Biochemical and Biophysical Research Communications **278,** 183–191 (2000)
doi:10.1006/bbrc.2000.3765, available online at http://www.idealibrary.com on IDEAL®

Proteoliposomes Colocalized with Endogenous Mitochondria in Mouse Fertilized Egg

Yutaka Inoki,*,† Yoji Hakamata,‡ Toshiro Hamamoto,* Tadatoshi Kinouchi,* Shoji Yamazaki,* Yasuo Kagawa,*,§ and Hitoshi Endo*,[1]

**Department of Biochemistry and ‡Laboratory of Experimental Medicine, Jichi Medical School, Minamikawachi-machi, Kawachi-gun, Tochigi 329-0498, Japan; §Department of Medical Chemistry, Women's University of Nutrition, Chiyoda, Sakado, Saitama 350-0288, Japan; and †Research Institute of Life Science, Snow Brand Milk Products Co., Ltd., Ishibashi-machi, Shimotsuga-gun, Tochigi 329-0512, Japan*

Received September 30, 2000

Colocalization of mitochondria is the first step of intermitochondrial interaction or fusion in a cell. Here, we showed colocalization between exogenous mitochondria and endogenous ones or between exogenous proteoliposomes and endogenous mitochondria in mouse fertilized eggs by confocal laser microscopy. Isolated mitochondria from mouse liver and proteoliposomes containing mitochondrial membrane were directly labeled with red fluorescent aliphatic marker, PKH26, which is incorporated into lipid membrane, and then were microinjected into fertilized mouse eggs. Exogenous mitochondria appeared to be almost colocalized with endogenous mitochondria at the 4- and 8-cell stages, when mitochondria were stained with Rhodamine 123 (green fluorescent marker). On the contrary, when liposomes consisted of soy bean phospholipid were microinjected into the eggs as a control, their localization was different from that of endogenous mitochondria. Next, the submitochondrial particles and proteoliposomes were microinjected. Both the proteoliposomes and the submitochondrial particles appeared to colocalize with endogenous mitochondria at the 4-cell stage. These results suggest the existence of a factor that makes liposomes colocalize with mitochondria. Such a proteoliposome would be useful for the development of mitochondrial gene transfer techniques. © 2000 Academic Press

Key Words: **mitochondria; liposome; proteoliposome; microinjection; fertilized egg; PKH26; Rhodamine 123.**

A mammalian cell contains hundreds of independent mitochondria, each containing several copies of their own circular mitochondrial DNA (1–5). Intermitochondrial interaction within the cell was initially suggested by morphological (6, 7) and genetic (8, 9) findings in yeast. In mammalian cells, giant mitochondria in hepatocytes have been reported to appear as cylindrical mitochondria linked together (10). Genetically, translational complementation of mitochondrial rRNA was observed in heteroplasmic cells with chloramphenicol-sensitive and -resistant mtDNA (11, 12), and translational complementation of mitochondrial tRNA was observed in heteroplasmic cells with mitochondrial DNA disease. In addition, using a cybrid technique, intermitochondrial interaction has been reported between wild-type and DNA-lacking mitochondria, and between wild-type mitochondria and deletion-mutant mtDNA in a HeLa cell (13, 14).

Recently, microinjection technique of intact mitochondria into mouse fertilized egg have been established, and showed that sperm mitochondria were eliminated in fertilized eggs, but somatic mitochondria were not eliminated (15). In addition, transmitochondrial transgenic mice have been established (16). However, any visual evidence of intermitochondrial interaction between exogenous and endogenous mitochondria in a cell have not been reported.

In the present report, we directly labeled mitochondria and proteoliposomes using red fluorescent marker (PKH26) with enhanced stability, and then microinjected them into fertilized eggs. We indicated the visual evidence of intermitochondrial interaction between PKH26-labeled mitochondria and endogenous mitochondria stained with Rh123 by confocal laser microscopy. In addition, we constructed proteoliposomes including mitochondrial membrane, which colocalized with endogenous mitochondria in preimplantation embryo.

[1] To whom correspondence should be addressed. Fax: +81-285-44-1827. E-mail: hendo@jichi.ac.jp.

