Stress Response in *Caenorhabditis elegans* Caused by Optical Tweezers: Wavelength, Power, and Time Dependence

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ABSTRACT Optical tweezers have emerged as a powerful technique for micromanipulation of living cells. Although the technique often has been claimed to be nonintrusive, evidence has appeared that this is not always the case. This work presents evidence that near-infrared continuous-wave laser light from optical tweezers can produce stress in *Caenorhabditis elegans*. A transgenic strain of *C. elegans*, carrying an integrated heat-shock-responsive reporter gene, has been exposed to laser light under a variety of illumination conditions. It was found that gene expression was most often induced by light of 760 nm, and least by 810 nm. The stress response increased with laser power and irradiation time. At 810 nm, significant gene expression could be observed at 360 mW of illumination, which is more than one order of magnitude above that normally used in optical tweezers. In the 700–760-nm range, the results show that the stress response is caused by photochemical processes, whereas at 810 nm, it mainly has a photothermal origin. These results give further evidence that the 700–760-nm wavelength region is unsuitable for optical tweezers and suggest that work at 810 nm at normal laser powers does not cause stress at the cellular level.

INTRODUCTION

Optical tweezers, also often referred to either as laser tweezers or the single-gradient optical trap, are increasingly used for noninvasive micromanipulation of living cells (Berns et al., 1992; Ashkin, 1997; Berns et al., 1998). An intense light gradient near the focal region of a near-infrared (NIR) continuous-wave (cw) laser beam gives rise to forces that make possible optical trapping and manipulation of a variety of micron-sized objects, including cells and organelles (Ashkin et al., 1987; Svoboda and Block, 1994; Greulich and Pilarczyk, 1998; Greulich, 1999).

Trapping of smaller objects, e.g., polystyrene beads or *Escherichia coli*, can be made with the light from a weak (a few milliwatts) HeNe laser whereas trapping of larger or irregularly shaped object often requires somewhat (although not exceptionally) higher laser powers. If trapping is done intracellularly or in the interior of living organisms considerably higher laser powers (many hundreds of milliwatts) are needed for successful optical micromanipulation due to the high viscous resistance of the cytoplasm or the extracellular matrix. High laser powers are also needed when forces in biological systems are to be measured by optical tweezers.

In many of these situations, there is a potential risk that the high laser powers used can affect the object under study, e.g., by inducing stress-response reactions. It is therefore of importance to assess the effects of NIR cw laser light on various types of biological systems. This work constitutes a contribution to the ongoing work regarding this by a study of cellular stress in a particular strain of *Caenorhabditis elegans*.

Stress responses in cells are often not visible by a direct microscopic observation, nor is cell viability easily defined in terms of a single physiological or morphological parameter. A certain transgenic strain of *C. elegans* (PC72) has previously been used as a sensitive biomonitor responsive to various external types of stress (Candido and Jones, 1996; Jones and Candido, 1999). This particular strain carries a reporter gene (*E. coli lacZ*) that is under the transcriptional control of a specific heat shock promoter. Under conditions of stress, induced, for example, by microwaves (Daniells et al., 1998), metal ions (Dennis et al., 1997), fungicides (Guven et al., 1999), immunological attack (Nowell et al., 1999), or soil and water pollution (Power et al., 1998), the gene promoter activates the transcription of *lacZ* leading to the production of β-galactosidase protein (β-gal), which can be readily detected in situ by histochemical staining (Candido et al., 1989; Stringham et al., 1992; Fire, 1992). We have, in this work, used this particular strain of *C. elegans* to monitor stress induced by the NIR cw laser light employed by optical tweezers to investigate the potential risks of using the optical tweezers technique in biology in general and to *C. elegans* in particular. This work thus constitutes a more direct monitoring of the influence of harmful effects of NIR cw light from optical tweezers on a living organism than just a life-death investigation, e.g., as previously has been performed by König et al. (1996).

MATERIALS AND METHODS

C. elegans and stress induction

The nematode *C. elegans*, which is ~1 mm in length and nearly transparent, is a common model system for a wide range of developmental studies

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worldwide (Brenner, 1974; Sulston and Horvitz, 1977; Riddle et al., 1997; The *C. elegans* Sequencing Consortium, 1998). A temperature increase to 29°C induces the synthesis of several heat-shock proteins in *C. elegans* whereas the synthesis of most other proteins present before the heat shock is suppressed (Snutch and Baillie, 1983). Although not all heat-shock proteins are stress inducible, the four small 16-kDa heat-shock proteins (hsp16) in *C. elegans,* which are coupled to a heat-shock promoter, are induced and expressed only under stress conditions (Russnak et al., 1983; Stringham and Candido, 1993). The *hsp16* promoter therefore provides a reliable and efficient means to detect the effects of stress on cells in general, and by laser light in particular, in this animal model system (Stringham and Candido, 1993).

