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Platelet-mediated Transformation of mtDNA-less Human Cells: Analysis of Phenotypic Variability among Clones from Normal Individuals—and Complementation Behavior of the $tRNA^{Lys}$ Mutation Causing Myoclonic Epilepsy and Ragged Red Fibers

Anne Chomyn,* Susan T. Lai,* Rebecca Shakeley,* Nereo Bresolin,† Guglielmo Scarlato,† and Giuseppe Attardi*

*Division of Biology, California Institute of Technology, Pasadena; and †Institute of Clinical Neurology, University of Milan, Milan

Summary

In the present work, we demonstrate the possibility of using human blood platelets as mitochondrial donors for the repopulation of mtDNA-less (ρ^0) cells. The noninvasive nature of platelet isolation, combined with the prolonged viability of platelet mitochondria and the simplicity and efficiency of the mitochondria-transfer procedure, has substantially increased the applicability of the ρ^0 cell transformation approach for mitochondrial genetic analysis and for the study of mtDNA-linked diseases. This approach has been applied to platelets from several normal human individuals and one individual affected by the myoclonic-epilepsy-and-ragged-red-fibers (MERRF) encephalomyopathy. A certain variability in respiratory capacity was observed among the platelet-derived ρ^0 cell transformants from a given normal subject, and it was shown to be unrelated to their mtDNA content. The results of sequential transfer of mitochondria from selected transformants into a ρ^0 cell line different from the first ρ^0 acceptor strongly suggest that this variability reflected, at least in part, differences in nuclear gene content and/or activity among the original recipient cells. A much greater variability in respiratory capacity was observed among the transformants derived from the MERRF patient and was found to be related to the presence and amount of the mitochondrial $tRNA^{Lys}$ mutation associated with the MERRF syndrome. An analysis of the relationship between proportion of mtDNA carrying the MERRF mutation and degree of respiratory activity in various transformants derived from the MERRF patient revealed an unusual complementation behavior of the $tRNA^{Lys}$ mutation, possibly reflecting the distribution of mutant mtDNA among the platelet mitochondria.

Introduction

The development of a generalized approach for mitochondria-mediated transformation of human cells, which utilizes mtDNA-less (ρ^0) cells as recipients (King and Attardi 1989), has opened the way to the analysis of the functional capacity of mtDNA from normal individuals or from individuals carrying an mtDNA mutation(s). This approach has already proved to be very useful for the study of mtDNA-linked diseases (Chomyn et al. 1991, 1992; Hayashi et al. 1991; King et al. 1992; Yoneda et al. 1992). However, a limitation in its use is the availability of cultures derived from cells (fibroblasts, myoblasts, or lymphoblasts) removed from an individual. The possibility of directly using, as mitochondrial donors, blood cells taken from the individual under investigation would substantially increase the utility of the ρ^0 cell transformation system. In the present report, this approach has been adapted to the use of blood platelets, the anucleate derivatives of megakaryocytes, as mitochondrial donors. Platelets have been

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Address for correspondence and reprints: Dr. Giuseppe Attardi, Division of Biology 156-29, California Institute of Technology, 1201 East California Boulevard, Pasadena, CA 91125.

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shown to contain, on average, four mitochondria per platelet and one mtDNA molecule per organelle (Shuster et al. 1988).

The results of the experiments described here have clearly indicated that the isolation of *pla*telet-derived ρ^0 cell transformants (ROPLA lines) represents a noninvasive approach, applicable to a large base population not accessible to biopsy and tissue-culture probing, for "cloning" the mtDNA of normal individuals or patients affected by mtDNA-linked diseases, for functional and genetic studies. The use of this approach has provided an insight into the genetic origin of the variability in respiratory competence exhibited by different clones derived from a given normal individual and has revealed an unusual pattern of complementation by wild-type mtDNA of the $tRNA^{Lys}$ mutation carried in platelet mtDNA from an individual affected by the MERRF encephalomyopathy.

Material and Methods

Cell Lines and Media

The $\rho^0$206 cell line (King and Attardi 1989), a derivative of 143B.TK$^-$ cells, was plated at a low density, and a fast-growing clone was isolated with a ring and was greatly expanded, and several hundred vials derived from it were frozen. This clone (Cl-3) was grown in Dulbecco's modified Eagle's medium (DMEM) containing 50 μg of uridine/ml and 100 μg of 5-bromodeoxyuridine (BrdU)/ml and was supplemented with 5% dialyzed fetal bovine serum (FBS). The $\rho^0$13 cell line, obtained by long-term exposure, to ethidium bromide, of an adenine phosphoribosyltransferase (APRT)-less mutant of 143B.TK$^-$ (Chomyn et al. 1991), was grown in DMEM with 10% FBS, 50 μg of uridine/ml, 100 μg of BrdU/ml, and 50 μg of 8-azaadenine (selective agent for the APRT-less genotype)/ml. 143B.TK$^-$ cells were grown as described elsewhere (King and Attardi 1989).

