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Cytoplasmic Transfer of Platelet mtDNA from Elderly Patients with Parkinson's Disease to mtDNA-less HeLa Cells Restores Complete Mitochondrial Respiratory Function

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For determination of whether platelet mtDNA in patients with Parkinson's disease (PD) possesses some lesions to reduce respiratory enzyme activities, platelet mtDNA was transferred into mtDNA-less (ρ^0) HeLa cells from aged PD patients and age-matched normal subjects, since their activities were controlled by both mitochondrial and nuclear genomes. The resultant mtDNA-repopulated cybrid clones containing the HeLa nuclear genome as a common background were used for comparison of respiratory enzyme activities. Remarkable variations of the enzyme activities were observed in the cybrid clones, irrespective of whether their mtDNA was transferred from normal subjects or PD patients, and some of them showed 20% reduction of average activities. Thus, the mtDNA mutations responsible for inducing 20% reduction should be polymorphic rather than pathogenic. On the other hand, pathogenic control cybrid clones possessing mtDNA mutations from patients with mitochondrial disorders showed significant and specific decline of respiratory enzyme complex I activity beyond the normal range of the variations. These observations warrant reassessment of the conventional concept that complex I activity in platelets of PD patients is defective due to mtDNA mutations.

There have been many reports that human mitochondrial respiration capacity decreases with age or with development of neurodegenerative diseases (1–6). Recently, this age-related decline in energy production capacity was proposed to be caused by accumulation of somatic mtDNA mutations, which have been shown to cause various kinds of mitochondrial diseases (2, 7–9). Besides these acquired pathogenic mtDNA mutations, maternally transferable lesions in mtDNA have also been suggested to be involved in the pathogeneses of neurodegenerative diseases (10–14). For example, transfer of platelet mtDNA from patients with Parkinson's disease (PD) and Alzheimer's disease (AD) to ρ^0 neuroblastoma cells consistently resulted in depression of the activities of respiratory enzymes complex I (11) and complex IV (12, 13), respectively. However, there is as yet no convincing evidence that mutations of mtDNA from the mother and/or acquired mutations of mtDNA during life are causal genetic factors of mitochondrial dysfunction in aged subjects and patients with neurodegenerative diseases.

Recently, we reported that the observed age-related reduction of respiratory enzyme activities in cultured human skin fibroblasts isolated from donors of various ages (0–97 years) (4) was not due to mtDNA mutations (4, 15), since isolation of cybrids by mtDNA transfer from fibroblasts to ρ^0 HeLa cells (16) and isolation of nuclear hybrids by nuclei transfer from ρ^0 HeLa cells to fibroblasts (17) provided convincing evidence that the age-related disorders were due to nuclear-recessive mutations of factors required for translation in mitochondria (15). Moreover, our conclusions generated from studies on human fibroblasts could be extended to human brain tissues showing much higher oxidative activities than fibroblasts (18): mtDNA in autopsied brain tissues from aged subjects with AD was rescued in ρ^0 HeLa cells by fusion of brain synaptosomal frac-

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TABLE 1

Characterization of Cybrids Possessing Imported mtDNA from Platelets of Normal Subjects and Patients with PD and with Mitochondrial Diseases

Parents and cybrids	Fusion	Pathogenic mutations
Parents		
Nuclear donors (mtDNA recipients)		
ρ^0 HeLa cells		
mtDNA donors		
PD1–PD10 (platelets from PD patients 1–10)		
N1–N10 (platelets from normal subjects 1–10)		
HeLa cells		
TIG102 (fibroblasts from an aged subject)		
P1 (CM4269, platelets from a CM patient)		
P2 (MELAS3243, fibroblasts from a MELAS patient)		
P3 (CM3394, platelets from a CM patient)		
P4 (DM14577, platelets from a DM patient)		
Cybrid clones		
CyPD1–PD10	ρ^0 HeLa cells × PD1–PD10	
CyN1–N10	ρ^0 HeLa cells × N1–N10	
CyHeEB	ρ^0 HeLa cells × HeLa cells	
CyAg1	ρ^0 HeLa cells × TIG102	
CyP1	ρ^0 HeLa cells × P1	4269 A/G in *tRNA*^{Ile}
CyP2	ρ^0 HeLa cells × P2	3243 A/G in *tRNA*^{LeuUUR}
CyP3	ρ^0 HeLa cells × P3	3394 T/C in *ND1*
CyP4	ρ^0 HeLa cells × P4	14577 T/C in *ND6*