0006-291X/00 $35.00

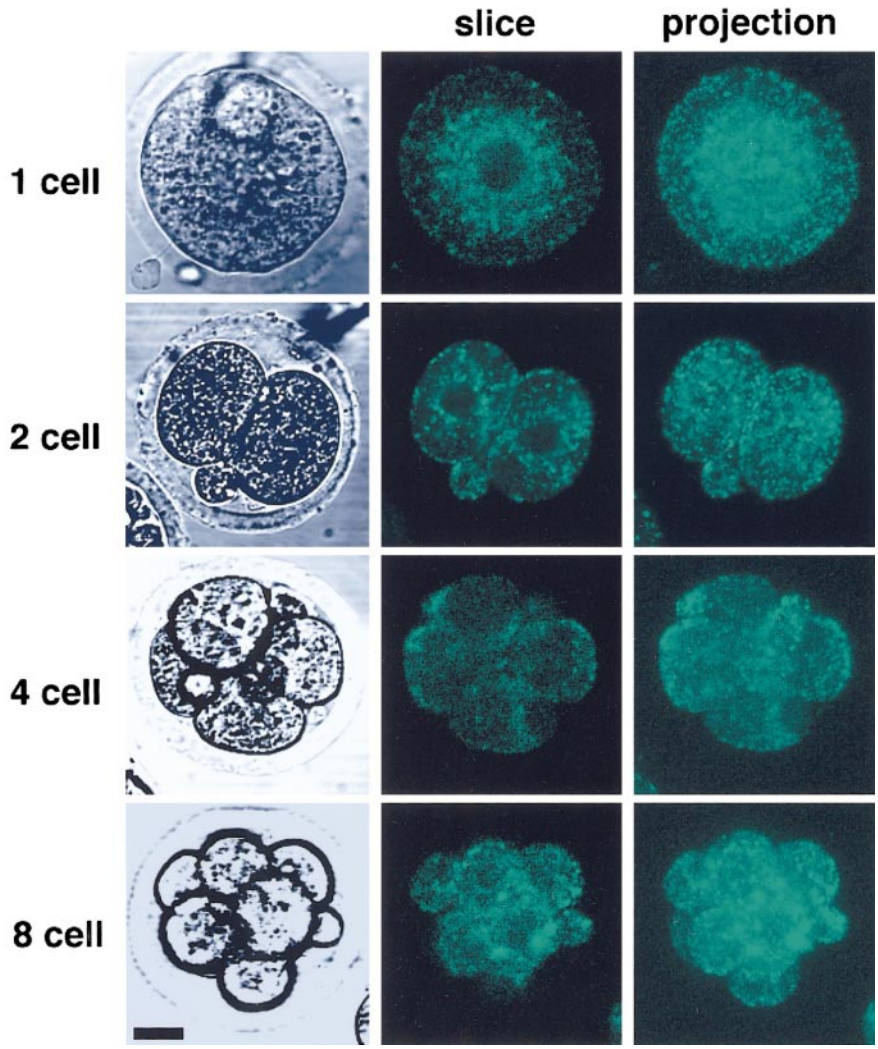

FIG. 1. The dynamics of endogenous mitochondria in 1- to 8-cell stage mouse embryos. Mitochondria in fertilized mouse eggs accumulated Rhodamine 123 (Rh123; green fluorescence) and observed using confocal laser microscopy. Left vertical line shows the transmission image. Middle and right lines show slice and projection images of Rh123-stained mitochondria, respectively, using confocal laser microscopy. All figures are to the same magnification. The scale bar represents 20 μm.

MATERIALS AND METHODS

Preparation of mitochondria and labeling with PKH26. Mitochondria were isolated from C57BL/6J mouse liver as described previously (17), suspended in 0.25 M sucrose with 0.2 mM EDTA (pH 7.4), and stored on ice for up to 8 h. Final concentration of mitochondrial protein was adjusted to approximately 20 mg/ml. The protein concentration was determined according to Lowry's method.

Direct labeling of mitochondrial membrane was performed using a PKH26 (excitation wavelength 551 nm, emission wavelength 567 nm) red fluorescent cell linker mini kit (Sigma Chemical Co., USA) (18). A 100 μl aliquot of isolated mitochondria solution was centrifuged at 5900*g* for 10 min, resuspended in 200 μl of Diluent C solution, and then mixed with 200 μl of Diluent C containing 2 μl of 1 mM PKH26 solution by gentle pipetting. After incubation at room temperature for 2 min, the labeled mitochondria mixture was supplemented with 500 μl of 75 mM KCl and 10 mM phosphate buffer (pH 7.0), and centrifuged at 5,900*g* for 10 min. The mitochondrial pellet was suspended in 200 μl of 75 mM KCl with 10 mM phosphate buffer (pH 7.0), and subjected to microinjection.

Preparation of PKH26-labeled submitochondrial particles. After labeling mitochondria with PKH26, 2 mg of labeled mitochondria suspended in 75 mM KCl with 10 mM phosphate buffer (pH 7.0) were frozen and thawed 3 times, and then sonicated to form submitochondrial particles.

Preparation of PKH26-labeled liposomes and proteoliposomes. For preparation of liposomes, 10 mg of soybean phospholipid (Asolectin) was dissolved in 1 ml of chloroform and then mixed with 5 μl of 1 mM PKH26. A 50 μl aliquot of this solution was evaporated with N_2 flow. Asolection was purchased from Associate Concentrates (Woodside, NY) and partially purified as described previously (19). The pellet was hydrated with 100 μl of 75 mM KCl and 10 mM phosphate buffer (pH 7.0), and then frozen and thawed 3 times following filtration with 0.2 μm pore filter to form PKH26-labeled liposomes. The labeled liposomes were centrifuged at 100,000*g* for 20 min to remove free PKH26.

Equal volumes of PKH26-labeled liposomes and non-labeled mitochondria were mixed and then frozen, thawed, and sonicated for preparation of proteoliposomes containing mitochondrial membrane fraction.

Measurement of mitochondrial membrane potential. The membrane potential of isolated mitochondria was monitored by accumulation of Rh123, which was incorporated into mitochondria dependent on membrane potential. Accumulation of Rh123 was measured by a fluorescent spectrophotometer (F-2000, Hitachi, Tokyo, Japan) with an excitation wavelength 470 nm and emission monitored at 530 nm. PKH26-labeled mitochondria (50 μg protein) or PKH26-labeled submitochondrial particles (50 μg protein) were added to 450 μl of Buffer 1 (0.25 M sucrose, 10 mM KCl, 1.2 mM $MgCl_2$, 0.2 mM EDTA, 1 mM succinate, 1 mM potassium phosphate, 10 mM Hepes-NaOH, pH 7.4) supplemented with Rhodamine 123 (10 ng/ml).

Electron microscopy. Isolated mitochondria and submitochondrial particles were fixed with 2% glutaraldehyde and embedded into epoxy's resin. Thin sections were examined on a Hitachi H-7000 transmission electron microscope.

Preparation of fertilized eggs. C3H/HeJ female mice were treated at 5–6 weeks with i.p. injection of 5 IU of PMSG (Serotropin) (Teikoku Hormone Co., Tokyo, Japan) followed 48 h later with 5 IU of hCG (Gonatropin) (Teikoku Hormone Co., Tokyo, Japan). Females were mated to C3H/HeJ male mice immediately after injection of hCG. Eggs were collected by rupturing the oviducts approximately 18 h after injection of hCG, treated with 0.03% bovine testicular hyaluronidase (Sigma Chemical Co., USA) in M2 medium (20) to remove cumuli, and then rinsed and stored in M16 medium (20) for 3 h at 37°C under 5% CO_2 in air. After incubation, fertilized eggs with two pronuclei were selected for microinjection.