Isolation of Platelets

Isolation of platelets was carried out by a modification of a published procedure (Mann et al. 1992). In particular, venous blood (7–20 ml) was collected in heparin tubes and was kept at room temperature until ready for processing. One to four hours after collection (with the exception of the blood from the MERRF patient, which was transported from Italy and thus was kept for 22 h at room temperature before being processed), each sample was mixed with 1/10th vol of 0.15 M NaCl and 0.1 M trisodium citrate (pH 7.0) and was centrifuged for 14 min at ~150 g_{av} in an International centrifuge at 4°C. The top three-fourths of the platelet-rich plasma was removed and centrifuged for 30–40 min at 2,300 g_{av} at 4°C, while a sample was analyzed in the hemocytometer for platelet count. The pellet was resuspended in 10 ml of physiological saline (0.15 M NaCl and 0.015 M Tris-HCl buffer [pH 7.4], at 25°C).

Platelet-mediated Mitochondrial Transformation of $\rho^0$206.Cl-3 Cells

Samples of the washed platelet suspension (1.4×10^7–4×10^7) were centrifuged at 2,300 g_{av} for 20 min at 15°C, and each pellet was overlaid with 10^5 or 10^6 $\rho^0$206.Cl-3 cells in 2 ml of Ca^{++}-free DMEM. After centrifugation in a Clinical centrifuge at 160 *g* for 5–10 min, all of the supernatant was aspirated, and the pellet was resuspended thoroughly in 100 μl of polyethylene glycol (PEG) solution (5 g of PEG 1500 [BDH], 1 ml of 10% dimethylsulfoxide, and 4 ml of Ca^{++}-free DMEM). After 1 min at room temperature, the suspension was diluted with 10 ml of DMEM supplemented with 5% or 10% FBS, 50 μg of uridine/ml, and 100 μg of BrdU/ml and then was distributed in five 6-cm or 10-cm Petri dishes (2×10^4–2×10^5 ρ^0 cells/dish). Three to six days later, the uridine-containing medium was replaced with selective medium (DMEM supplemented with 10% dialyzed fetal bovine serum and 100 μg of BrdU/ml). In this medium, only fusion products of platelets and $\rho^0$206 cells were expected to survive, since $\rho^0$206 cells would not grow in the absence of added uridine (King and Attardi 1989), and, on the other hand, any hybrid between nucleated blood cells contaminating the platelet preparation and $\rho^0$206 cells would be killed by BrdU, converted to its toxic phosphorylated derivative by the thymidine kinase encoded in the blood cell nucleus. Individual clones were isolated 2–4 wk after fusion.

Second-Stage Transfer of ROPLA-Line Mitochondria into $\rho^0$13 Cells

Selected ROPLA cell lines were used as donors for mitochondrial transfer to the $\rho^0$13 cell line. For this purpose, cytoplasts from several thousand cells of each chosen ROPLA line were fused with a 10–20-fold excess of $\rho^0$13 cells, as described elsewhere (King and Attardi 1989), and transformants were isolated in DMEM containing 50 μg of 8-azaadenine/ml and 100 μg of BrdU/ml, supplemented with 10% dialyzed FBS either in 96-microwell plates or in 10-cm plates.

O_2 Consumption and Cytochrome c Oxidase (COX) Measurements

Rate of O_2 consumption was measured with a Gilson 5/6 oxygraph on samples of 5×10^6 cells in 1.85 ml of

DMEM lacking glucose, supplemented with 5% dialyzed bovine serum (King and Attardi 1989). COX activity was measured on samples of 5×10^4 cells treated with digitonin (Storrie and Attardi 1972). For this purpose, 9 μl of a 10% (w/v) solution of digitonin in dimethylsulfoxide were added to 141 μl of buffer (0.025 M Tris-HCl [pH7.5], at 25°C, 0.137 M NaCl, 0.005 M KCl, and 0.007 M Na_2HPO_4) containing 7×10^5 cells. After a 15-min incubation on ice, 10-μl samples of the suspension were used to measure spectrophotometrically the rate of KCN-sensitive oxidation of dithionite-reduced horse cytochrome *c* in a final volume of 1 ml, as described elsewhere (Mason et al. 1973).