tions with ρ^0 HeLa cells, and this mtDNA transfer resulted in complete restoration of mitochondrial respiratory function, suggesting functional integrity of the mtDNA in brain from the aged AD subjects.

In cases of PD, preferential decline of complex I activity was reported in platelets (19–21) and postmitotic tissues (22–26), whereas other reports suggested the absence of complex I deficiency (27–29). Recently, Swerdlow *et al.* (11) isolated cybrid clones by cytoplasmic transfer of platelet mtDNA from PD patients into ρ^0 human cells, and concluded that all PD patients consistently possessed putative mtDNA lesions that were responsible for a stable 20% reduction of complex I activity. Similar results were obtained by Gu *et al.* (30) and Trimmer *et al.* (31). However, none of these reports identified pathogenic mtDNA mutations causing complex I deficiency. On the other hand, cytoplasmic transmission of the stable and specific abnormality of complex I activity (11, 30, 31) represents homoplasmic mtDNA mutations responsible for the complex I deficiency of the PD patients. Considering strictly maternal inheritance of mtDNA (32, 33), both the putative mutant mtDNAs and the resultant disease phenotypes have to be transmitted maternally as they are transmitted cytoplasmically in cell cultures (11, 30, 31). However, most cases of the disease are sporadic and no reports unequivocally show its maternal transmission.

To address these controversial issues, we examined the limit of the normal variation of respiratory enzyme activities created by polymorphic mtDNA mutations using ten cybrid clones with mtDNAs from ten different individuals in the normal population. As pathogenic controls, we used cybrid clones with identified pathogenic mtDNA mutations responsible for specific reduction of complex I activity in patients with mitochondrial diseases (34–36), and found that platelet mtDNAs from PD patients are functionally intact.

MATERIALS AND METHODS

Cells and cell culture. In addition to cybrid clones CyN1–N10 isolated in this study, we used a cybrid clone CyHeEB (34), and a cybrid clone CyAg1 (4) (Table 1), as normal control cybrid clones with mtDNA from normal subjects, since they had been used as standards in our experiments for determination of the pathogenicity of mtDNA mutations, and were shown to be reliable in possessing average respiratory enzyme activities of cybrid clones with mtDNA from normal subjects (4, 18, 34–36). As pathogenic controls showing a decrease of only complex I activity leading to expression of the disease phenotypes, we used two cybrid clones, CyP3 and CyP4, possessing homoplasmic pathogenic mutant mtDNA with a 3394 A/G mutation in the ND1 gene from a patient with cardiomyopathy (CM) (35) and with a 14577 T/C mutation in the ND6 gene from a patient with diabetes mellitus (DM) (36), respectively. Furthermore, two overall respiration-deficient cybrid clones, CyP1 and CyP2, possessing predominantly mutant mtDNA with a 4269 A/G mutation in the tRNA^{Ile} gene from a patient with CM and with a 3243 A/G mutation in the tRNA^{LeuUUR} gene from a patient with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), respectively, were also used as pathogenic controls (Table 1). The ρ^0 HeLa ells (16) and all cybrid clones isolated by fusion of ρ^0 HeLa cells with platelets were grown in normal medium: RPMI 1640 (Nissui Seiyaku, Tokyo) containing 10% fetal calf serum, 50 mg/ml uridine and 0.1 mg/ml pyruvate.

