Fluorescent Dye Monitoring of Mitochondrial Changes Associated with Malignant Cell Transformation

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ABSTRACT. The fluorescent dyes, rhodamine 6G and 123, which specifically stain mitochondria, were used to examine changes in mitochondria that follow malignant transformation. The spatial distribution and shapes of mitochondria differ in untransformed and malignant-transformed cells. In untransformed C3H/10T1/2 clone 8 cells, the mitochondria were distributed radially around the nucleus, and each had a fibrous shape. In chemically transformed MCA clone 16 cells, the mitochondria were distributed randomly in the cytoplasm, and each was shaped like a short rod. Another important mitochondrial change after malignant transformation was the change in the time course of fluorescence emission from the rhodamine present in the mitochondria. A slow increase in fluorescence, which was instantaneous at the time of excitation irradiation, occurred in untransformed but not in transformed cells. This slow fluorescence emission, peculiar to untransformed cells, was affected by proton ionophore but not by calcium ionophore treatment. The difference in the time courses of fluorescence emission for untransformed and transformed cells may reflect differences in the quenching of the dye fluorescence. The data reported provide evidence that mitochondria are affected by malignant cell transformation.

Mitochondria are the main energy source in the cell; therefore, they are important in malignant transformation because tumor cells require a large amount of energy for rapid proliferation. Because tumor tissues appear to have defective respiration and abnormally high rates of glycolysis (42), investigators have attempted to obtain direct evidence that the morphological and metabolic characteristics of tumor mitochondria, especially those of rapidly proliferating tumor cells, differ from those characteristics of normal cells (31, 43). Differences between tumor and normal cell mitochondria recently have been reported for adenine nucleotides effluxes from mitochondria (22, 24), for glutamine oxidation pathways (25, 29, 32), and for the

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Abbreviations used: FCCP, carbonyl cyanide p-trifluoromethoxy-phenylhydrazone.
natural ATPase inhibitor content (26). During transformation by Rous sarcoma virus, the rate of mitochondrial DNA replication in chick embryo fibroblasts is increased (6, 7). Therefore, it has been suggested that mitochondria may be the main target in carcinogenesis (1, 3, 30, 43) and in Rous sarcoma virus transformation (6, 7).

Recently, Johnson et al. (17) succeeded in observing the mitochondria of living cultured cells by staining the cells with the fluorescent dye, rhodamine 123. It also has been reported that mitochondria stained by rhodamine 123 are visible only in living cells (8) and that the fluorescence produced by this dye is altered because of the transmembrane potential of the mitochondria (18). Use of this fluorescent rhodamine 123 has produced a novel method for observing the spatial distribution of the mitochondria and for monitoring their membrane potentials in vivo. Consequently, differences in mitochondria and differences in the mitochondrial membrane of tumor and normal cells can be observed in situ by combining rhodamine staining with fluorescent microscopy.

To clarify the differences in the mitochondrial characteristics of tumor and normal cells in situ, we used a mouse embryo fibroblast line, C3H/10T1/2 clone 8 (10T1/2), as the normal cells and its 3-methylcholanthrene transformed cell line, MCA clone 16 (MCA CL 16), as the model of malignant transformation. In our experiments, mitochondria in living, cultured cells differed in their spatial distribution and time-dependent fluorescence emission dynamics for 10T1/2 cells and MCA CL 16 cells stained with rhodamine 6G or 123. Our data suggest that the characteristics of the mitochondrial membrane are changed by malignant cell transformation, and that this may be a phenotype of carcinogenesis.

MATERIALS AND METHODS

Cells. The mouse embryo cell line C3H/10T1/2 clone 8 (10T1/2) and its 3-methylcholanthrene transformed MCA clone 16 (MCA CL 16) cells were supplied by the University of Southern California Cancer Center, Los Angeles. All the 10T1/2 cells used in the experiments were taken from passages 8 to 15. Both cell lines were grown at 37°C in a mixture of Ham's F-12 medium (Grand Island Biological Co., N.Y.) with 8% fetal calf serum (Armour-pharmaceutical Co., N.Y.) in humidified incubators in an atmosphere of 5% CO2 in air. Both were ascertained as being free of mycoplasma.