The strain of *C. elegans* used in this work (PC72) has the *hsp16* promoter coupled to a reporter gene (*E. coli lacZ*) that leads to the production of β-gal, which can be readily detected in situ by histochemical staining (Candido et al., 1989; Stringham et al., 1992; Fire, 1992). This particular strain of *C. elegans* was kindly provided by Eve G. Stringham and is described in more detail by Stringham et al. (1992).

Handling and mounting

C. elegans worms were maintained at room temperature (~21°C) on nematode growth medium (NGM) agar plates with *E. coli* strain OP50 as a food source (Lewis and Fleming, 1995).

Sodium azide has previously been used as anesthetic for work with *C. elegans*. It was found in this work, however, that this substance gave rise to uncontrolled gene expression (i.e., staining) under certain conditions (in illuminated as well as in reference animals). The animals were therefore instead anesthetized with levamisole (L[-]-2,3,5,6-tetrahydro-6-phenylimidazo{2,1-b}thiazole; Sigma-Aldrich, Milwaukee, WI), and 4 μl of 0.5 mM levamisole in M9 buffer (Kimble, 1998) was placed onto a 0.5-mm layer of 3.0% agar noble (Difco Laboratories, Detroit, MI) flattened out on a microscope slide.

Individual animals (mainly L2 to L4 larval stages or young adults) were selected and transferred to this liquid drop, using a thin brush slightly moistened with pure water (W 3500; Sigma-Aldrich). This allowed rapid and gentle transfer of the worms. The sample was then covered by a thin microscope coverslip and transferred onto the microscope stage for laser irradiation.

Although only animals that had stopped moving were selected for irradiation, it was found that they were not completely immobilized by the anesthetic. Sudden body movements in otherwise calm animals could sometimes be triggered by the laser light itself, especially when higher laser powers were applied. The animals could therefore occasionally move or roll around its body axis while being irradiated. This was more likely to happen during longer irradiation times (up to several minutes). These movements were assumed to be initiated by the animal's thermosensory system (Mori and Ohshima, 1997). The irradiation of such an animal was then temporarily interrupted until the animal had ceased moving.

Animals could be recovered even after prolonged exposure to levamisole, and the majority survived. No staining was observed in animals mounted in levamisole but not irradiated, indicating that all expression of the transgene was laser induced.

Optical tweezers setup

Cells were irradiated with an argon ion laser-pumped titanium-sapphire laser (model 2060–10SAH and 3900S, respectively; Spectra-Physics, Mountain View, CA) with a tuning range from 675 to 980 nm and a maximum power of 2.3 W. The expanded laser beam (in TEM_{00} mode) was directed into an inverted microscope (Olympus IX 70) and focused to a diffraction-limited spot in the specimen plane by a high numerical aperture (NA) microscope objective (Ultra-plan 100/NA 1.35). The setup was the same as that previously described for the dual-trap optical tweezers system (Fällman and Axner, 1997) with the exception that the polarizing beam-splitting cube and thereby one of the arms were not applied. The object was moved in the object plane relative to the laser focus by a motor-driven scanning stage (Scan IM 100 × 100; Märzhäuser, Wetzlar, Germany). The microscope was combined with an image processor and a video camera (Argus-20 and C2400–75i; Hamamatsu, Hamamatsu-City, Japan), which facilitated the identification of cells in the nematode by increasing the microscope's effective sensitivity and resolution. The experiments were documented by microphotography.

TABLE 1 Characteristics of the focused laser beam and the microscope objective

λ (nm)	Transmission (%)	w_0 (μm)	I at 100 mW (MW/cm^2)	Absorption (cm^{-1})	ΔT (K/100 mW)
700	67	0.20	77	0.0067	0.19
760	57	0.22	66	0.0286	0.79
810	48	0.24	58	0.0219	0.60
850	48	0.25	53	0.042	1.15
1064	—	0.31	33	0.143	3.84

λ, Wavelength of the laser light; transmission, the percentage of light transmitted through the microscope objective; w_0, beam radius at the focal point; I, the mean intensity within the diffraction-limited spot, i.e. for beam radius $\leq w_0$; absorption, the absorption coefficient of light in water (Kou et al., 1983); ΔT; calculated temperature rise due to illumination of 100 mW of Gaussian-shaped laser radiation.