mtDNA donors. Blood samples provided with informed consent from 10 patients with PD and 10 normal subjects were used as mtDNA donors. The ten PD patients, PD1–PD10, were female/64 yr,

female/82 yr, female/65 yr, female/74 yr, female/57 yr, male/63 yr, female/79 yr, female/59 yr, female/70 yr, male/75 yr, respectively. All patients had at least two of the three cardinal features of PD, i.e., resting tremor, rigidity, or akinesia, and showed a good response to levodopa. The average age of the patients was 68.8 ± 27. Ten age-matched normal subjects, N1–N5, were a female/43 yr, female/67 yr, female/76 yr, female/85 yr, female/85 yr, male/74 yr, male/66 yr, female/59 yr, male/64 yr, and female/81 yr, respectively. The average age of the normal subjects was 70.0 ± 27. mtDNAs of CyAg1, P1, CyP2(34), CyP3, and CyP4 were derived from a 97 yr normal subject (4), a patient with fatal CM (34), and a patient with mitochondrial myopathy, encephalopathy, lactic acidosis, and stroke-like episodes (MELAS), a patient with familial cardiomyopathy (CM) (35), a patient with maternally inherited type 2 diabetes mellitus (DM) (36).

Introduction of platelet mtDNA into ρ^0 HeLa cells. We recently developed a simple procedure for preparation of a platelet-rich fraction from a very small amount (about 1 ml) of peripheral blood (18). The platelet-rich fraction was used for fusion to ρ^0 HeLa cells, resulting in isolation of mtDNA repopulated cybrid clones. Fusion of platelets with ρ^0 HeLa cells was carried out in the presence of 50% (w/v) polyethylene glycol 1500 (Boehringer Mannheim, Germany). The fusion mixture was cultivated in selection medium RPMI 1640 without pyruvate and uridine, in which even cybrids with very low COX activity have been shown to grow (34). On day 14–30 after fusion, the cybrid clones growing in the medium were isolated clonally by the cylinder method. Then cybrid clones with mtDNA were screened by PCR analysis, so that even cybrids with no COX activity could be isolated.

Biochemical measurement of respiratory enzyme activities. Cells in log-phase growth were harvested, and complex I, complex II+III, and complex IV activities were measured as described before (34).

Analysis of mitochondrial translation products. Mitochondrial translation products were labeled with [^{35}S]methionine as described previously (34). Proteins in the mitochondrial fraction were separated by 0.85% SDS, 12% polyacrylamide gel electrophoresis. For quantitative estimation of [^{35}S]methionine-labeled polypeptides, the dried gel was exposed to an imaging plate for 12 h and the radioactivities of polypeptides were measured with a bioimaging analyzer, Fujix BAS 5000 (Fuji Film, Japan).