Mitochondria specific dye staining. Samples were prepared for staining as described by Johnson et al. (17) with slight modifications. Cells were grown on 15-mm round cover slips then stained with rhodamine 6G or 123. Both rhodamine 6G (commercial name rhodamine 6GO, Chroma-Gesellschaft, Stuttgart-Unterürkheim) and rhodamine 123 (laser-grade, Eastman Organic Chemicals, Rochester, N.Y.) were prepared as stock solutions in distilled water at concentrations of 1 mg/ml and kept at 4°C in the dark. Rhodamine 6G was diluted in Eagle's solution (123 mM NaCl, 5.4 mM KCl, 1.4 mM CaCl2, 0.8 mM MgSO4, 1.0 mM NaH2PO4, 13.1 mM NaHCO3 and 5.6 mM glucose, pH 7.3) to 0.5 μg/ml (1.04 μM) then added to cells which were incubated at room temperature (16-22°C) for 2 min. The rhodamine 123 stock solution was diluted in Eagle's solution to 5 μg/ml (13.3 μM) then incubated with cells at 37°C for 20 min. These stained cells were rinsed twice with dye-free Eagle's solution to remove nonspecific fluorescence. A chamber prepared from a piece of parafilm (Sealon Film, Fuji Photo Film Co., Japan) punched with a 10-mm diameter hole was pressed on a standard microscope slide. The cover slip with the stained cells then was
mounted in Eagle's solution on the chamber of the slide glass.

**Morphological observation.** Stained cells were examined by epifluorescent microscopy with an Olympus BH-2 equipped with epifluorescent optics BH2-RFL (Olympus, Japan). When the rhodamine dyes were excited by blue light ($\lambda_{\text{max}}=490$ nm), rhodamine 6G emitted a yellow fluorescence and rhodamine 123 a green fluorescence. When excited by green light ($\lambda_{\text{max}}=545$ nm), both emitted red fluorescence. Green light excitation, however, was not used in this study because the fluorescence emitted by rhodamine 123 was very dim and the intensity of the rhodamine 6G fluorescence faded rapidly.

Photographs were made on Kodak Tri-X or Fuji Neopan 400 film with an Olympus automatic exposure control unit, PM-10-A (Olympus, Japan), set at ISO 1600. Films were developed for 11 min at 20°C in Fuji Pandol.

**Drug treatment.** After being stained by rhodamine, the cells were treated with carbonyl cyanide p-trifluoromethoxy-phenylhydrazone (FCCP, 10 $\mu$M), or A23187 (10 $\mu$M), in Eagle's solution. After 1 to 6 min of incubation with one of these drugs, the sample was rinsed with drug-free Eagle's solution, and the reversibility of the effects of drug treatment examined.

**Measurement of fluorescence intensity change.** The system for measuring fluorescence was similar to that used for the morphological observations. Fluorescence was detected with a photodiode, PIN 10 MWO 2975 (United Detector Technology Inc., Santa Monica, USA), behind an ocular lens. The photodiode signal current was translated to voltage by an I-V

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Fig. 1. Mitochondria stained by rhodamine 123 in living mouse embryo fibroblasts. a: 10T1/2 cells; b: MCA CL 16 cells. Bar=50 $\mu$m.
Morphological differences of mitochondria. Subconfluent cultured 10T1/2 cells formed a monolayer that showed contact inhibition (Fig. 1a). Mitochondria were concentrated around the nucleus in a radial distribution. Each mitochondrion had a fibrous shape and a diameter estimated to be about 0.8 μm, but the length varied randomly up to 10 μm (Fig. 2a). When a cell reached the confluent phase the number of mitochondria decreased, those remaining being shorter and aligned more randomly. This growth phase-dependent change in mitochondrial shape is very similar to that seen in human fibroblasts (13).

MCA CL 16 cells were spherical (Fig. 1b), their mitochondria being somewhat less concentrated around the nuclei as compared to the 10T1/2 cells. Each mitochondrion had a short-rod shape about 0.5 μm in diameter and 2–3 μm long (Fig. 2b). Some MCA CL 16 cells were giant ones that had large or multiple nuclei. The mitochondria in these cells often were very small and showed no change in

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**Fig. 2.** Mitochondria stained by rhodamine 123 in living mouse embryo fibroblasts. a: 10T1/2 cells; b: MCA CL 16 cells. Bar = 10 μm.
Exposure to excitation light for a few minutes diffused the mitochondria-staining dye (6G or 123) into the cytoplasm, so that the outline of the fluorescent mitochondria became indistinct as irradiation time increased (Fig. 3a, b). The viability of rhodamine-treated cells was examined by the ethidium bromide-staining method reported by Cohen et al. (5). Few ethidium bromide-stained nuclei were observed during the first 30 min in both the rhodamine 6G- and 123-treated cells. This confirmed that rhodamine was not cytotoxic during the short staining period used in our experiment.