Irradiation level determination

To assess correctly any potentially harmful conditions for the optical tweezers technique, it is of importance to determine with a high degree of accuracy the laser power to which to the objects under study are exposed. It is, however, nontrivial to determine the amount of light that exits a high-NA microscope objective. The main reason for this is the high divergence of the light that results when objectives with high NA (especially those exceeding unity) are being used. The amount of light to which the animals were exposed was therefore calculated as the product of the laser power before the objective, the proportion of laser power that passes the entrance pupil of the objective, and the objective transmission. The former was measured with a cw-laser power meter (model 407A; Spectra-Physics) whereas the other two were determined by a new technique for measurement of the transmission of objectives that was recently developed by Fällman and Axner (manuscript in preparation). This technique includes, among other things, the construction of a dummy objective with an aperture of the same size as the entrance pupil of the objective. As is shown in Table 1, it was found that the objective transmission for the particular objective used in this work varied between 48% and 67% in the 700–850-nm wavelength region. In the wavelength-dependence studies made, the power of the laser system was therefore adjusted for each wavelength so that the animals were irradiated with an accurately determined and constant laser power. This implied in practice, for the experiments in which the animals were exposed to a power of 360 mW in the specimen plane (see below), that 520, 615, and 750 mW of laser light were passed through the dummy aperture (and thereby the entrance pupil of the microscope objective) for the wavelengths 700, 760, and above 800 nm, respectively. Measurements of the laser light wavelength were made by a laser wavelength meter (model LWM-6500B with an OMH-6370B measurement head; ILX Lightwave, Bozeman, MT).

Modes of illumination

As is presented in detail below, a study of the influence of irradiation time and wavelengths (for a given specimen illumination) on gene expression was performed at four different wavelengths (700, 760, 810, and 850 nm), whereas a more detailed study of the influence of laser power and irradi-

ation time was made for the wavelength for which the lowest frequency of gene expressed was expressed, i.e., 810 nm. The irradiation time was controlled by an external trigger to an electronic shutter driver system with internal timer (model SDT 16560; JML Optical Industries, Rochester, NY).

Before the experiments were initiated, the quality of the optical trapping, i.e., the beam alignment, was tested by moving 3-μm latex beads in the specimen (x-y) plane as well as in the axial (z) direction using external optics described previously (Fällman and Axner, 1997).

Laser heat-shock application and *C. elegans* treatment

The laser radiation was preferentially focused on the relative large excretory cell near the pharynx. When the laser radiation was focused on this cell, its nucleus was drawn into the center of the laser focus volume where it became trapped. The nucleus could be slightly moved around in the cytoplasm by the optical tweezers. The exact location of the laser beam could therefore be determined by observation of the position and movement of the nucleus. The depth of the focal region of the optical tweezers varied from measurement to measurement because of variations of the position of the animal as well as the position of the excretory cell within the animal, but was estimated to be around 10-μm. No visible sign of damage was observed by optical trapping of the cell nucleus.

Following exposure, the worms were subsequently removed from the paralytic mount and placed on NGM petri dishes with separate thinly spread out areas of *E. coli* OP50. The animals were allowed to recover for 1–4 h and were then transferred with a 32-gauge platinum wire pick into a small droplet of M9 buffer on diagnostic microscope slides with numbered, separate chambers with diameter 6 mm (Menzel, Braunschweig, Germany). This allowed us to follow, analyze, and identify individual animals throughout the process of laser heat shock, recovery, and subsequent staining for detection of reporter gene expression.

Positive and negative control animals were mounted in the same way as described above. They were then either heat shocked at 37°C for 1–2 h and not subjected to laser radiation or not exposed to any heat-shock treatment at all.

Fixation and staining

After recovery, the specimens were cryofixed by bringing them rapidly in contact with a cold aluminum block precooled to ~−76°C with solid carbon dioxide. The animals were then dried for several hours in vacuum. The freeze-dried animals were subsequently permeabilized in cold acetone and assayed overnight for β-gal activity in a humidified chamber in the dark at an incubation temperature of 37°C by applying the indole derivative X-gal (5-bromo-4-chloro-3-indolyl-β-D-galactoside) as perceptible substrate, which gives rise to a bright blue color (Fire, 1992).