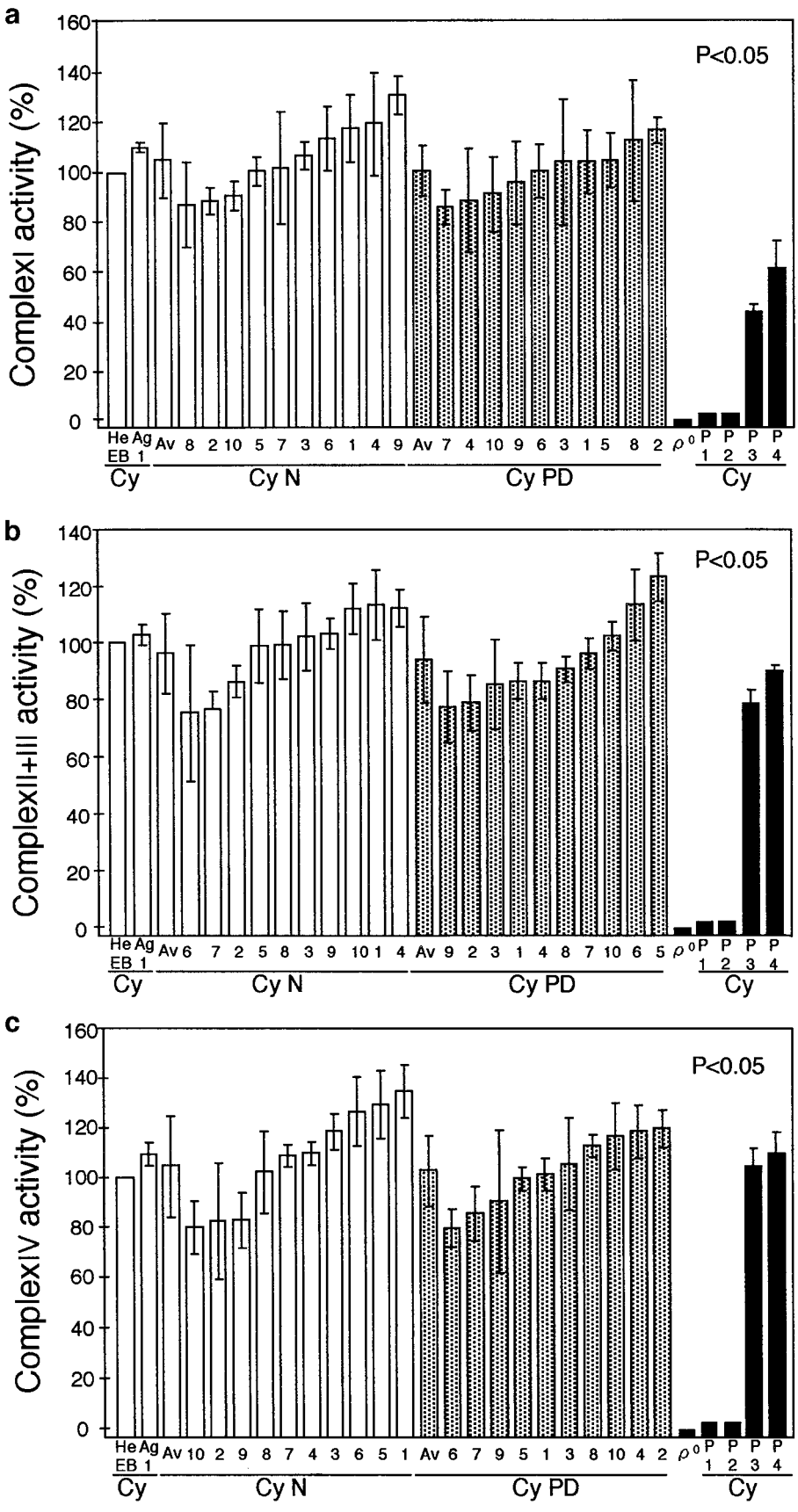
Measurements of oxygen consumption. The rate of oxygen consumption was measured by trypsinizing cells, incubating the suspension in phosphate-buffered saline, and recording oxygen consumption in a polarographic cell (1.0 ml) at 37°C with a Clark-type oxygen electrode (Yellow Springs Instruments, OH) (36).

RESULTS

Since nuclear DNA mutations as well as mtDNA mutations could affect respiratory complex activities, we isolated mtDNA repopulated cybrid clones with the same nuclear background, and compared the enzyme activities of cybrids with mtDNA from PD patients and from normal subjects for determination of the involvement of mtDNA mutations in reduction of the enzyme activities. In this study, only 1 ml blood samples from PD patients and from age-matched normal subjects were used for isolation of 10 cybrid clones with mtDNA from 10 PD patients (CyPD1–CyPD10) and 10 cybrid clones with mtDNA from 10 normal subjects (CyN1–CyN10), respectively (Table 1). Using all these cybrid clones containing the HeLa nuclear genome as common nuclear background, we compared the mitochondrial respiratory enzyme activities of complex I, complex II+III, and complex IV of cybrids with mtDNA from PD patients and from age-matched normal subjects (Fig. 1), because these enzyme activities were possibly affected by mtDNA mutations.