**Time course of fluorescence emission.** During continuous excitation by light irradiation, the fluorescence of the rhodamine 6G in each mitochondrion of the 10T1/2 cells increased in intensity, with superimposed visible fluctuations, reached

![Fig. 3. Changes in the fluorescent image of a 10T1/2 cell. The photographs show the distribution and intensity of rhodamine 6G fluorescence in a cell at the beginning of excitation by light irradiation (a), and after fluorescence scattering by continuous blue light ($\lambda_{\text{max}}=490\ \text{nm}$; 40 sec) irradiation (b). Objective lens, Olympus UVFL 20 (NA 0.65); ocular lens, Olympus FK 5X; exposure time for photographs, 15 sec. Bar=20 $\mu\text{m}$.](image-url)

![Fig. 4. Time courses of fluorescence emission. a: 10T1/2 cells; b: MCA CL 16 cells. Cells were stained with rhodamine 6G then rinsed with dye-free Eagle's solution and excited by green light ($\lambda_{\text{max}}=545\ \text{nm}$). Arrows: beginning of excitation light irradiation.](image-url)
a maximum, then faded gradually and was diffused into the cytoplasm. The fluorescence of MCA CL 16 cells, however, neither increased nor fluctuated. It decreased gradually with continued exposure, also being diffused into the cytoplasm.

The typical time course of the fluorescence change of the entire field, as recorded by a photodiode, is shown in Fig. 4. In 10T1/2 cells, the initial fluorescence from rhodamine 6G was about half the peak value; it peaked somewhat slowly (rising phase) then decayed gradually (Fig. 4a). Fluorescence fluctuations of individual mitochondria were not recorded because fluorescence was averaged for the entire field. Fluorescence from the MCA CL 16 cells decreased from a plateau reached at the maximum intensity (Fig. 4b).

Fluorescence changes after staining with rhodamine 123 were similar to those obtained with 6G, but, the time course of fluorescence was longer. There was no fluctuation as seen with 6G.

To analyze the fluorescence dynamics, we introduced the parameters of the intensity curves: $V_0$—fluorescence intensity (expressed in mV) at the beginning of excitation; $V_m$—maximum intensity (in mV); $\Delta V$—difference between $V_m$ and $V_0$; and $t_d$—time constant of fluorescence decay (Fig. 5). The mean values of the three parameters and their standard deviations for the number of measurements indicated are shown in Table 1. In 10T1/2 cells the fluorescence increase ratio ($\Delta V/V_0$) was 70%, whereas it was close to 0% for MCA CL 16 cells. The value of $t_d$ for 10T1/2 cells was smaller than for MCA CL 16 cells. The difference between the values of $V_0$ for 10T1/2 and MCA CL 16 cells could not compared directly because the numbers of cells for which fluorescence was measured differed for each line (about 4 in 10T1/2 cells and about 10 in MCA CL 16 cells per detected field). Differences in the fluorescence time courses of the two cell lines were very clear and were ascribed to alteration of the

![Fig. 5. Parameters of the intensity curve used to assess fluorescence characteristics (for the definition of each parameter, see text).](image)

**TABLE 1. PARAMETERS OF THE TIME COURSE OF FLUORESCENCE EMISSION**

Differences in the time courses of three parameters (see Fig. 5) of fluorescence emission from 10T1/2 (untransformed cell line) and MCA CL 16 (malignant transformed cell line) cells.

<table>
<thead>
<tr>
<th></th>
<th>10T1/2 cells</th>
<th>MCA CL 16 cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>$V_0$ (mV)</td>
<td>12.61 ± 5.71</td>
<td>16.63 ± 7.64</td>
</tr>
<tr>
<td>$\Delta V/V_0$ (%)</td>
<td>70.6 ± 36.4</td>
<td>2.7 ± 5.2</td>
</tr>
<tr>
<td>$t_d$ (s)</td>
<td>11.92 ± 1.99</td>
<td>17.29 ± 1.39</td>
</tr>
</tbody>
</table>

* $V_0$ values of 10T1/2 cells and MCA CL 16 cells could not be compared because the numbers of cells in the detected fields differed for each cell line.