Microinjection into fertilized mouse eggs. A micromanipulator (Leitz) equipped with a Piezomicropipette driving unit (Model PMM-01, Prima Meat Packers, Ibaraki, Japan) was used. The micropippetes (~7 μm tip diameter) were pulled from borosilicate glass capillary tubing (1.0 mm outside diameter, 0.75 mm inside diameter) (Sutter Instruments, Novate, CA), and contained mercury columns to maximize penetration efficiency.

Confocal laser microscopy. Active mitochondria of fertilized embryos at various preimplantation stages were stained with HECM-3 containing Rh123 (final concentration 0.2 μg/ml) for 20 min, washed with HECM-3, and then transferred onto a glass-bottom dish. Active mitochondria accumulating Rh123 was observed using confocal microscopy (MicroRadiance, Bio-Rad Laboratories Inc., CA) under 488 nm excitation light with a 515/30 filter for emission. Exogenous mitochondria, submitochondrial particles, liposomes, and proteoliposomes labeled with PKH26 was also observed under 514 nm excitation light with a 570 long pass filter for emission. A computer equipped with Bio-Rad software (LaserSharp ver. 3.4) was used for operating the system and for processing of images.

RESULTS

Distribution of mitochondria in fertilized mouse eggs. First, we studied the dynamics of endogenous mitochondria in fertilized mouse eggs from the 1- to 8-cell embryonic developmental stages under confocal laser microscopy after staining with Rhodamine 123 (Rh123) (Fig. 1). Mitochondria were observed in a perinuclear pattern at the 1-cell stage, and then distributed throughout the cytoplasm at the 2-cell stage. After distribution, mitochondria were partially localized in interfaces between cells. The diffuse pattern of mitochondria distribution was observed from the 2- through 8-cell stages. Thus, the mechanism of mitochondrial movement from the perinuclear to diffuse pattern is active between the 1- and 2-cell stages. These results

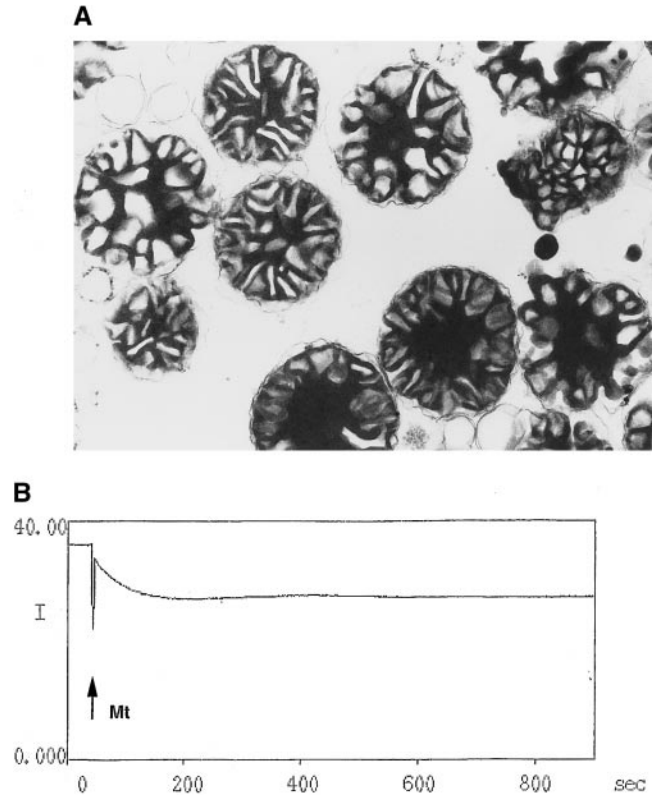

FIG. 2. Electron microscopy and measurement of membrane potential of isolated mitochondria. (A) Electron microscopy image of isolated mitochondria. Mouse liver mitochondria were purified according to the sucrose-gradient method described under Materials and Methods, and then subjected to electron microscopy, ×26,700. (B) Measurement of the membrane potential of PKH26-labeled mitochondria was performed using Rh123 in a fluorescent spectrophotometer. Y-axis is the intensity of fluorescence monitored at 530 nm, and the point of addition of PKH26-labeled mitochondria is indicated with an arrow.

are consistent with those reported previously in hamster embryos (21).

Inspection of isolated mitochondria. To test the quality of the sample for microinjection, a mitochondria fraction isolated from mouse liver by the sucrose gradient method was observed under electron microscopy (Fig. 2A). Most structures in the fraction were shown to be intact mitochondria with cristae, and the fraction was contaminated with a small amount of degraded mitochondria, microsomes, and cell debris. In addition, the membrane potential of the mitochondria after labeling with PKH26, a membrane-labeling fluorescent marker, was measured by the accumulation of Rh123 in mitochondria using fluorescent spectrophotometer. Figure 2B shows that PKH26-labeled mitochondria possessed membrane potential, and therefore that the labeling process with the fluorescent marker did not damage the membrane potential of mitochondria.