DNA Analysis

Total DNA was isolated from cells by using an Applied Biosystems 340A DNA extractor. Platelet DNA from individual S13 was extracted from a pelleted platelet sample, isolated from 0.3 ml plasma, by lysing it in 80 μl of PCR buffer (10 mM Tris-HCl [pH8.4], 50 mM KCl, and 1.5 mM $MgCl_2$) containing 100 μg of proteinase K/ml and 0.5% Tween 20 (Kawasaki 1990), then digesting it at 55°C for 80 min, and finally heating it at 95°C for 20 min. For the quantitation of mtDNA, in some experiments, total DNA samples were digested to completion with *Hae*II in the presence of 10 μg of RNase A/ml, were ethanol precipitated, and were dissolved in 10^{-2} M Tris-HCl (pH 7.4) and 10^{-3} M EDTA; after O.D.260 reading, equal samples (0.2 or 2 μg) were electrophoresed on a 0.8% agarose gel, transferred by capillarity onto a Zetaprobe membrane (BioRad), and probed with total HeLa cell mtDNA, ^{32}P-labeled by random priming (Feinberg and Vogelstein 1983). In other experiments, the quantitation of DNA was carried out by slot blot hybridization, by using the clone pTZ18-K4 (containing the *Eco*RI-*Sac*I fragment of human mtDNA between positions 41 and 2578), labeled by random priming, as a probe. To correct for possible quantitative variations among different DNA samples, the same membrane was probed with a nuclear 28S rRNA gene fragment, which was constructed by PCR amplification using oligonucleotides corresponding to positions 1503–1522 and 1981–2000 in the 28S rRNA gene (Gonzalez et al. 1985), purified on agarose gel, and ^{32}P-labeled by random priming. Quantitation of the hybridizations was carried out by scanning the autoradiograms with an LKB laser densitometer or by analyzing the slot blots in a Phosphorimager (Molecular Dynamics).

For the detection of the MERRF A→G transition in the $tRNA^{Lys}$ gene (Shoffner et al. 1990; Yoneda et al. 1990) at position 8344 in the Cambridge sequence (Anderson et al. 1981), a DNA segment encompassing the mutation was amplified by PCR, using a mismatched primer that generates a new *Nae*I site over the mutated template, and then was digested with *Nae*I (Tanno et al. 1991), in the presence of the 1,075-bp *Xmn*I-*Kpn*I fragment of pBluescript II KS+ as an internal marker for completion of digestion. Equal samples of the various mixtures were analyzed by electrophoresis through a 5% polyacrylamide gel for the detection and quantitation of the MERRF mutation; to check that the internal marker had been completely digested, other aliquots of the same mixtures were run on a 2.5% agarose gel, which gave a better resolution of the digestion products of the marker. The proportion of digested and undigested amplification product was determined by laser densitometry after ethidium bromide staining and was corrected for resistance to digestion of heteroduplexes of wild-type and mutant mtDNA on the basis of a mixed-template standard curve (Shoffner et al. 1990; Yoneda et al. 1992). The latter curve was constructed by subjecting the mixtures of mutant and wild-type reference mtDNA fragments, in different proportions, to PCR amplification and *Nae*I digestion, running the products on a 5% polyacrylamide/7 M urea gel, and determining the proportion of digested and undigested molecules by laser densitometry (M. Yoneda et al., in press).

Results and Discussion

Phenotype and mtDNA Content of ROPLA Lines

All donors were healthy, normal individuals, except donor S13, who was the brother of a previously investigated patient affected by the MERRF encephalomyopathy (Chomyn et al. 1991). This individual was 43 years old at the time of blood sample removal and exhibited muscle weakness, mild psychosis, high serum lactate and pyruvate levels, and the histopathological picture of a mitochondrial myopathy, with numerous ragged red fibers and COX deficiency. Table 1 shows the list of blood donors and the frequency of transformation of $\rho^0$206.Cl-3 with platelet mitochondria. One can see that, with the exception of the experiment with the C1 donor, in which a new batch of PEG, which proved to be more toxic, was used, the frequency of transformants was between 1.0 and $>3.3/10^4$ recipient cells. The low frequency of transformants obtained relative to the number of platelets suggests either that there is a

Table 1

List of Platelet Donors and Frequency of Transformation

Subject	Sex (age in years)	Frequency of Transformation (no./10^4 ρ^0 cells)
D20	F (25)	3.1
B3	F (40)	1.0
H1	M (67)	>3.3
C1	F (73)	>.3
S13	M (43)	1.2

NOTE.—Samples of 1.4×10^7–4×10^7 platelets from each donor were fused, as described in Material and Methods, to 10^5 $\rho^0$206.Cl-3 cells.

low probability, per platelet, of a productive interaction with a ρ^0 cell or that multiple fusions of platelets with the same ρ^0 cell are necessary for the phenotypic rescue of the recipient cell.