Calculation of the temperature increase due to heating by cw laser light

To correlate the measurement to the expected behavior of a pure photothermal process, a calculation of the heating of the irradiated cell by the cw laser light was made.

Liu et al. (1995) have shown that a thermal equilibrium will be attained in the laser focal volume within the first 10 s. Because the laser irradiation was applied continuously and for rather long periods (30–240 s), it was assumed that a steady-state situation prevailed in our experiments. The temperature of the irradiated cell will therefore depend on a balance between the amount of energy absorbed and the flow of heat into the surroundings.

The amount of energy absorbed can depend on both the intracellular absorption of the sample and the absorption of water. Because most proteins and DNA show weak absorbance in the red to NIR range (600–1200 nm) most cells do the same. The heat absorbance of the system is therefore presumably dominated by that of water. The flow of heat into the surrounding is likewise assumed to be mainly given by the thermal conductivity of water.

This implies that the steady-state temperature distribution in the laser focal volume can be calculated from the time-independent heat equation:

$$\nabla(k\nabla T) - h = 0, \tag{1}$$

where k is the thermal conductivity of water, 0.6 W m^{-1} K^{-1}, and h the applied heat (in units of W m^{-3}), in our case given by the wavelength-dependent absorption of laser light in water (Kou et al., 1993).

Simulations were performed by the finite element method using the programming language FlexPDE Lite (PDE Solutions, Antioch, CA). The simulations were based on the assumption that the incoming laser light had a Gaussian intensity distribution and that the beam propagation follows the theory of Gaussian beams (Milonni and Eberly, 1988). An accurate calculation of the temperature increase in the closest proximity to the focal region of optical tweezers requires in general knowledge about the amount of spherical aberration at the particular depth used. Because such information is far from trivial to obtain (E. Fällman and O. Axner, submitted for publication), for the estimate of the temperature in the focal region we have, in this work, simply assumed that the light is being focused to a diffraction-limited spot. Because the temperature increase is largest for the most tightly focused conditions, such a calculation will provide an upper limit of the temperature increase. Moreover, preliminary investigations of spherical aberration (E. Fällman and O. Axner, submitted for publication) and the temperature distribution in laser focal volumes in water (Fällman and Axner, in preparation) have shown that the temperature increase in the focal region of a laser beam subjected to the amount of spherical aberration that occurs at a focal depth of 10 μm is not severely affected by the spherical aberration phenomenon. This implies that the temperature calculations performed still are expected to be fairly accurate.

The radius of the diffraction-limited spot from beam with a Gaussian intensity distribution, w_0 is related to the divergence angle of the light beam, θ, by the wavelength of the light, λ, through the relation:

$$w_0 = \frac{\lambda}{\pi\theta} \tag{2}$$

Because the divergence angle of the light beam can be related to the numerical aperture, *NA,* and the index of refraction of the surrounding media, n, according to:

$$NA = n\sin(\theta), \tag{3}$$

for an optical tweezers instrumentation, the diffraction-limited spot radius is given by the expression:

$$w_0 = \frac{\lambda}{\pi \sin^{-1}\left(\frac{NA}{n}\right)} \tag{4}$$

To not be affected by spherical aberration effects (E. Fällman and O. Axner, submitted for publication), the focal spot was assumed to be positioned at the intersection between the cover glass and the water in the simulations. The simulations assumed that the index of refraction of the glass, n, is 1.522 and that $NA = 1.35$. It was assumed that all absorbed light is transferred to heat. The actual absorption coefficients used are given in Table 1.

The simulations show that the temperature will rise between 0.2°C and 1.15°C per 100 mW of laser light in the 700–850-nm wavelength range (see Table 1). To facilitate comparison with previously published work, much of which has reported the effects of light with a wavelength of 1064 nm, the simulated temperature rise at 1064 nm was also calculated and

TABLE 2 Gene expression (percent) as a function of irradiation time and wavelength at a laser power of 360 mW

Irradiation time (s)	Wavelength (nm)			
	700	760	810	850
30	25 (17)	56 (20)	—*	—*
60	49 (18)	87 (14)	—*	20 (18)
120	50 (20)	100 (0)	4 (8)	39 (23)
240	60 (19)	100 (0)	37 (22)	95 (11)

The values within parentheses represent a 95% confidence interval calculated according to Eq. 5.

*No gene expression was detected for irradiatin times of 30 s at wavelengths above 800 nm or for 60 s at 810 m in a pre-study. Those conditions were therefore not included in the final study (whose results are presented here).