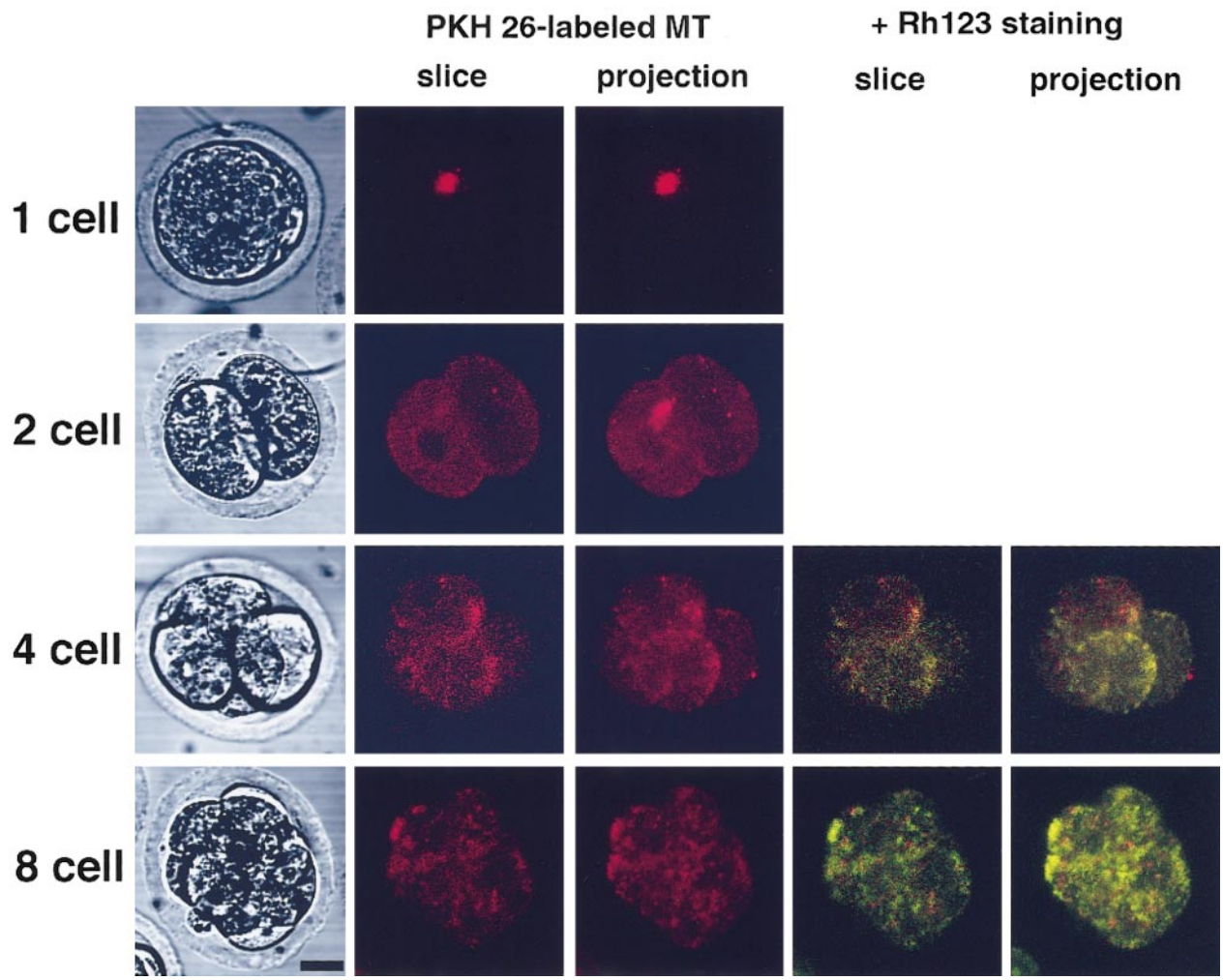

FIG. 3. The dynamics of exogenous mitochondria labeled with PKH26 in fertilized mouse eggs from the 1- to 8-cell embryonic development stages. At the 4- and 8-cell stages, mitochondria are stained with Rh123 (fourth and fifth vertical lines). First vertical line shows transmission images. Second and third lines show slice and projection images of PKH26-labeled mitochondria, respectively, using confocal laser microscopy. Fourth and fifth lines show slice and projection images of double staining with PKH26 and Rh123, respectively, using confocal laser microscopy. All figures are to the same magnification. The scale bar represents 20 μm.

Mitochondria from mouse liver colocalized with endogenous mitochondria. Microinjection of PKH26-labeled mitochondria into mouse fertilized egg was performed to study the dynamics of exogenous mitochondria in preimplantation embryos. Exogenous mitochondria were observed from the 1- to 8-cell embryonic development stages under a confocal laser microscope. As shown in Fig. 3, exogenous mitochondria (red fluorescence) remained at the injected site nearby nuclei at the 1-cell stage, and then distributed over the cytoplasm at the 2-cell stage. This behavior was basically consistent with that of endogenous mitochondria (Fig. 1). At the 4- and 8-cell stages, when mitochondria were stained with Rh123 to visualize the interaction between exogenous and endogenous mitochondria, exogenous (red) and total active (green) mitochondria appeared to be overlaid. Thus, exogenous mitochondria isolated from mouse liver colocalized with endogenous mitochondria in the eggs.

Liposomes did not colocalize with mitochondria. As a negative control, liposomes, which consisted of soybean phospholipid, was used. Figure 4 shows that PKH26-labeled liposomes (red) were distributed immediately after microinjection, in contrast with both endogenous and exogenous mitochondria (Figs. 1 and 3). In addition, when endogenous mitochondria were stained with Rh123, liposomes (red) appeared in a speckle pattern, the localization of which was totally different from the locus of endogenous mitochondria. These findings indicate that the mitochondrial colocalization shown in Fig. 3 was specific to mitochondria, and that a mitochondrial component was necessary for colocalization with endogenous mitochondria.

