of the factors regulating cardiac cell contractility. Morphologically distinct lesions can be placed in individual mitochondria and the subsequent contractile, electrical, and morphological responses of the cell can be analyzed (Figs. 15 and 16). Salet *et al.* (*31*) appear to have demonstrated that the laser light energy can be trapped and converted directly to adenosine triphosphate by the irradiated organelle; the irradiated cells also undergo a transient increase in beat rate. In other studies, cells have been impaled with microelectrodes prior to selective irradiation, and a distinct depolarization of the cell membrane has been demonstrated after irradiation of one mitochondrion. Only those cells with the classic "pacemaker" action potential (*32*) can be shown to enter a fibrillatory state after irradiation. The nonpacemaker cells exhibit a laser-induced depolarization but maintain normal electrical and contractile activity. In all of the irradiated cells, the cell membrane eventually returns to its normal membrane resting potential, thus suggesting that the laser effect on the cell membrane is transient, probably resulting in a temporary alteration of membrane permeability to specific ions. This kind of investigation permits precise alteration in cardiac cell contractility by producing a well-defined lesion at a predetermined subcellular site. Subsequent repair, recovery, and pharmacologic control of beat arrhythmia is thus studied in a precisely controlled situation.

Myofilaments, stress fibers, and 100-angstrom filaments. The laser microbeam can be used to study other motility-related cytoplasmic cell structures. For example, individual myofibers can be microirradiated at specific subfilament points. It is possible to damage a single Z line or A band in an actively contracting cell (*33*) and then analyze the changes in both contractile pattern and myofilament structure (Figs. 17 and 18).

The cytoplasmic stress fibers of cultured endothelial cells are amenable to selective microirradiation (*34*). It is possible to sever a single stress fiber and observe its repair and regeneration (Figs. 19 and 20). Selective alteration of a specific number of stress fibers at specific locations within the cytoplasm permit detailed studies on the role of these cytoskeletal elements in cell migration and cell shape changes.

Intracellular motility patterns have been studied by placing multiple 0.25-μm lesions in preselected regions of bands of 100-Å filaments and then examining the relative movement of the lesion sites with respect to each other (*33*) (Figs. 21 and 22).

Plant Cell Development—Chloroplast Irradiation

Though no detailed microbeam studies have been conducted on the chloroplasts in plant cells, the potential for such studies is great. Cells with large chloroplasts or multiple distinct chloroplasts would be particularly amenable to study. Entire chloroplasts, parts of a chloroplast, or specific ultrastructural elements of a chloroplast could be selectively damaged by appropriate matching of laser wavelength and chloroplast pigment.