* $p<0.01$ (10T1/2 versus MCA CL 16)

Data are means ± S.D.; numbers in parentheses are the numbers of measurements.
mitochondrial membrane by malignant transformation.

**Effects of ionophores on fluorescence characteristics.** We applied proton and calcium ionophores extracellularly to determine the cause of the differences in the fluorescence characteristics of 10T1/2 and MCA CL 16 cells.

**Effects of proton ionophore.** The proton ionophore used was FCCP. The fluorescence of rhodamine 6G began to diffuse from the mitochondria to the cytoplasm immediately after treatment with FCCP. Fig. 6 (b and e) shows the time courses of fluorescence intensity in the presence of FCCP (10 μM). The time courses of the two cell lines were very similar after FCCP treatment. After FCCP treatment, the fluorescence intensity decreased without inflection of $V_0$. The rising phase ($\Delta V$) in 10T1/2 cells and the plateau in MCA CL 16 cells both disappeared. It should be noted that $V_0$ was elevated by FCCP treatment of 10T1/2 cells. The visible fluctuation of fluorescence in the 10T1/2 cells disappeared during FCCP treatment.

After the drugs had been washed out with ionophore-free Eagle’s solution, the distribution pattern of fluorescence in the 10T1/2 cells was restored almost to the initial image, and visible fluctuation of the fluorescence of individual mitochondria reappeared. The effects of FCCP on mitochondrial shape and distribution were negligible for both cell lines. Although the features of the time course of the 10T1/2 cells were restored, $V_m$ was smaller (Fig. 6c), and the plateau of the MCA CL 16 cells was only partially restored on the removal of FCCP (Fig. 6f). Reversibility was clear for the 10T1/2 cells, but not for the MCA CL 16 cells. Another proton ionophore, 2,4-dinitrophenol (1 mM), had effects similar to those of FCCP (data not shown). The fluorescence parameters in the presence of FCCP are compared with those of the controls in Table 2.

**Effects of calcium ionophore.** After adding A23187 (10 μM) to the incubation medium, a slight diffusion of fluorescence from the mitochondria to the cytoplasm was observed in the 10T1/2 cells, whereas none was present in the MCA CL 16 cells. Thus, the fluorescence changes produced by A23187 treatment were minor.

The time courses of fluorescence changes in the presence of A23187 are given in Fig. 7b and e. Although the $V_0$ and $V_m$ of the 10T1/2 cells were reduced, a slow rising phase of fluorescence ($\Delta V$) was clear. For MCA CL 16 cells, the plateau period was shorter, but the prolonged decrease phase remained. The effects of A23187 appeared to be irreversibly toxic even though treatment was limited to 5 min; $V_0$ values
Table 2 shows the changes produced in the fluorescence parameters by ionophores. For 10T1/2 cells, \( t_d \) was decreased by treatment with FCCP, but restored when the drug was washed out. With MCA CL 16 cells, \( t_d \) also was decreased by FCCP, but decreased as the incubation time increased, and some cells of both the 10T1/2 and MCA CL 16 lines peeled off during the washing out of A23187.

Table 2 shows the changes produced in the fluorescence parameters by ionophores. For 10T1/2 cells, \( t_d \) was decreased by treatment with FCCP, but restored when the drug was washed out. With MCA CL 16 cells, \( t_d \) also was decreased by FCCP, but decreased as the incubation time increased, and some cells of both the 10T1/2 and MCA CL 16 lines peeled off during the washing out of A23187.

Table 2. Parameters of time courses of fluorescence emission during proton and calcium ionophore treatments, and after ionophore wash out