Figure 1*a* shows the O_2 consumption rate in several ROPLA lines derived from each of the platelet donors. A certain variability in O_2 consumption rate was observed among the transformants derived from some of the normal donors, in particular D20 and C1. A much more marked variability occurred among the transformants derived from donor S13. The latter was the MERRF patient, and 5 of the 10 transformants obtained from his platelets that were analyzed exhibited the $tRNA^{Lys}$ gene mutation associated with the MERRF syndrome (fig. 2). It is clear from figure 1*a* that these five transformants were the lowest respirers among the S13 clones, as well as in the whole set of 27 ROPLA lines analyzed.

Figure 1*b* shows the COX activity measured in the same transformants as are analyzed for O_2 consumption in figure 1*a*. There was, in general, a good correlation between O_2 consumption rate and COX activity of the individual ROPLA lines. Exceptions were represented by the clone S13-5.1, which exhibited a relatively high COX activity, and the clone H1-6, which showed a low COX activity. Figure 1*c* shows the mtDNA content measured in the same transformants. The mtDNA level in the ROPLA lines was, in general, 50%–100% of the level found in 143B.TK$^-$ (~9,100 molecules/cell; King and Attardi 1989). However, there appeared to be no correlation between O_2 consumption rate and mtDNA content in the various transformants, with the possible exception of clones B3-4.4 and S13-5.1, which exhibited, respectively, ~25% and ~30% of the mtDNA level measured in 143B.TK$^-$.

$tRNA^{Lys}$ Mutation in ROPLA Lines Derived from the MERRF Patient

As mentioned above, only 5 of the 10 S13 transformants analyzed exhibited the $tRNA^{Lys}$ mutation. In particular, as shown in figure 2, three clones (S13-2.2, S13-2.4, and S13-6.2) exhibited the MERRF mutation in strongly predominant form, and two others (S13-1.2 and S13-5.2) exhibited it in a substantial proportion (~50%) of the mtDNA complement. On the other hand, the mutant mtDNA content in the original S13 platelet preparation was 81% (fig. 2). The great variability observed among the transformants derived from the MERRF patient, as concerns the presence and amount of the $tRNA^{Lys}$ mutation, suggested a marked heterogeneity in mutant mtDNA content among the original platelets. Furthermore, the lower-than-expected proportion of transformants exhibiting the mutation pointed to selection against transformants containing only mutant mtDNA, due to the lack of pyrimidine biosynthesis and/or to a propagative advantage of the wild-type mtDNA.

The diagram in figure 3 illustrates the relationship between proportion of mtDNA carrying the MERRF mutation and degree of respiratory activity in the ROPLA lines derived from the MERRF patient. There appears to be a sharp threshold, at ~5%, in wild-type mtDNA proportion, above which this DNA gives partial protection against the phenotypic effects of the mutation. Thus, the rate of O_2 consumption reaches ~2 fmol/min/cell already with ~10% wild-type mtDNA, and it remains close to this level in transformants containing ≤63% wild-type mtDNA (see legend to fig. 3). The observation that levels of wild-type mtDNA as high as 63% failed to produce full recovery of respiration is in contrast to what has recently been found in ρ^0 cell transformants derived from a different MERRF patient's myoblasts. In the latter transformants, levels of wild-type mtDNA >10% exerted full protective effect (Yoneda et al., in press), similar to what previous studies had shown in heteroplasmic myotubes formed by fusion of MERRF mutation–containing and wild-type myoblasts (Boulet et al. 1992) and in MELAS mutation–containing ρ^0 cell transformants derived from patient myoblasts (Chomyn et al. 1992). This discrepancy may be related to the very low copy number of mtDNA molecules per mitochondrion in the donor platelets (average of one molecule per organelle; Shuster et al. 1988). In fact, recent data have revealed an important role of intramitochondrial heteroplasmy in the protective effect of wild-type mtDNA