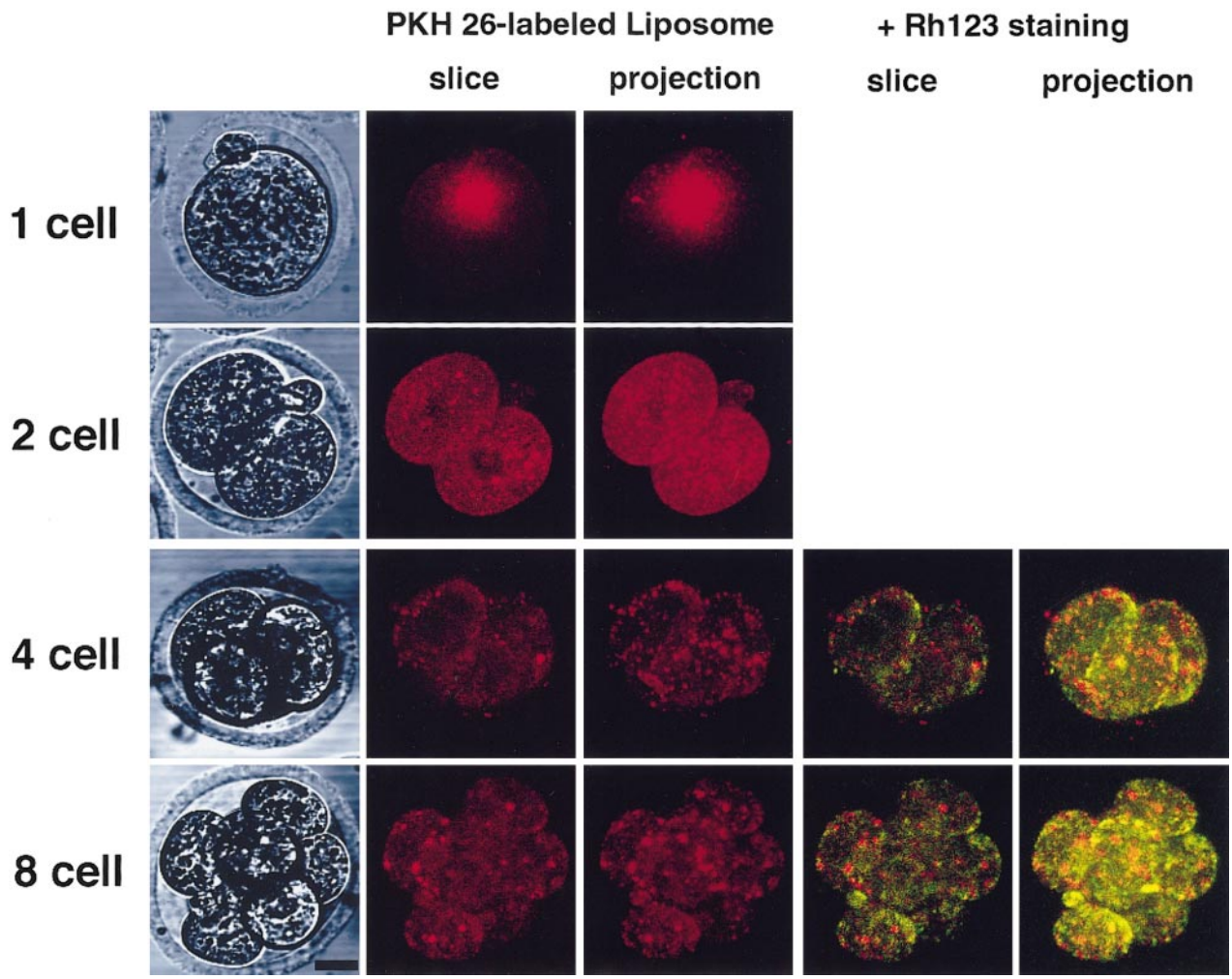

FIG. 4. The dynamics of liposomes labeled with PKH26 in fertilized mouse eggs from the 1- to 8-cell embryonic development stages. At the 4- and 8-cell stages, mitochondria are stained with Rh123 (fourth and fifth vertical lines). First vertical line shows transmission images. Second and third lines show slice and projection images of PKH26-labeled liposomes, respectively, using confocal laser microscopy. Fourth and fifth lines show slice and projection images of double staining with PKH26 and Rh123, respectively, using confocal laser microscopy. All figures are to the same magnification. The scale bar represents 20 μm.

Submitochondrial particles colocalized with mitochondria. To investigate whether submitochondrial particles are sufficient for the colocalization of mitochondria in a cell, submitochondrial particles were microinjected into fertilized eggs. To test the quality of the injection sample, the submitochondrial particles were analyzed under electron microscopy. Figure 5A shows that most of the mitochondria appeared to fragment into submitochondrial particles as a single membrane structure. In addition, the measurement of the membrane potential showed that samples of submitochondrial particles contained very few intact mitochondria (Fig. 5B).

PKH26-labeled submitochondrial particles were microinjected into fertilized eggs, and the dynamics of the particles were monitored by confocal laser microscopy from the 1- to 4-cell stage of embryonic development (Fig. 6). PKH26-labeled submitochondrial particles (red) showed distribution at the 1-cell stage, although the extent of this distribution was clearly different from the speckle pattern of liposomes shown in Fig. 4. When a 4-cell embryo was stained with Rh123, PKH26-labeled submitochondrial particles (red) appeared to be colocalized with endogenous mitochondria (green). Note that submitochondrial particles does not accumulate Rh123, the appearance of the submitochondrial particles at the 4-cell stage was consistent with that of intact exogenous mitochondria (Fig. 3). According to these results, colocalization of mitochondria in a cell was not dependent upon any components of the mitochondria matrix, nor did it depend upon the membrane potential of mitochondria.

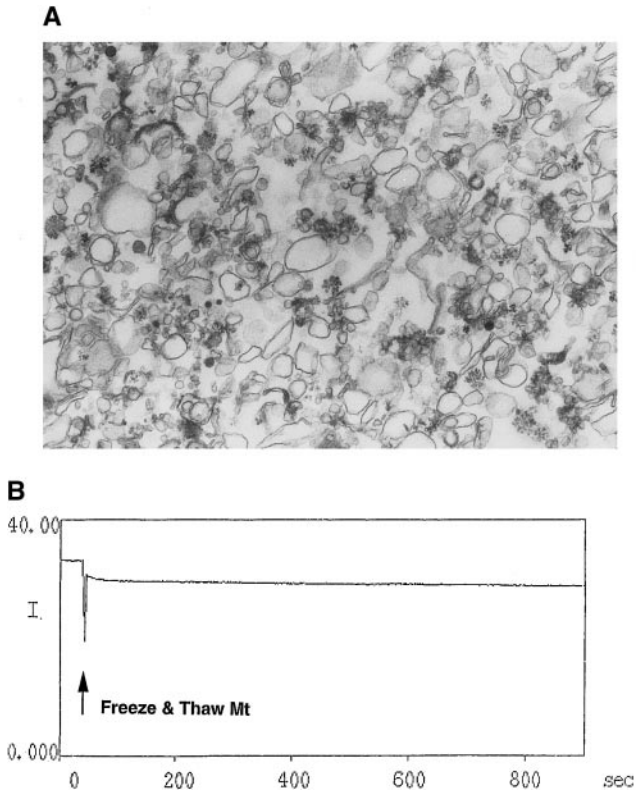

FIG. 5. Electron microscopy and measurement of membrane potential of submitochondrial particles. (A) Electron microscopy image of submitochondrial particles. Magnification is the same as that in the legend to Fig. 2A, ×32,000. (B) Measurement of membrane potential of submitochondrial particle fraction using Rh123 in a fluorescent spectrophotometer. Y-axis is the intensity of fluorescence of incorporated Rh123 monitored at 530 nm. The point of addition of submitochondrial particle fraction is indicated with an arrow.