TABLE 3 Gene expression (percent) as a function of irradiation time and laser power at a laser wavelength of 810 nm

Irradiation time (s)	Laser power (mW)		
	240	360	480
60	—*	—*	0 (0)
120	5 (10)	4 (8)	41 (23)
240	0 (0)	37 (22)	91 (17)

The values within parentheses represent a 95% confidence interval calculated according to Eq. 5.

*No gene expression was detected for irradiation times of 60 s at a wavelength of 810 nm in a pre-study; therefore, only the highest irradiation condition (480 mW) was included in the final study.

included in the Table. Because the absorption of water increases with wavelength (although with a shallow local minimum around 810 nm), the highest temperatures will be obtained for the longest wavelengths and the lowest for the shortest. Moreover, because an energy balance determines the temperature rise, the temperature will increase linearly with applied laser power.

A comparison with previous temperature calculations shows that our calculations of the temperature rise (corrected for the different absorption values of water at the various wavelength used) are in excellent agreement (within 5%) with those of Liu et al. (1995), which were performed for a wavelength of 1064 nm and evaluated at a position in the peripheral of the laser focal volume. The calculation of Liu et al. is, in turn, in reasonable agreement with measurements performed using the fluorescent dye Laurdan as a probe of the physical state of a thermosensitive phospholipid (Liu et al., 1995).

RESULTS

The expression of the *lacZ*-reporter gene was characterized after illumination of the excretory cell in individual animals as a function of laser power, irradiation time, and laser wavelength. The proportion of animals that showed gene expression in at least one cell in the illuminated region was used as a measure of the stress response. It was found that this proportion varied significantly with both wavelength and irradiation time.

Table 2 shows four sets of measurements, representing the data from four different wavelengths (700, 760, 810, and 850 nm) for a variety of irradiation times (30 s to 4 min) for a fixed power in the specimen plane (360 mW). With the exception for those situations that gave rise to gene expression in 100% of the irradiated animals, the entries in the table are based upon an average of 22 animals (ranging between 18 and 31). The values within parentheses represent a 95% confidence interval for a binomial distribution, calculated as:

$$1.96\sqrt{\frac{p(1-p)}{n}}, \tag{5}$$

where p is the proportion of animals showing gene expression and n is the number of animals studied (Mendenhall and Sincich, 1992).

It was found that the highest frequency of induction of the *hsp16-lacZ* transgene and subsequent β-gal expression was observed for 760-nm laser radiation, followed by 700 nm. These two wavelengths were the only ones for which gene expression could be observed after 30 s of illumination of 360 mW of laser light. Furthermore, all of the animals that were irradiated with 760-nm light for 120 s (or more) expressed *lacZ*. The lowest frequency of expression was observed for 810-nm light.

Table 3 shows the frequency of reporter gene induction for three different laser powers (240, 360, and 480 mW) at the wavelength that showed the least induction of gene expression (i.e., 810 nm) for a variety of illumination times (1–4 min). It can be concluded that virtually no animals expressed *lacZ* at powers below 240 mW at this wavelength, not even for the longest illumination times. It is here of importance to note that 240 mW is a power that is more than 10 times higher than that required to manipulate free micron-sized objects by the optical tweezers. An increase of the power to 360 mW resulted, however, in a significant frequency of gene expression for the longest illumination period (240 s); around one third of the animals (37%) expressed *lacZ*. A final increase of the laser power to 480 mW gave rise to a significant increase in the proportion of animals expressing *lacZ*. At the longest illumination time (240 s), almost all animals (>90%) showed gene expression.

DISCUSSION

Induction of heat-shock-responsive gene expression in *C. elegans* by laser light

These results show indisputably that a few hundred milliwatts of NIR cw light from optical tweezers can induce gene expression in transgenic strains of *C. elegans* that carry a heat-shock-responsive *hsp16-lacZ* transgene. Because it has previously been demonstrated that the expression of the *hsp16* genes is uniquely triggered by a stress response (Russnak et al., 1983; Jones and Candido, 1999; Link et al.,

1999) these findings indicate clearly that light from optical tweezers can induce stress in *C. elegans*.

Stringham and Candido (1993) have shown that the *hsp16* genes in *C. elegans* can be expressed by a stress response by pulsed ultraviolet laser light. The activation of the *hsp16-lacZ* transgene in this work has thus shown that a stress response in *C. elegans* can also be induced by NIR cw laser radiation.