Results showed the presence of significant variations in all complex I, complex II+III, and complex IV enzyme activities even in cybrid clones with mtDNA from age-matched normal subjects (Fig. 1). For example, 20% reduction of complex I activity was observed in three cybrid clones, CyN-8, -2, and -10 (Fig. 1a), whereas other clones showed more than 20% reduction of complex II+III or complex IV activity (Figs. 1b and 1c). Moreover, comparison of different respiratory enzyme activities of the same cybrid clones showed the absence of their coordinated reduction in CyN1–10 (Fig. 2). Similar results were obtained from cybrid clones with mtDNA from patients with PD (CyPD1–CyPD10). Cybrid clones CyPD1–CyPD10 restored mitochondrial respiration activity to almost comparable levels to those of cybrid clones CyN1–CyN10 with mtDNA from age-matched normal subjects (Fig. 1). Although CyPD1–CyPD10 showed significant variations in respiration enzyme activities, the ranges of the variations were within those of the variations obtained from the cybrid clones with mtDNA from normal subjects, and no preferential reduction of complex I activity or other complex activities was observed in these cybrid clones (Fig. 2).

On the other hand, two pathogenic cybrid clones, CyP3 and CyP4, showed remarkably reduced complex I activity beyond the normal variation ranges, whereas their other respiratory complex activities were within normal ranges (Figs. 1 and 2). These cybrid clones were used as pathogenic controls, since their mtDNA mutations 3394 and 14577 have been shown to be pathogenic for development of CM (35) and DM (36), respectively. We also used two completely respiration-deficient cybrid clones, CyP1 and CyP2, possessing predominantly mtDNA with a 4269 mutation and with a 3243 mutation, respectively. These pathogenic control cybrid clones showed coordinated reduction of activities of all respiratory complexes (Fig. 2), which would be due to overall reduction of mitochondrial translation (cf. Fig. 3a) as a consequence of pathogenic mutations in the tRNA genes required for mitochondrial translation. Therefore, it is unlikely that we selected only respiration-competent cybrid clones possessing mtDNA from PD patients, because we used a selection procedure that selectively excluded cells without respiration activity, such as unfused parental ρ^0 HeLa cells, but did not exclude pathogenic control cybrid clones, even when they possessed extremely low respiration activity (Fig. 1). These observations suggest that none of the 10 PD patients we examined possessed pathogenic mtDNA mutations that could specifically reduce complex I activity or overall complex activities beyond the normal variation ranges.


a
ComplexI activity (%)
P<0.05
He Ag EB 1
Av 8 2 10 5 7 3 6 1 4 9
Av 7 4 10 9 6 3 1 5 8 2
ρ0
P 1 P 2 P 3 P 4
Cy
Cy N
Cy PD
Cy
b
ComplexII+III activity (%)
P<0.05
He Ag EB 1
Av 6 7 2 5 8 3 9 10 1 4
Av 9 2 3 1 4 8 7 10 6 5
ρ0
P 1 P 2 P 3 P 4
Cy
Cy N
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ComplexIV activity (%)
P<0.05
He Ag EB 1
Av 10 2 9 8 7 4 3 6 5 1
Av 6 7 9 5 1 3 8 10 4 2
ρ0
P 1 P 2 P 3 P 4
Cy
Cy N
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Cy

For testing the overall functional integrity of platelet mtDNAs from PD patients, we examined the translation in mitochondria and O_2 consumption rates using CyN8, CyN9, CyPD7, and CyPD2 cybrid clones possessing the lowest and highest complex I activities of CyN and CyPD clones (Fig. 1a), respectively (Fig. 3). The amounts and molecular sizes of all mtDNA-encoded polypeptides of CyPD cybrids deduced from their intensities of [^{35}S]methionine incorporation and their mobilities on electrophoresis were almost comparable to those of all mtDNA-encoded polypeptides of CyN cybrids (Figs. 3a and 3b). Similar results were obtained when O_2 consumption rates were compared (Fig. 3c). On the other hand, pathogenic control CyP3 cybrids showed normal [^{35}S]methionine incorporation (Figs. 3a and 3b), but reduced O_2 consumption rates (Fig. 3c), suggesting that a 3394 T/C mutation in the ND1 gene is responsible for the production of inactive ND1 polypeptide that simultaneously reduces complex I activity and the O_2 consumption rates. Therefore, the amounts and functions of the mitochondrial translation products of PD patients are sufficient to maintain normal respiratory function, suggesting the complete functional integrity of platelet mtDNAs from PD patients.