Proteoliposomes containing mitochondrial membrane colocalized with mitochondria. Based on the above results, the existence of a factor for mitochondrial colocalization on the mitochondrial membrane was strongly suggested. We attempted to prove the existence of such a factor by construction of proteoliposomes containing mitochondrial membrane. PKH26-labeled liposomes and non-labeled mitochondria were mixed and then frozen, thawed, and sonicated for preparation of proteoliposomes containing mitochondrial membrane fraction. Proteoliposomes microinjected into fertilized mouse eggs were monitored using confocal laser microscopy from the 1- to 4-cell stages of embryonic development (Fig. 7). At the 1-cell stage, proteoliposomes containing mitochondrial membrane (red) were more widely distributed than submitochondrial particles, but less than liposomes containing only soybean phospholipid (Figs. 4 and 6). When a 4-cell embryo was stained with Rh123, patterns of colocalization between proteoliposomes (red) and endogenous mitochondria (green) were obtained. These results indicate that a factor for mitochondrial colocalization exists on the mitochondrial membrane, and that the factor derived from mature mouse liver was active even in fertilized eggs, where cytoplasm conditions are different from those of liver cells.

DISCUSSION

In the present report, we traced the exogenous mitochondria, submitochondrial particles, and proteoliposomes that were labeled with aliphatic fluorescent marker, PKH26. They were prepared from mouse liver, microinjected into fertilized mouse eggs, and observed by confocal laser microscopy to examine intermitochondrial interaction between them and active mitochondria in a cell. We showed that exogenous mitochondria colocalized with endogenous mitochondria in the preimplantation embryo, although the liposomes did not colocalize with endogenous mitochondria. In addition, we also showed that submitochondrial particles or proteoliposomes containing mitochondrial membrane proteins colocalized with endogenous mitochondria, indicating that a factor for colocalization of mitochondria existed in mitochondrial membrane.

Technique of microinjection of mitochondria into eggs has been established using a Piezomicropipette driving unit, and enabled to be reported that sperm mitochondria were eliminated in fertilized eggs by the detection of Rh123-stained sperm mitochondria using a confocal microscopy (22). Recently, it is suggested that proteolytic embryonic factors detect nuclear-encoded proteins of the sperm mitochondrial sheath or associated elements (23). In contrast, recent reports have reported that exogenous mitochondrial DNA from somatic cells are not eliminated from the fertilized egg after microinjection using PCR method (15). Mitochondria isolated from somatic cells likely lack the signal that would induce their destruction in the fertilized egg. We indicated that somatic mitochondria retained in fertilized eggs until 8-cell stage using long-term detection marker. This result supported that mitochondria from liver cells are protected from the selective destruction of sperm mitochondria in the fertilized egg.

Mitochondrial DNA (mtDNA) shows higher sensitivity to mutation because of an inaccurate DNA repair system and the absence of protective histone. Mutations on mtDNA cause various mitochondrial disorders such as diabetes, neuropathy, and myopathy (24). Mutant mtDNA is usually mixed with wild-type mtDNA in a heteroplasmic cell. According to the segregation theory (24), when a heteroplasmic cell is divided, the mutant and wild-type mtDNAs are randomly distributed into the daughter cells, and ultimately a cell with predominantly mutant mtDNA is produced. This process, known as replicative segregation, is associated with mitochondrial diseases. If mitochondrial fusion in