<table>
<thead>
<tr>
<th>Ionophore</th>
<th>cell</th>
<th>Before treatment</th>
<th>Under drug treatment</th>
<th>After treatment (washout)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>( V_0 ) (mV)</td>
<td>( \Delta V/V_0 ) (%)</td>
<td>( t_d ) (s)</td>
</tr>
<tr>
<td>FCCP</td>
<td>10T1/2</td>
<td>13.72±2.01 (6)</td>
<td>22.40±2.70 (6)</td>
<td>9.47±0.90 (7)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCA CL 16</td>
<td></td>
<td>20.47±8.27 (5)</td>
<td>24.10±4.56 (6)</td>
<td>19.23±4.91 (7)</td>
</tr>
<tr>
<td>FCCP</td>
<td>10T1/2</td>
<td>15.67±1.08 (5)</td>
<td>12.61±1.07 (6)</td>
<td>11.28±2.37 (7)</td>
</tr>
<tr>
<td>A23187</td>
<td>MCA CL 16</td>
<td>17.45±1.49 (2)</td>
<td>11.99±2.36 (5)</td>
<td>16.45±3.08 (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>88.3±18.0 (2)</td>
<td>35.6±20.6 (5)</td>
<td>14.4±6.3 (4)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>11.6 (1)</td>
<td>11.77±0.25 (3)</td>
<td>10.91±0.66 (3)</td>
</tr>
<tr>
<td>A23187</td>
<td>MCA CL 16</td>
<td>17.96±3.47 (3)</td>
<td>14.97±3.29 (7)</td>
<td>11.70±0.36 (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0±0 (3)</td>
<td>0.5±1.2 (7)</td>
<td>13.3±0.65 (3)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>18.50±0.56 (3)</td>
<td>16.00±0.74 (7)</td>
<td></td>
</tr>
</tbody>
</table>

\( a \ldots \) not definable
\( b \) p<0.01 (before treatment vs. under drug treatment)
\( c \) p<0.05 (before treatment vs. under drug treatment)
\( d \) p<0.01 (under drug treatment vs. after treatment)
\( e \) p<0.05 (under drug treatment vs. after treatment)
\( f \) p<0.01 (before treatment vs. after treatment)
\( g \) p<0.05 (before treatment vs. after treatment)

Data are means±S.D.; numbers in parentheses are the numbers of measurements.

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Fig. 7. Effects of the calcium ionophore, A23187 (10 \( \mu \)M), on time courses of fluorescence emission. Left: traces (a-c) from 10T1/2 cells; right: traces (d-f) from MCA CL 16 cells. Traces a and d, before A23187 treatment; b and e, during A23187 treatment; c and f, after the wash out of A23187.
there was no recovery. Calcium ionophore treatment decreased the values of $V_0$ and $t_d$ in both cell lines and of $\Delta V/V_0$ in the 10T1/2 cells.

**DISCUSSION**

We have shown that the spatial distribution and shape of the mitochondria, as well as the time course of fluorescence emission from mitochondria-staining dye differ markedly for 10T1/2 cells and chemically transformed MCA CL 16 cells. The 10T1/2 cells form a flat monolayer on the surface of the dish and are highly sensitive to post confluence inhibition of cell division. They have an extremely low incidence of spontaneous transformation and are not tumorigenic in mice when injected subcutaneously (14, 33, 34). But malignant transformation studies that use 10T1/2 cells have the advantage of showing clear transformations by chemicals (28, 34), radiation (14, 21, 40) and transfection with oncogenes (15). MCA CL 16 cells show malignant transformation-related phenotypes in their morphology, ability to grow in soft agarose, less serum dependence (14, 23) and tumorigenicity in mice (14, 34). Thus the 10T1/2 cell line and its transformed cell line, MCA CL 16, should prove useful for detecting mitochondrial changes produced by malignant transformation.

Rhodamine 123 has been used in many studies as a mitochondria-specific optical probe in living cells because of its minimal cytotoxicity. Continuous exposure to rhodamine 123 (above 0.25 $\mu$g/ml), however, has been shown to reduce the growth rate and cell viability of human leukemia cells in culture (16). Colony formation in some carcinoma cell lines was reduced by rhodamine 123 treatment (2–5 $\mu$g/ml for 24 h), but clonal growth was not affected even at a higher concentration of rhodamine 123 when the exposure time was short (10$\mu$g/ml for 10 min) (2). In contrast, rhodamine 6G is a potent inhibitor of mitochondrial oxidative phosphorylation (12), but has only a small effect on myocardial cells stained briefly (30 min) with a dye concentration of less than 3 $\mu$M (35). Because we used rhodamine 6G at a concentration of 1 $\mu$M and for only 2 min, we found no cytotoxic effect on living cells monitored by ethidium bromide staining of their nuclei (5). Use of rhodamine 6G staining therefore has the advantages of a shorter staining time, a lower concentration of dye and a more intense fluorescence.