DISCUSSION

In this study, the normal limits of the variation of mitochondrial respiratory enzyme activities created by polymorphic mtDNA mutations in various individuals from a normal population were carefully examined before determination of the presence of pathogenic mtDNA mutations in PD patients, since candidate mtDNA mutations being responsible for the pathogenesis were not identified in PD patients. In cases in which mtDNA mutations were already determined, and existed as heteroplasmy, i.e. when mutant and wild type mtDNA coexisted within the same patients, the pathogenicity of the mtDNA mutations could be easily proved by the lower respiration enzyme activity in cybrids with predominantly the mutant mtDNA than in those with wild type mtDNA from the same patients (16, 17, 34). On the other hand, when mtDNA mutations were homoplasmic or were not yet determined as cases of PD patients, respiratory enzyme activities of cybrid clones with mtDNA from patients and from normal subjects had to be compared. Although each human individual possesses homogeneous mtDNA sequences, many different polymorphic mutations are present in different individuals, and some of them could affect respiratory enzyme activities. Therefore, even when cybrid clones show decreased enzyme activities, it is very difficult to determine whether the decrease is due to polymorphic or pathogenic mtDNA mutations. We carefully examined the normal limits of the variation of mitochondrial respiratory enzyme activities, and clearly showed that none of the 10 PD patients we tested possessed pathogenic mtDNA mutations that could specifically reduce complex I activity

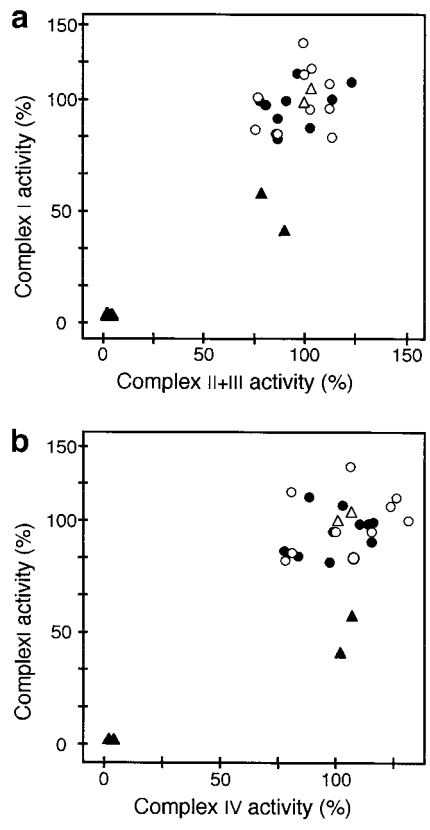

FIG. 2. Relationship between activities of complex I and complex II+III (a), and complex I and complex IV (b) in cybrid clones. Open circles, cybrids with mtDNA from normal subjects (CyN1–CyN10); open triangles, cybrids with mtDNA from normal subjects (CyHeEB, CyAg1); closed circles, cybrids with mtDNA from PD patients (CyPD1–CyPD10); closed triangles, cybrids with mtDNA from patients expressing mitochondrial disorders (CyP1, CyP2, CyP3, CyP4). Complex I activity of cybrids with mtDNA from patients expressing mitochondrial disorders was reduced beyond the normal range, while that of cybrids with mtDNA from PD patients were within the normal range.

FIG. 1. Comparison of respiratory enzyme activities in cybrids possessing imported mtDNA from normal subjects and patients with PD or with mitochondrial diseases. a, complex I activity; b, complex II+III activity; c, complex IV activity. CyHeEB, cybrids with imported HeLa mtDNA; CyAg1, cybrids with fibroblast TIG102 mtDNA; CyN1–CyN10, cybrids with platelet mtDNA from normal subjects 1–10; CyPD1–CyPD19, cybrids with platelet mtDNA from PD patients 1–10; ρ^0, ρ^0 HeLa cells; CyNAv and CyPDAv are average activities of CyN1–CyN10 (open bars) and CyPD1–CyPD10 (dotted bars), respectively. Open bars, cybrids with mtDNA from normal subjects (CyHeEB, CyAg1, CyN1–CyN10); dotted bars, cybrids with mtDNA from PD patients (CyPD1–CyPD10); solid bars, ρ^0 HeLa cells and cybrids with mtDNA from patients expressing mitochondrial disorders (CyP1, CyP2, CyP3, CyP4).