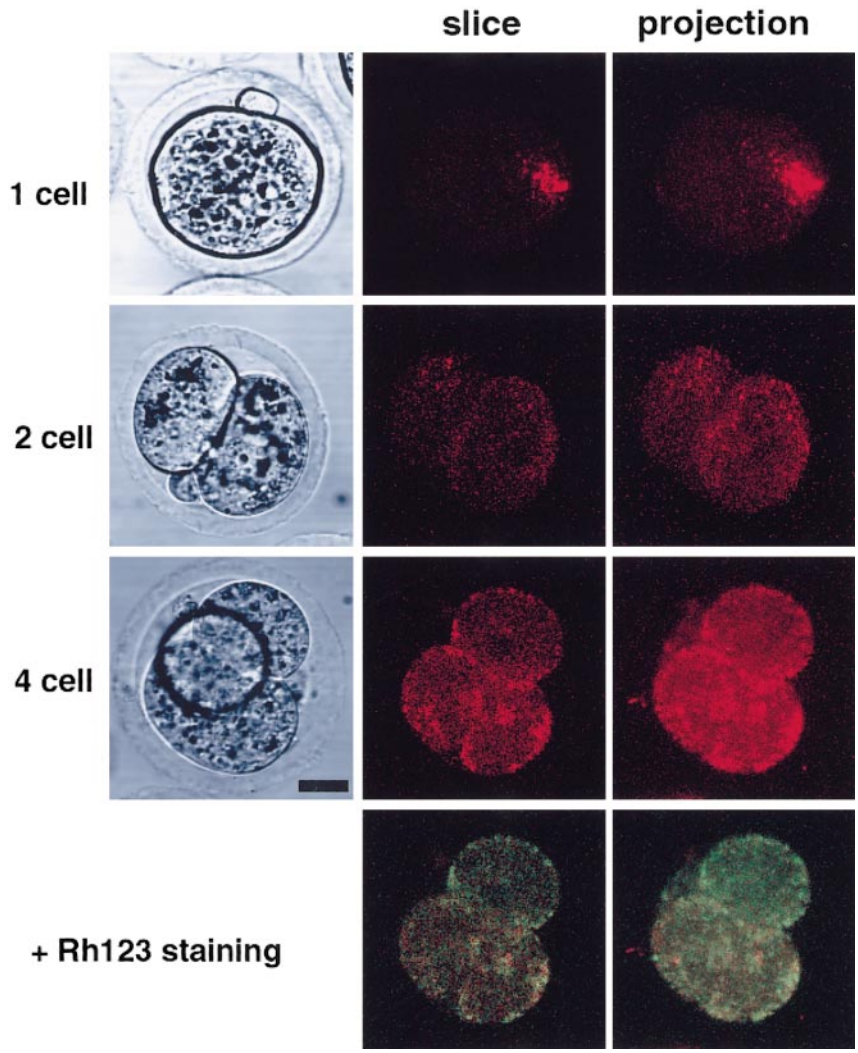

FIG. 6. The dynamics of submitochondrial particles labeled with PKH26 in fertilized mouse eggs from the 1- to 4-cell embryonic development stages. At the 4-cell stage, endogenous mitochondria are stained with Rh123 (fourth row). First vertical line shows transmission images. Second and third lines show slice and projection images of PKH26-labeled submitochondrial particles, respectively, using confocal laser microscopy. Fourth row shows slice and projection images of double staining with PKH26 and Rh123 using confocal laser microscopy. All figures are to the same magnification. The scale bar represents 20 μm.

a cell occurs to the point of genotypical homogeniety, it would be difficult to explain the pathogenesis of mitochondrial diseases using the segregation theory. Figure 3 shows that exogenous mitochondria were not distributed homogeneously into daughter cells between the 1- and 2-cell embryonic stages. These results indicate that intermitochondrial interaction occurs within a cell, and also that the distribution of mitochondria during cell division is not homogenous in the embryo, which agrees with the segregation theory to some extent.

We also showed that exogenous mitochondria and proteoliposomes containing mitochondrial membrane fraction colocalized with endogenous mitochondria in an egg by confocal laser microscope. Colocalization of mitochondria is the first step of intermitochondrial interaction (25). We indicated the visual evidence using microinjection of proteoliposomes that a factor for mitochondrial colocalization existed in mitochondrial membrane. Previously, partial purification of mitochondrial fusiogenic protein from mouse liver has been reported (26). Such a factor accelerating intermitochondrial interaction would exist on mitochondrial membrane from mouse liver.

In spite of numerous reports about mitochondrial diseases, a fundamental technique for gene therapy of mitochondrial disorders has not been sufficiently developed. The current study shows that the proteoliposomes colocalized with endogenous mitochondria in a cell, and also showed a possibility that such a proteo-

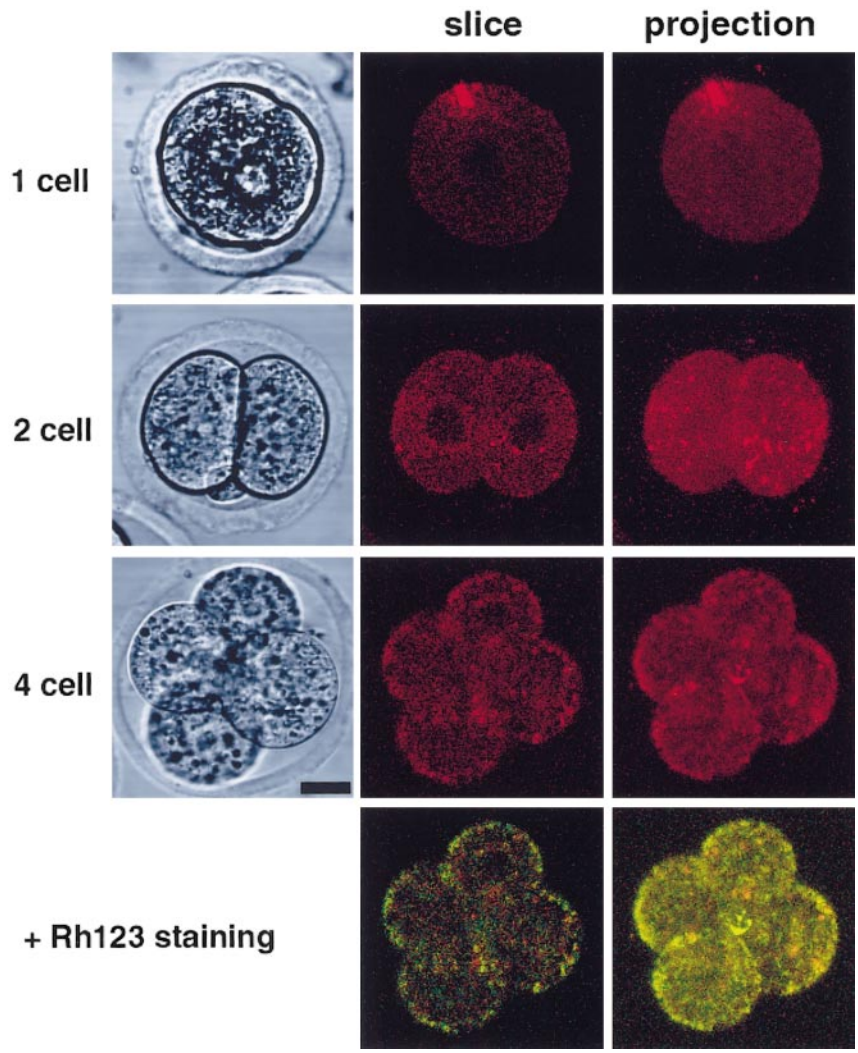

FIG. 7. The dynamics of PKH26-labeled proteoliposomes containing mitochondria membrane in fertilized mouse eggs from the 1- to 4-cell embryonic development stages. At the 4-cell stage, mitochondria are stained with Rh123 (fourth row). First vertical line shows transmission images. Second and third lines show slice and projection images of PKH26-labeled proteoliposomes, respectively, using confocal laser microscopy. Fourth row shows slice and projection images of double staining with PKH26 and Rh123 using confocal laser microscopy. All figures are to the same magnification. The scale bar represents 20 μm.

liposome is useful for developing a gene transfer vector of foreign mitochondria. The investigation of their dynamics in fertilized eggs can be important clues to establish therapeutic techniques for mitochondrial diseases.