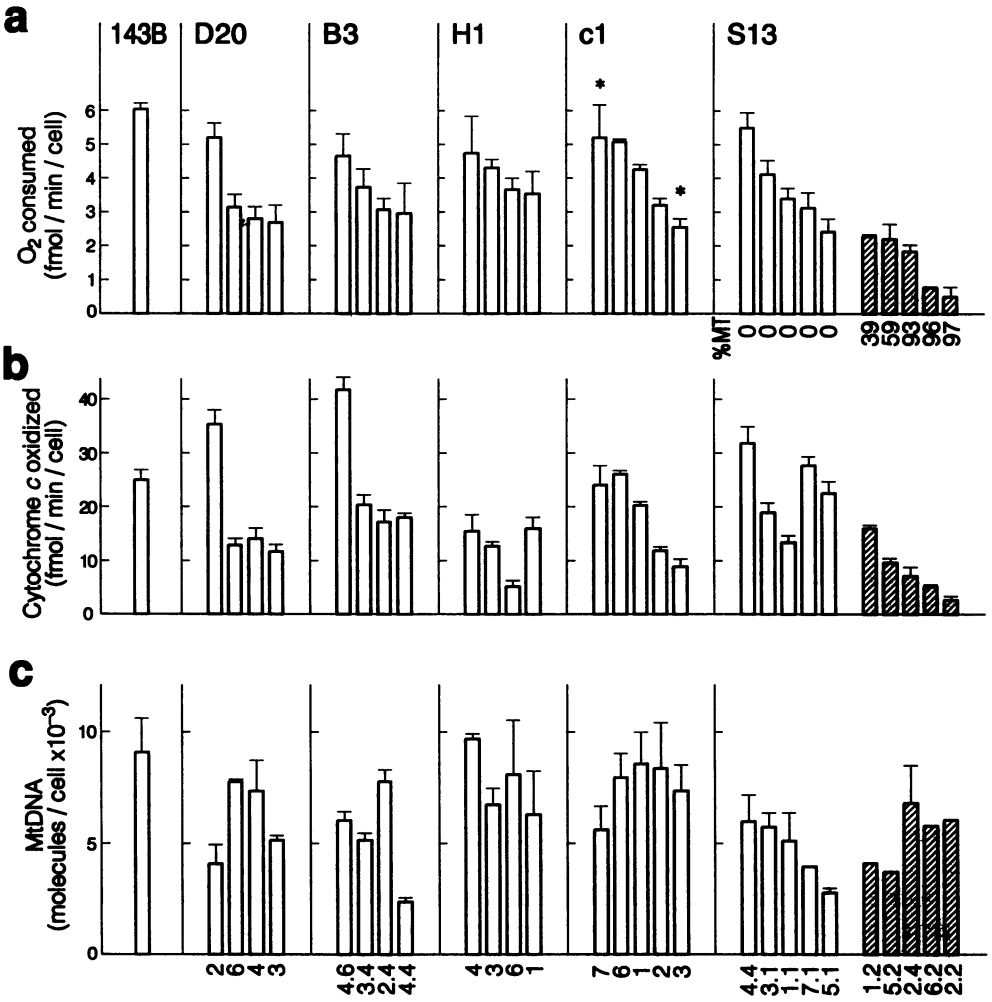

Figure 1 Rates of O_2 consumption (*a*), levels of COX activity (*b*), and mtDNA contents (*c*), both in ROPLA lines obtained by mitochondria transfer from platelets of different human individuals into $\rho^0$206.Cl-3 cells and, for comparison, in 143B.TK$^-$ cells. The mtDNA contents of the individual transformants were determined by Southern blot and/or slot blot analysis, as described in Material and Methods, and were converted to number of mtDNA molecules per cell, on the basis of the comparison with the 143B.TK$^-$ cell line, whose mtDNA content had been determined elsewhere (9,1000 molecules/cell; King and Attardi 1989). Transformants were tested for O_2 consumption rate, COX activity, and mtDNA content 7–16 wk, 5–14 wk, and 7–14 wk, respectively, after fusion. The time of respiration measurement corresponded, in general, within 1 and 2 wk, to the time of DNA analysis and COX activity determination, respectively. The error bars represent two standard errors. The S13-derived transformants represented by cross-hatched bars exhibited the MERRF mutation. The asterisks indicate the ROPLA lines used as donors for mitochondrial transfer to the $\rho^0$13 cell line (see fig. 4). %MT = percentage of mtDNA that carries the MERRF mutation.

against the phenotypic effects of the MERRF mutation in myoblast-derived cybrids, and, furthermore, substantial evidence has pointed to the genetic independence of individual mitochondria introduced into ρ^0 cells (Yoneda et al., in press). Since a considerable proportion of mutant molecules presumably resided in pure form in the individual organelles of the S13 patient platelets, if the conclusions mentioned above apply to the present system, it has to be expected that these mutant molecules would not be subject to intramitochondrial complementation in the derived transformants. However, further work is needed to ascertain whether the unusual pattern of complementation by wild-type mtDNA of the MERRF mutation carried in platelet-derived transformants is specifically related to the low copy number of mtDNA molecules per organelle in platelets or whether it can be found also in cybrids derived from other cell types.