Possible causes of stress

It is not a priori clear which type of stress causes the *hsp16-lacZ* gene expression under laser light illumination. A common feature of agents that induce stress response is thought to be their ability to denature proteins (Hightower, 1980; Ananthan et al., 1986; Parsell and Lindquist, 1993; Stringham and Candido, 1993; Feder and Hofmann, 1999; Cotto and Morimoto, 1999). Laser irradiation might act to damage proteins directly by heating, or indirectly by generation of free radicals (which can give rise to oxidative damage). It is clear that cw laser light from optical tweezers can generate light intensities in the tens of megawatts per square centimeter range due to diffraction-limited focusing (see Table 1). Such high light intensities can cause a significant temperature increase (Liu et al., 1995) and give rise to harmful photochemically induced processes (Vorobjev et al., 1993).

Photothermal versus photochemical effects

It has previously been shown that the expression of the *hsp16-lacZ* transgene has a temperature dependence (Stringham et al., 1992). The activation temperature of the *hsp16* promoter is between 29°C and 31°C, with a stable expression at 33°C. Because the background temperature at the microscope stage in our setup is close to 25°C (mainly originating from heating by the microscope objective, which in turn is heated by the light from the microscope illumination), it can be estimated that a temperature rise in the target cell of a few degrees (~4–6°C) would be required for an activation of the *hsp* promoter by the photothermal effect.

The calculations presented in Table 1 show that the expected temperature increase for an illumination of 360 mW in the 700–850-nm region ranges between 0.7°C and 4.1°C, with the highest temperatures for the longest wavelengths and the lowest for the shortest. The calculated temperature rises are falling slightly short of those required for activation of the *hsp16* promoter. These calculations do therefore not give any direct and unambiguous evidence that the gene expression observed in our experiments is caused by a photothermal effect. As discussed below, however, they suggest that it is unlikely that photothermal effects account for the gene expression observed in animals exposed to 360 mW of the shortest wavelengths (below 800 nm), whereas they do not rule out the possibility for longer wavelengths (above 800 nm).

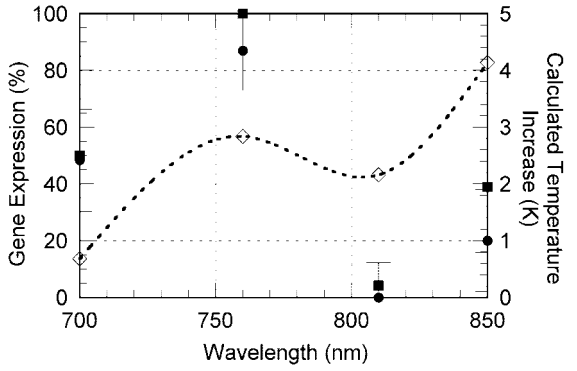

FIGURE 1 A comparison between the gene expression and calculated temperature increase as function of wavelength. The gene expression (*left axis*) is plotted from data in Table 2 for a laser power of 360 mW (measured in the specimen plane) for two different irradiation times: 120 s (●) and 240 s (■). The calculated temperature increase (◇, *right axis*) refers to the same laser power. The error bars represent a 95% confidence interval calculated according to Eq. 5.

Fig. 1 shows the proportion of animals expressing *lacZ* plotted against wavelength (solid markers and the left axis). The calculated steady-state temperature increase due to absorption of light by water has been inserted in the same figure (open diamonds and right axis). It is evident from the figure that there is a poor agreement between the proportion of animals expressing *lacZ* and the calculated temperature rise.

A scrutiny of the gene expression data and the calculated temperature increases at different wavelengths shows that the laser-induced gene expression at the two lowest wavelengths investigated (i.e., 700 and 760 nm) is unlikely to be explained solely by a photothermal effect. The clearest evidence for this is that the reporter gene expression shows a pronounced maximum at 760 nm. There is, for example, a higher gene expression at 760 nm than at 850 nm. The calculations show, however, that the increase in temperature is significantly lower at 760 nm than at 850 nm. This indicates that the gene expression at 760 nm is not predominantly caused by photothermal effects.

Furthermore, the temperature rise predicted for irradiation at 700 nm is expected to be considerably less than that necessary for activation of the heat-shock promoter. The temperature rise at 700 nm is in fact predicted to be less than that at 810 nm. Because the frequency of gene expression is considerably larger at 700 nm than at 810 nm, the data suggest that the gene expression observed at 700 nm also does not have a photothermal origin. These results therefore suggest that the stress response in the 700–760-nm region is predominantly caused by a photochemical effect.