An important mitochondrial change seen in malignant transformation was the change in time course of fluorescent emission from mitochondrial dyes. When MCA CL 16 cells were continuously excited, the initial fluorescence showed a brief plateau instead of the rising phase ($\Delta V$) seen in 10T1/2 cells. As these differences were very clear, we looked for the reason for the rising phase ($\Delta V$) present in the fluorescent emission of untransformed cells. The rising phase ($\Delta V$) of fluorescence intensity in 10T1/2 cells possibly is explainable by one, or both, of the following likelihoods: 1) The fluorescence intensity was increased transiently by a change in the molecular structure of the dye during excitation, or 2) The dye-mitochondria interaction in 10T1/2 cells differs from that in MCA CL 16 cells. The former probably is not valid because no slowly rising phase ($\Delta V$) was present in the MCA CL 16 cells. Consequently, the increase in fluorescence ($\Delta V$) would be caused only by alteration of the interaction between the rhodamine and the mitochondria. The second possibility is therefore the most probable.

These considerations are supported by visible fluorescence fluctuations seen in 10T1/2 cells but not in MCA CL 16 cells. Furthermore, these fluorescence fluctuations
disappeared upon treatment with FCCP. Because these fluctuations might reflect localized alterations in molecules or ions at the suborganelle level (35), the interaction of dyes with mitochondrial membranes should differ for 10T1/2 and transformed cells.

The change in fluorescence emission (Fig. 6) from the complex increase-decrease and plateau-decrease to a simple decrease upon treatment with FCCP suggests that the dye molecules normally are held in the mitochondrial membrane, their constraint being released by proton ionophores. Rhodamine dyes may accumulate in the mitochondrial membrane like cyanine dyes, as proposed by Johnson et al. (18), Cohen et al. (5) and Yoshikami and Okun (44). If this is true, rhodamine dyes would accumulate there in a hyperpolarizing condition by making a dimer, and their fluorescences would then be diminished by quenching (20, 36).

Decay of fluorescence under constant excitation in an aqueous solution is simple noninflected decay from \( V_0 \) (Fig. 6). When rhodamine dye molecules are highly concentrated in the membrane, the absolute value of the fluorescence intensity will be reduced due to quenching, with no change in the decay time constant. If membrane-bound dye molecules are exuded from the membrane and diffused by irradiation; e.g., because of injury by excited dye molecules (9, 27), the fluorescence intensity will gradually increase because dye molecules are released from the quenched state and, after reaching a peak, the intensity will decay exponentially. This is the pattern observed for the 10T1/2 cells (Fig. 4a).

In a recent study of rhodamine 123 on isolated rat liver mitochondria, Emaus et al. (11) reported that rhodamine uptake and fluorescence quenching occurred following mitochondrial energization, and depended especially on the mitochondrial membrane potential. It also has been suggested that membrane potential-independent uptake of rhodamine 123 functions in the selective staining of mitochondria (39). Although the factors that control rhodamine 123 uptake and quenching in the mitochondria of living cells are as yet unknown, our results indicate that fluorescence quenching occurs in the mitochondria of living 10T1/2 cells. The difference in quenching in 10T1/2 and MCA CL 16 cells may reflect differences produced by the alteration of mitochondria after malignant cell transformation.

Our morphological observations showed that the mitochondria in 10T1/2 cells were distributed radially around the nucleus, whereas those in MCA CL 16 cells were distributed randomly in the cytoplasm (Fig. 1, 2). Johnson et al. (17) and Brouthy-Boyé et al. (4) have reported on the cytoskeletal structures of cultured cells observed without fixation by using rhodamine 123 to stain the mitochondria associated with the cytoskeleton. Differences in the distribution of mitochondria may reflect cytoskeletal alterations caused by malignant transformation because microtubules and intermediate filaments are known to codistribute with mitochondria (38, 41). Rous sarcoma virus-transformed fibroblasts have been reported to deplete ordered microfilamental structures and microtubular arrays (10). Our present data indicate that the cytoskeleton of MCA CL 16 cells also may be disordered by chemical transformation. In addition after malignant transformation we observed shape changes of mitochondria from fibrous to short rod-like forms, but we can not be sure what caused these changes.

Alterations in the uptake and retention of rhodamine by mitochondria during malignant transformation have been reported (19, 37). We have shown that these alterations can be monitored for individual mitochondria by using continued excita-
Transformed Mitochondria Monitored by Rhodamine 535

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