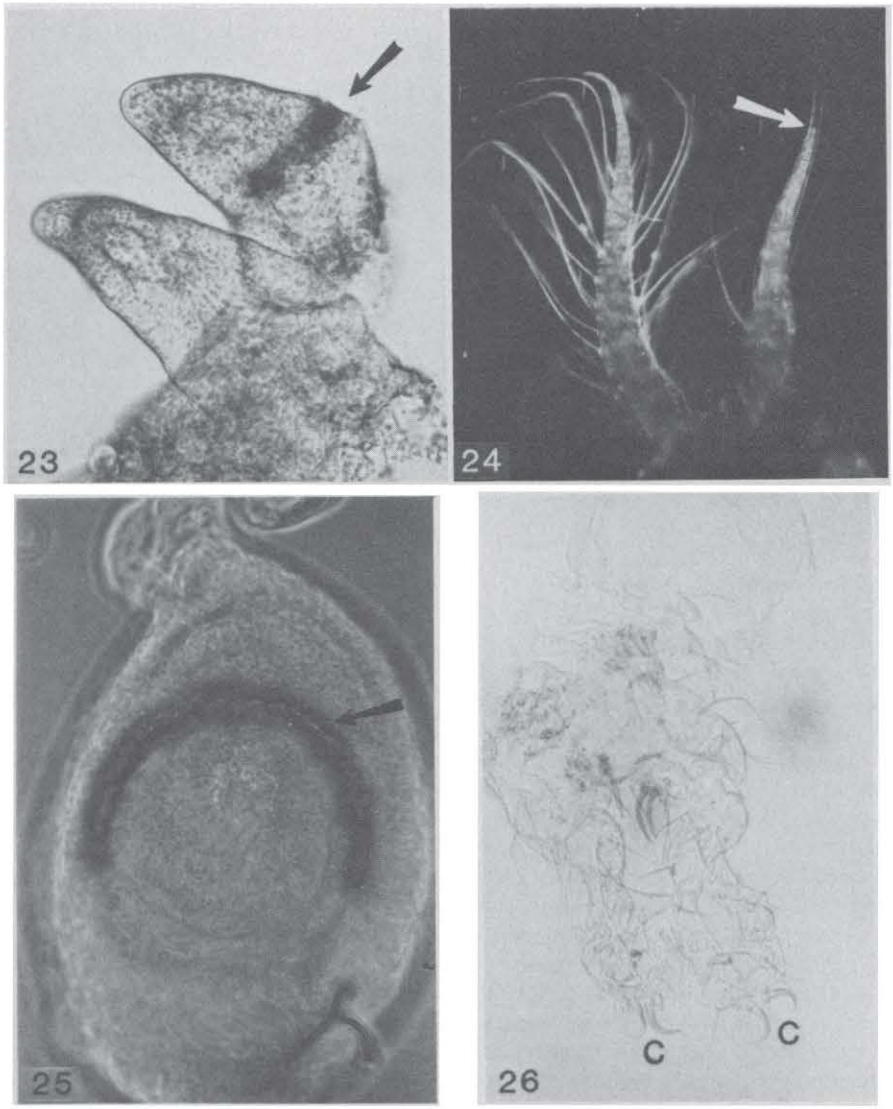


Fig. 23. Cricket embryo with embryonic rudiments of the cerci clearly visible at the rear of the animal. A band of cells approximately 25 μm in width has been killed with the 265-nm fourth harmonic of the YAG laser. The dead cells have been stained with trypan blue to illustrate the region of irradiation (arrow). (×170) Fig. 24. Cricket carried through to hatching with one cercus formed from a cercal rudiment that had been irradiated as indicated in Fig. 23. Note that the cercus developed from the irradiated embryonic rudiment is shorter and lacking in the fine hairs when compared to the cercus developed from the unirradiated cercal rudiment. Various degrees of final cercal differentiation occur depending upon the time, extent, and position of the irradiation. (×63) Fig. 25. Mesothoracic *Drosophila* imaginal disc after laser microirradiation at 265 nm in the lower tibia–upper tarsus region. The disc was stained by trypan blue immediately after irradiation to demonstrate the zone of dead cells corresponding to the lesion site (arrow). Fig. 26. The result of a prothoracic leg imaginal disc irradiated as in Fig. 25, implanted within the abdomen of an adult female for 10 days at 25°C, and then injected into a late third-instar larva for metamorphosis. Note the duplication of extra distal leg structures (claws), labeled *C*.

The efficient absorbance of the argon laser wavelengths (488 and 514 nm) by chloroplasts has been demonstrated in the green alga *Coleochaete* (*35*). In this alga, single cells in the developing multicellular thallus were destroyed by selective irradiation of the one large chloroplast in the cell.

A series of developmental studies was conducted in which a specific number of non-seta- (flagellum) bearing cells in the thallus were destroyed, and the subsequent mitotic and differentiative pattern of the thallus was studied (*35*). These studies revealed that mitosis could be stimulated in the thallus by merely reducing the number of cells in a given region. Mitosis apparently was stimulated when thallus cells were no longer contacted on all sides by other cells. The selective destruction of seta-bearing cells consistently resulted in new seta-bearing cells differentiating from non-seta-bearing cells, so that the number of these cells was always maintained. These studies demonstrated built-in self-regulatory developmental mechanisms for both seta cell differentiation and vegetative cell growth. It was possible to induce a differentiative process by selective removal of a specialized cell type (the seta cells).

Developmental Neurobiology

The laser microbeam is useful in developmental studies when precise destruction of specific cells or groups of cells in the embryo or larva is necessary. In an early study, the ruby laser microbeam was used to destroy the supraesophageal ganglion in spiders in order to analyze the altered web-building behavior (*36*). However, considerable microbeam work has been done on the nervous sytem of the nematode *Caenorhabditis elegans* (*37*). In these studies, specific cells in the embryonic or juvenile nervous systems were destroyed by laser microirradiation, and the subsequent nervous system development and behavior of the organism were analyzed.

In studies by Lors-Schardin *et al.* (*38*), the 257-nm wavelength of a frequency-doubled argon laser has been used to destroy selected regions of the developing *Drosophila* germ band and blastoderm. Up to 45 nuclei were destroyed with a 10- to 30-μm focused laser beam, and the subsequent defects were used to derive "defect maps." According to these investigators, the laser microbeam approach provides a more detailed and accurate developmental fate map than the earlier methods of lesion production did, perhaps because of the ability to selectively destroy a smaller group of cells in a specific target area. The ultraviolet laser system has also been used for studies of chromatin damage and repair (*39, 40*).

We have used the 265-nm fourth harmonic of the YAG laser and the 280-nm second harmonic of the YAG-pumped dye laser to study the development of a neurosensory system in the cricket (*41*). The hypothesis that pioneer fibers, which develop relatively early in the differentiation of insect appendages, serve to organize the peripheral sensory nerves was tested by ablating apical regions of the cercal rudiments in embryos of *Acheta domesticus*. Multiple nerve bundles, rather than the normal middorsal and midventral pair of nerves, were formed within the cercus after laser ablation of the cercal tip before pioneer fiber differentiation, but the cercal nerve was normal when lesions were made after formation of the pioneer fiber tracts and associated glia. These results indicate a necessary morphogenetic role for the pioneer fibers (Figs. 23 and 24).