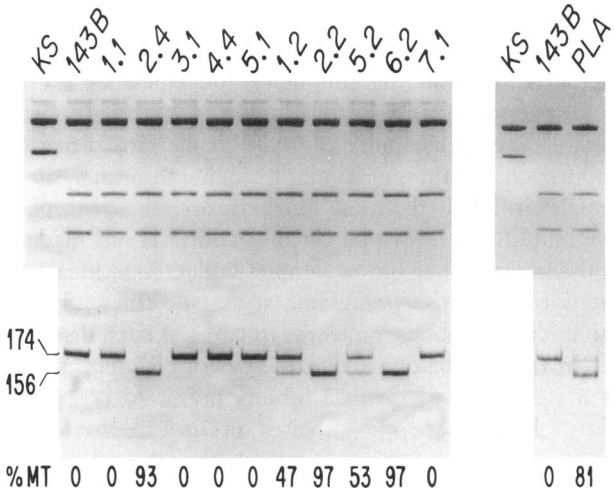

Figure 2 Detection of the MERRF mutation in 10 ROPLA lines obtained by transfer of platelet mitochondria from MERRF patient S13 into $\rho^0$206.Cl-3 cells (shown in fig. 1). The analysis was carried out on samples collected 6–14 wk after fusion. The upper portion of the figure shows the *Nae*I digestion products of the internal marker *Xmn*I-*Kpn*I fragment of pBluescript II KS(+), separated on a 2.5% agarose gel. The lower portion of the figure shows the *Nae*I digestion products of the DNA samples from the 10 S13-derived transformants and from 143.TK$^-$, separated on a 5% polyacrylamide gel; indicated are the expected sizes of the PCR product (174 bp) and of the larger of the two fragments produced by *Nae*I digestion of the MERRF mutation-containing PCR product (156 and 18 bp). KS = undigested *Xmn*I-*Kpn*I fragments of pBluescript II KS(+); PLA = platelet mtDNA; and %MT = percentage of mtDNA that carries the MERRF mutation.

Analysis of Phenotype Variability among the Normal ROPLA Lines

The variability in O_2 consumption rate observed among the transformants derived from some of the normal platelet donors could reflect nuclear differences among the recipient $\rho^0$206.Cl-3 cells or, alternatively, could result from mtDNA heterogeneity in the platelet population. In order to distinguish between these two possibilities, a second-stage transfer of mitochondria from selected $\rho^0$206.Cl-3-derived ROPLA lines into $\rho^0$13 cells was performed. The highest and lowest respirers among the C1 clones (C1-7 and C1-3, respectively) (fig. 1*a*) were chosen for this second-stage mitochondrial transformation.

As shown in figure 4*a*, in each of the two sets of second-stage transformants, the degree of heterogeneity in O_2 consumption rate was similar to that observed in the set of the original C1 ROPLA lines (fig. 1*a*). However, the most significant result of this set of experiments was that both the ranges and the averages of the O_2 consumption values obtained for the C1-7 and C1-3 second-stage transformants were substantially identical (fig. 4*a*). By contrast, the original C1-7 and C1-3 clones, kept in culture in parallel to the secondary transformants, maintained the substantial difference in O_2 consumption rate exhibited at the time of the second-stage transfer of their mitochondria into $\rho^0$13 cells (fig. 4*b*). No significant mtDNA-content difference between the two sets of second-stage transformants was observed (data not shown). The results described above pointed to the role of variability in nuclear gene content and/or activity among the recipient $\rho^0$206.Cl-3 cells in accounting for the difference in O_2 consumption rate observed between the original C1-7 and C1-3 clones. The alternative possibility—i.e., of an unequal transfer into ρ^0 cells of genotypically variable platelet mitochondria at the first transformation step, followed by a nonrandom second-stage transfer of organelles into the second-type recipient cells—seems less likely but cannot be absolutely excluded.

A variability in respiratory competence had previously been observed among cybrids obtained by fusion of $\rho^0$206 cells with cytoplasts derived either from an established cell line, HT1080 (King and Attardi 1989), or from normal myoblasts of the same individual (Chomyn et al. 1991, 1992). However, it had not been established whether this variability reflected nuclear heterogeneity among the recipient ρ^0 cells or mtDNA structural differences among the donor cells. The experiments described above have provided the first evidence pointing to a role of the recipient cells in deter-

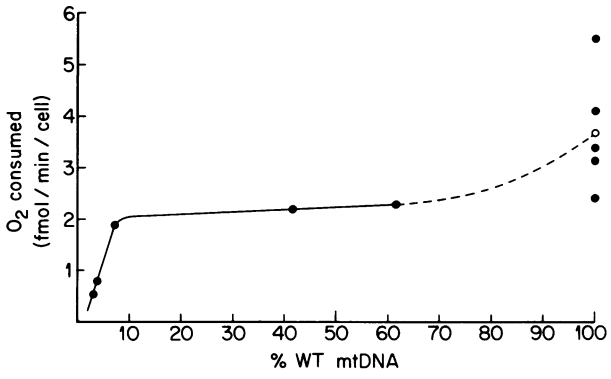

Figure 3 Relationship between respiratory activity of the 10 ROPLA lines derived from the MERRF patient S13 and their wild-type mtDNA content. The genotype of each transformant was determined either at the same time as or, in two cases, within 8 d of determination of its O_2 consumption rate. The slight difference from fig. 2, in the amount of mutant mtDNA in some of the samples, is due to sampling the cells 1–2 wk later.