These results are in agreement with other studies that show that 760-nm cw light can cause significant cell dam-

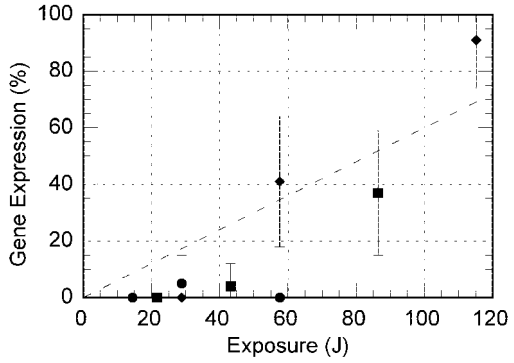

FIGURE 2 Gene expression as function of exposure (defined as laser power times irradiation time) for irradiation by 810-nm light. The data are taken from Table 3 and represent three different laser powers: 240, 360, and 480 mW, (●, ■, and ♦, respectively). The straight line is the best linear fit to the data that passes origin. The error bars represent a 95% confidence interval calculated according to Eq. 5.

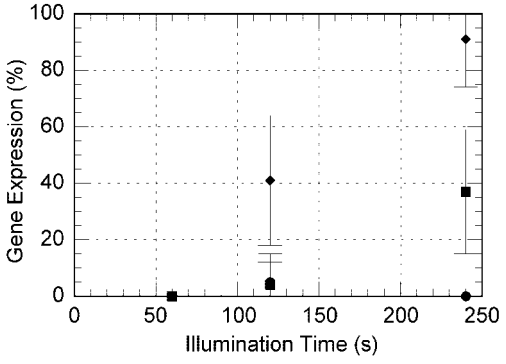

FIGURE 3 Gene expression as function of illumination time for irradiation by 810-nm light. The data are taken from Table 3 and represent three different laser powers: 240, 360, and 480 mW (●, ■, and ♦, respectively). The error bars represent a 95% confidence interval calculated according to Eq. 5.

age (Vorobjev et al., 1993; König et al., 1997; Liang et al., 1997). They also support the previous finding that optical tweezers employing light in the 760-nm region can cause damage to cells and that this damage results from photochemical effects (Neumann et al., 1999). It is not possible, however, to determine the exact nature of the damage from the work presented in this paper.

For damage caused by a photochemical effect, it seems reasonable to assume that the proportion of animals expressing *lacZ* would increase with increasing photochemical damage. It is also likely that the amount of photochemical damage at a given wavelength increases with exposure (i.e., the total number of photons to which the cell is exposed because each photon has the same probability of inducing a photochemical damage) and, further, that the damage would be the same for a given exposure irrespective of the time over which the exposure occurs (assuming that no protein-repairing mechanism takes place in the cell during the time of illumination). Thus, for a given wavelength, if the damage were solely of a photochemical origin, the proportion of animals showing expression would be the same for animals receiving the same exposure. The data presented in Fig. 2, however, show that at 810 nm this is not the case. In this figure the proportion of animals showing expression is plotted against exposure. If the stress were due to a photochemical process, the data would line up on a common line. On the other hand, if it were due to a photothermal process, the exposures made with the highest intensity would consistently give rise to higher frequencies of gene expression than those made by lower-intensity light.

The best linear fit that passes the origin has been inserted as a dashed line in the figure. The figure shows that the agreement between the data and the fitted line is poor. The only two data points lying above the fit originate from exposures in which the highest intensity has been used. Furthermore, the two different modes of illumination giving rise to an exposure of 58 J give rise to significantly different frequencies of gene expression, 40% for an illumination of 480 mW for 120 s and 0% for 240-mW irradiation for 240 s, respectively. The fact that illumination with a high laser power gives rise to a significantly higher gene expression than with a low power (for a given total exposure) indicates that the gene expression at 810 nm cannot be predominantly of a photochemical origin. This observation suggests instead that at this wavelength the gene expression is caused mainly by a photothermal effect, e.g., by light absorption by water.