Pattern Formation

The laser microbeam has been used to induce specific pattern abnormalities by the production of small areas of localized cell death in individual imaginal discs of *Drosophila* larvae. Specific regions of dissected discs were treated with the 265-nm fourth harmonic wavelength of a YAG laser to induce cell death. The effects were then analyzed by culture in vivo and induced metamorphosis to detect pattern duplications and triplications. The key feature of this system was the ability to confine effects of the laser treatment to selected regions of the discs. The irradiated discs were incubated in vitro for a short time after irradiation and then transplanted into the abdomens of host larvae, which were then observed through metamorphosis. This method has made it possible to determine the potential for pattern regulation of a small group of cells in situ, with a resolution much greater than in previous studies (Figs. 25 and 26).

Conclusion

Laser microbeam irradiation has already contributed to the resolution of specific problems in cell and developmental biology. In other investigations, laser microsurgery is just beginning to be applied, and the ultimate contributions of this approach have yet to be realized. A new approach to optical microscopy in which a high-sensitivity television system is combined with an image array processing computer appears to extend greatly the capabilities of laser microbeams and optical microscopy in general. A diffraction-limited focused laser beam could be used to stimulate spectral emissions (such as fluorescence, Raman spectra, resonance Raman spectra) in restricted regions of living cells. This should yield precise physical-chemical data on the structure and organization of the living cell.

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Biomass as a Source of Chemical Feedstocks: An Economic Evaluation

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Summary. It is suggested that the raw materials and technology exist for basing a major fraction of the U.S. chemical industry on four fermentation products, used in the proper portions: ethanol, isopropanol, *n*-butanol, and 2,3-butanediol. The primary route for introduction of these materials is dehydration of the alcohols and diols to olefins, which would cause little disruption of the existing industry downstream from the olefins. The proposed substitution has the advantages that it would provide a smooth transition toward renewable feedstocks, while decreasing dependence on fossil sources of organic material and use of toxic materials. However, to make these materials attractive as feedstocks or intermediates in chemical production, their current prices must be substantially reduced. Even with the optimum mix, their large-scale utilization will only occur at about 20 to 40 percent of their estimated chemical prices.

The chemical industry provides the synthetic materials used by industrial societies today. Its operations can be viewed as a continuum of chemical transformations, starting from basic raw materials and yielding finished products. The raw materials used currently by the industry are mostly hydrocarbons, derived from natural gas and crude oil, and synthesis gas, derived from coal. At present, the industry consumes a significant fraction of these commodities, and its consumption is expected to increase in the future.

Fermentation-derived fuels and chemicals have recently received much attention as a result of drastic price increases for oil and gas and forecasts of future shortages. Here we will not discuss biomass-derived fuels, but note that there is serious doubt about the adequacy of supply for this very large use. Instead, we will concentrate on biomass as an alternative source of chemical feedstocks. We believe that this is the more promising use of biomass, both because the supply of raw materials is unlikely to be limiting and because chemical feedstocks generally command a higher price than fuel. As a rule, the chemical value of a reactive compound is about three times its fuel value.

Our approach is to seek effective points of entry into the chemical industry, diagrammed in Fig. 1a. Shown in a schematic way is the progression from relatively low-cost raw materials at the left to much more valuable consumer products at the right. Figure 1b shows current production and prices for 14 of the largest volume intermediates at their approximate position in the progression. Greatly facilitating our task is the remarkable flexibility of the industry, which stems basically from the fact that there are many alternative routes to a given product. This has led the industry to a continuous search for the most economical feedstocks, and all we need to do is join the search process.

There are several good reasons for believing that biomass is an attractive source of organic materials on a long-term basis. Perhaps most important is the fact that biomass production can be sustained indefinitely in many different regions of the world and in both terrestrial and aquatic environments. For many countries then, and not least the United States, there is a prospect for decreased dependence on imports from expensive and frequently insecure sources. There are also potentially important social and ecological benefits.

1) Biomass production is relatively labor-intensive and can employ unskilled and semiskilled workers, who are currently in surplus, not only in developing countries but also in many industrialized ones.

2) Producing facilities would tend to be small relative to petrochemical complexes and could be dispersed, at least to some extent, according to social needs.

3) In addition to conserving nonrenewable resources, use of biomass would have ecological benefits. For example, carbon dioxide is abstracted from the atmosphere through photosynthesis, heat release to the environment is reduced, and formation of toxicants such as benzene can frequently be eliminated from process sequences.

4) It may be possible to upgrade municipal, industrial, and agricultural wastes, which can no longer be discarded without expensive processing; their negative economic value can have a significant effect on process costs.

These benefits are particularly important because, as we will see, the strictly economic advantages of biomass-based processes are still doubtful.

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