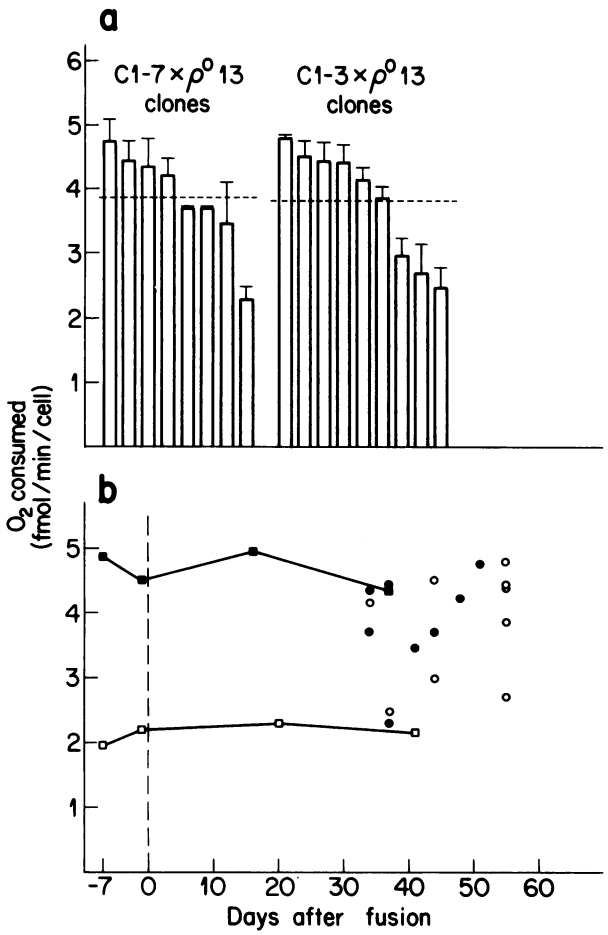

Figure 4 *a*, Rates of O_2 consumption in second-stage transformants obtained by mitochondria transfer from selected $\rho^0$206.Cl-3-derived ROPLA lines (C1-7 and C1-3 in fig. 1) into $\rho^0$13 cells. *b*, Rates of O_2 consumption in the original ROPLA lines used as donors, at the time of the second-stage transformation and at different times before and after (■ = C1-7; and □ = C1-3), compared with those of the second-stage cybrids shown in panel *a* (● = C1-7 derivatives; and ○ = C1-3 derivatives). The horizontal dashed lines in panel *a* represent the average O_2 consumption rate in each set of second-stage transformants. The respiration rates of these transformants were measured 34–55 d after fusion.

mining the phenotypic differences among ρ^0 cell transformants. Furthermore, they have identified a level of ~2 fmol/min/cell as that below which a respiration defect in the platelet-derived transformants obtained with the ρ^0 cells utilized here is likely to be due to mtDNA structural alteration(s).

Potential Use of ROPLA Lines

The present paper has demonstrated the possibility of using human platelets as mitochondrial donors for the repopulation of mtDNA-less cells (King and Attardi 1989). The noninvasive nature of the method of platelet isolation and the simplicity and efficiency of the mitochondria transfer procedure have extended considerably the applicability of the ρ^0 cell transformation approach for the genetic and biochemical analysis of mtDNA-linked diseases. Furthermore, the prolonged viability (≥22 h) of platelet mitochondria has made it possible to utilize blood samples collected at locations remote from the processing site, increasing substantially the possibility of access to cases of such diseases.