For situations in which the stress is induced by photothermal effects, certain predictions can be made about the way in which the proportion of animals that show gene expression should vary with laser power and illumination time. For low laser powers, it is expected that no photothermally induced stress will occur, irrespective of the illumination time (the laser power is not sufficient to increase the temperature to the activation temperature of the promoter). As the laser power is increased above a certain level (i.e., for powers that bring the cell temperature up to the region in which the gene transcription starts), the frequency of gene expression is expected to increase with both laser power and illumination time. These qualitative behaviors correlate well with the data taken at 810 nm, as can be seen from Fig. 3, which displays the proportion of animals showing gene expression as a function of illumination time for three different laser powers (data taken from Table 3). Although Fig. 3 does not give any indisputable proof that the laser-induced stress at 810 nm has a photothermal origin, the general form of the three sets of data agree with what is expected from a thermally induced gene expression: no laser-induced stress at low laser powers (240 mW), irrespective of the illumination time, and a gene expression that increases with both laser power and illumination time for higher laser powers on a time scale that is similar to that of

pure thermally induced heat shock (i.e., a few minutes). This is again in contrast to the time dependence of the gene expression at 760 nm, which shows a significant expression (56%) already after 30 s.

To investigate further whether the gene expression observed at 810 nm might be of purely a photothermal origin, we have measured the length of time required to induce a heat-shock response in simple temperature-shift experiments (i.e., in the absence of laser irradiation). When whole worms were placed on preheated agar plates for given amounts of time, it was found that whereas all worms placed at 29°C for 10 min showed evidence of *lacZ* induction, none did so after just 5 min at 29°C. In contrast, in worms irradiated with 360 mW of 810-nm light (which, assuming absorption by water, is calculated to give rise to a temperature rise of just 2°C, i.e., to a temperature of ~28°C) *lacZ* expression was sometimes observed after only 2 min. This result indicates either that light of 810 nm results in a photochemical stimulus that lowers the threshold for heat-induced gene expression or that the temperature rise at 810 nm is actually greater than 2°C because substances other than water can absorb light of this wavelength. It is noteworthy in this respect that whole worms placed at 32°C begin to induce lacZ expression after just 90 s. Thus, if absorption of light in our experiments is more efficient than that calculated, the actual temperature rise could be sufficient alone to induce a heat shock.

Similar arguments can be made for irradiation by 850-nm light except that the heating effect is likely to be greater at this wavelength.

SUMMARY AND CONCLUSIONS

Consequences for users of optical tweezers

This work has clearly demonstrated evidence of a laser-induced stress in *C. elegans* caused by NIR cw laser used for optical tweezers. It has been shown that stress can be induced by a few hundreds of milliwatts of NIR cw laser light.

The stress response observed varied significantly with wavelength, laser power, and irradiation time. It could be concluded, from a comparison between calculated temperature rise values and the experimental results, that the stress response could not be explained solely by a photothermal effect. It was found that a stress response was more often induced by wavelengths below than above 800 nm for a given laser power although the amount of heating from water is higher above 800 nm than below. Stress response occurred most frequently at 760 nm. A high frequency of stress induction in *C. elegans* at 760 nm is in agreement with results from other studies showing increased laser-induced damages in other biological systems at this particular wavelength (Vorobjev et al., 1993; König et al., 1997; Liang et al., 1997).

At 810 nm, on the other hand, the frequency of stress induction was much lower. The data suggest that the gene expression at 810 nm mainly originates from a photothermal process, possibly in combination with a laser-light-induced lowering of the threshold for a photothermal response. This conclusion is primarily based upon the combination of two findings. Laser-irradiated animals show a behavior that is fully consistent with a thermal response, e.g., that a significant gene expression was obtained for 480 mW of irradiation whereas virtually no animals expressed *lacZ* at 240 mW, irrespective of the illumination time. Laser-induced gene expression takes place faster and at a slightly lower temperature (after 2 min at ~28°C or 4 min at ~27°C) than gene expression induced by thermal heating of whole animals (no animals showed any gene expression for a 5-min exposure to a temperature of 29°C, whereas a majority of the animals expressed the gene after 10 min of exposure). It was furthermore argued that the stress response seen at 850 nm also originates from a photothermal process.

It is yet not known whether optical tweezers can induce a stress response in cells of other plants or animals. If this is the case, however (which seems likely), then our results show that the combination of high laser powers (above a few hundred milliwatts in the specimen plane) and the wavelength region between 700 and 760 nm should be avoided in optical tweezers instrumentation for biological applications.

In summary, this work constitutes a contribution to the work ongoing to assess the degree to which NIR cw laser light used in micromanipulation of cells by the optical tweezers technique is noninvasive. It also describes a sensitive assay for the evaluation of cellular stress in optical trapping experiments.

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