ACKNOWLEDGMENTS

We thank members of our laboratory for their helpful comments. This work was supported by Grants-in-Aid from the Ministry of Education, Science, and Culture of Japan.

REFERENCES

1. 1. Borst, P., and Kroon, A. M. (1969) Mitochondrial DNA: Physicochemical properties, replication, and genetic function. *Int. Rev. Cytol.* **26,** 107–190.
2. Nas, M. M. K. (1969) Mitochondrial DNA: Advances, problems, and goals. *Science* **165,** 25–35.
3. Clayton, D. A. (1984) Transcription of the mammalian mitochondrial genome. *Annu. Rev. Biochem.* **53,** 573–594.
4. Attardi, G., and Schatz, G. (1988) Biogenesis of mitochondria. *Annu. Rev. Cell Biol.* **4,** 289–333.
5. Wallace, D. C. (1992) Mitochondrial genetics: A paradigm for aging and degenerative diseases? *Science* **256,** 628–632.
6. Stevens, B. (1981) Mitochondrial structure. *In* The Molecular Biology of the Yeast Saccharomyces (Strathern, J. N., Jhohns, E. W., and Broach, J. R., Eds.), pp. 471–504, Cold Spring Harbor Laboratory Press, New York.
7. Miyakawa, I., Aoi, H., Sando, N., and Kuroiwa, T. (1984) Fluorescence microscopic studies of mitochondrial nucleoids during meiosis and sporulation in the yeast, *Saccharomyces cerevisiae. J. Cell Sci.* **66,** 21–38.
8. Dujon, B., Slonimski, P. P., and Weill, L. (1974) Mitochondrial

genetics IX: A model for recombination and segregation of mitochondrial genomes in *Saccharomyces cerevisiae. Genetics* **78,** 415–437.

9. Clark-Walker, G. D., and Miklos, G. L. G. (1975) Complementation in cytoplasmic petite Mutants of yeast to form respiratory competent cells. *Proc. Natl. Acad. Sci. USA* **72,** 372–375.
10. Tandler, B., and Hoppel, C. L. (1986) Studies on giant mitochondria. *Ann. NY Acad. Sci.* **488,** 65–81.
11. Oliver, N. A., and Wallace, D. C. (1982) Assignment of two mitochondrially synthesized polypeptides to human mitochondrial DNA and their use in the study of intracellular mitochondrial interaction. *Mol. Cell. Biol.* **2,** 30–41.
12. Gillespie, F. P., Hong, T.-H., and Eisenstadt, J. M. (1986) Transcription and translation of mitochondrial DNA in interspecific somatic cell hybrids. *Mol. Cell. Biol.* **6,**1951–1957.
13. Hayashi, J.-I., Ohta, S., Kikuchi, A., Takemitsu, M., Goto, Y., and Nonaka, I. (1991) Introduction of disease-related mitochondrial DNA deletions into HeLa cells lacking mitochondrial DNA results in mitochondrial dysfunction. *Proc. Natl. Acad. Sci. USA* **88,** 10614–10618.
14. Hayashi, J.-I., Takemitsu, M., Goto, Y.-I., and Nonaka, I. (1994) Human mitochondria and mitochondrial genome function as a single dynamic cellular unit. *J. Cell. Biol.* **125,** 43–50.
15. Pinkert, C. A., Irwin, M. H., Johnson, L. W., and Moffatt, R. J. (1997) Mitochondria transfer into mouse ova by microinjection. *Transgenic Res.* **6,** 379–383.
16. Marchington, D. R., Barlow, D., and Poulton, J. (1999) *Nature Med.* **5,** 957–960.
17. Hamamoto, T., Ohno, K., and Kagawa, Y. (1982) Net adenosine triphosphate synthesis driven by an external electric field in rat liver mitochondria. *J. Biochem.* **91,** 1759–1766.
18. Wallace, P. K., Palmer, L. D., Perry-Lalley, D., Bolton, E. S., Alexander, R. B., Horan, P. K., Yang, J. C., and Muirhead, K. A. (1993) Mechanisms of adoptive immunotherapy: improved methods for in vivo tracking of tumor-infiltrating lymphocytes and lymphokine-activated killer cells. *Cancer Res.* **53,** 2358–2367.
19. Hirata, H., Ohno, K., Sone, N., Kagawa, Y., and Hamamoto, T. (1986) Direct measurement of the electrogenicity of the H^+-ATPase from thermophilic bacterium PS3 reconstituted in planar phospholipid bilayers. *J. Biol. Chem.* **261,** 9839–9843.
20. Hogan, B., Beddington, R., Costantini, F., and Lacy, E. (1994) *in* Manipulating the Mouse Embryo, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
21. Tokura, T., Noda, Y., Goto, Y., and Mori, T. (1984) *Am. J. Anat.* **171,** 335–355.
22. Cummis, J. M., Wakayama, T., and Yanagimachi, R. (1998) Fate of microinjected spermatid mitochondria in the mouse oocyte and embryo. *Zygote* **6,** 213–222.
23. Sutovsky, P., Moreno, R. D., Ramalho-Santos, J., Dominko, T., Simerly, C., and Schatten, G. (1999) Ubiquitin tag for sperm mitochondria. *Nature* **402,** 371–372.
24. Wallace, D. C. (1999) Mitochondrial diseases in man and mouse. *Science* **283,** 1482–1488
25. Yaffe, M. P. (1999) The machinery of mitochondrial inheritance and behavior. *Science* **283,** 1493–1497.
26. Cortese, J. D. (1998) Stimulation of rat liver mitochondrial fusion by an outer membrane-derived aluminum fluoride-sensitive protein fraction. *Exp. Cell Res.* **240,** 122–133.