In view of the unusual properties of platelets as concerns both the small number of mitochondria per platelet and the low copy number of mtDNA molecules per organelle, it is pertinent to ask whether the cybrids derived from platelets represent a valid model for the functional and genetic analysis of mtDNA and for the study of mtDNA-linked diseases, as has been shown for cybrids derived from other cell types, such as myoblasts, fibroblasts, and transformed cell lines (King and Attardi 1989; Chomyn et al. 1991, 1992; Hayashi et al. 1991; King et al. 1992). In this connection, it would be useful to compare the cybrids derived from platelets with those derived from another cell type of either the same individual or the same maternal lineage. This comparison is indeed already possible in the case of the S13-derived ROPLA lines. In fact, cybrids were previously constructed using $\rho^0$206 cells and myoblasts derived from a MERRF patient maternally related to the S13 donor and, therefore, carrying the same mtDNA as does the latter individual (Chomyn et al. 1991). In this case, five transformants were isolated, three homoplasmic or near homoplasmic for the MERRF mutation, which exhibited severe protein synthesis and respiration defects, and two containing wild-type mtDNA in pure or near pure form, which exhibited normal phenotype. The lack of transformants containing intermediate mixtures of wild-type and mutant genomes in the work mentioned above presumably reflected the distribution of the two genomes in the patient myoblast population, as has been subsequently shown for myoblasts from other MERRF patients (Boulet et al. 1992). In the case of the ROPLA lines, the occurrence of mixed genomes in some of the transformants may have resulted (a) from fusion of multiple platelets containing or lacking the MERRF mutation with the same ρ^0 cell and/or (*b*) from coexistence of the mutant and wild-type genomes in the same platelet. In any case, it is clear that, for the analysis of both the pathogenetic mechanism of a disease-causing mtDNA mutation and the role of the nuclear genome in the manifestation of the biochemical

defect(s) associated with the mutation, the possibility of producing cell lines containing in pure form the mutant genome or the wild-type genome makes the use of platelets as mitochondrial donors a perfectly valid approach. On the other hand, the difference, in the pattern of complementation of the MERRF mutation, between the ROPLA lines and both the myoblast-derived ρ^0 cell transformants (Yoneda et al., in press) and heteroplasmic myotubes from MERRF patients (Boulet et al. 1992) indicates that the complementation and segregation behavior of a mtDNA mutation may vary between different cell types and derived transformants, reflecting the different distribution of the mutation among the organelles and the capacity of the mutant and wild-type genomes to interact with each other (Yoneda et al., in press).

A report of a specific dysfunction of complex I of the respiratory chain in platelets from Parkinson disease patients (Parker et al. 1989) has been confirmed recently in several laboratories (Krige et al. 1992; Yoshino et al. 1992; Benecke et al. 1993). Similarly, there have been reports of COX deficiency in platelets from Alzheimer disease patients (Parker et al. 1990). While the genetic basis of these dysfunctions has not been clarified, the possibility exists that a particular mtDNA haplotype, conceivably combined with aging-related mtDNA alterations, has at least a contributing role in their etiology. In fact, attention recently has been called to the existence of distinctive mtDNA haplotypes in some patients affected by Parkinson and Alzheimer diseases (Ozawa et al. 1991; Shoffner et al. 1993); furthermore, the observed occurrence of dysfunctions of respiratory complexes in muscles of Parkinson patients (Bindoff et al. 1991) has emphasized the presence of a genetic component in this disorder. It should also be noted that platelets derive from postmitotic cells, the megakaryocytes, which, as such or as their precursors, may accumulate aging-related mutations throughout the life of the organism.

The above-mentioned considerations are relevant to a possible use of the platelet-mediated mitochondrial transformation of ρ^0 cells for the analysis of the role of inherited or oxygen radical–produced mutations of the mitochondrial genome in neurodegenerative disorders, such as Alzheimer or Parkinson disease, or in aging. While the present work has established a baseline for the detection of any disease- or aging-related mtDNA-linked respiratory defects in the platelet-derived transformants produced with the ρ^0 cells utilized here, it has to be expected that further improvements of the technique will increase the sensitivity of variant-type detection at the level of total respiration, activity of different enzymes of the oxidative phosphorylation apparatus, mitochondrial protein synthesis, etc. In particular, an increase in efficiency of platelet-mediated transformation of ρ^0 cells (e.g., by electroporation) should allow the use of smaller and more homogeneous clonal populations of recipient cells—and thus decrease significantly any background variation resulting from nuclear differences among ρ^0 cells. Furthermore, the application to ROPLA lines of the presently available rapid screening methods for detection of structural alterations of mtDNA—such as the SSCP (Orita et al. 1989) and the GC-clamp denaturing gradient gel electrophoresis (DGGE) (Abrams and Stanton 1992) methods—may lead to the identification of the mtDNA mutations responsible for the defective phenotypes observed in ρ^0 cell transformants derived either from patients affected by neurodegenerative diseases or from aged individuals. Recent work has shown that the DGGE method has a sensitivity adequate for detection of mtDNA mutations present in a proportion as low as 1% of the mtDNA complement (G. Hofhaus and G. Attardi, unpublished observations).

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