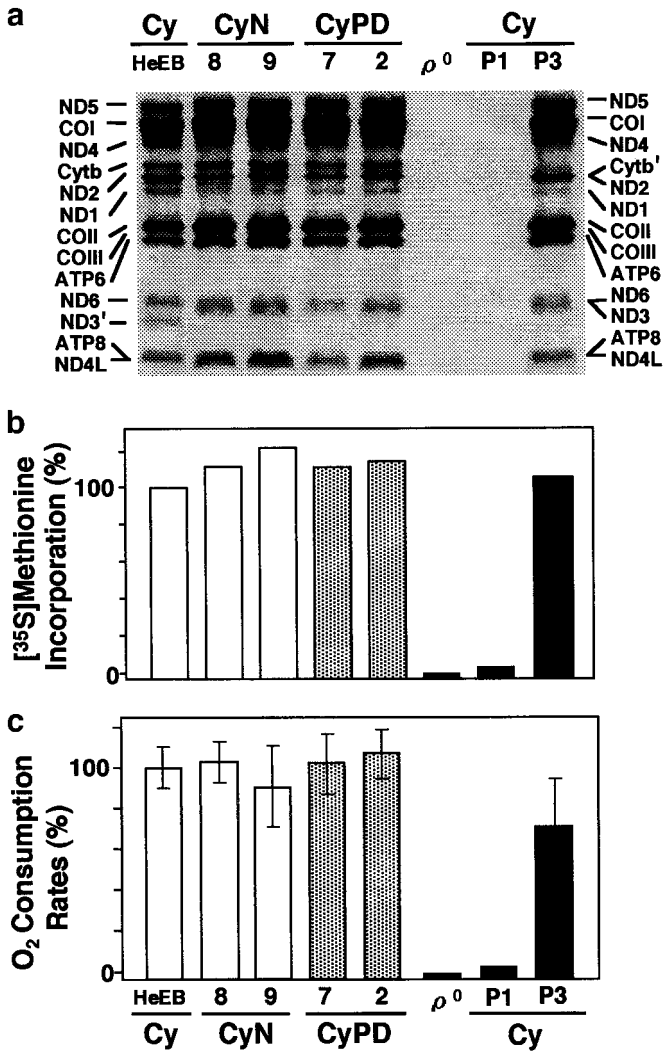

FIG. 3. Comparison of mitochondrial translation and O_2 consumption in cybrids possessing imported mtDNA from normal subjects and patients with PD or with mitochondrial diseases. (a) Protein synthesis in mitochondria was examined after [^{35}S]methionine-labeling of mitochondrial translation products in the presence of emetine (0.2 mg/ml) to protect translation in the cytoplasm. Proteins of the mitochondrial fraction (50 mg/lane) were separated by SDS–polyacrylamide gel electrophoresis. ND5, COI, ND4, Cytb ND2, ND1, COII, COIII, ATP6, ND6, ND3, ATP8, and ND4L are polypeptides assigned to mtDNA genes. Note that the mobilities of ND3 in Cy-HeEB cybrids (ND3′) and Cytb in CyP3 cybrids (Cytb′) were slightly faster than those in other cybrids, probably due to polymorphic mutations in mtDNA. (b) Quantitative estimation of [^{35}S]methionine-labeling of mitochondrial translation products. (c) O_2 consumption rates. CyHeEB, cybrids with imported HeLa mtDNA; CyN8 and CyN9, CyN cybrids expressing the lowest and highest complex I activity, respectively; CyPD7 and CyPD2, CyPD cybrids expressing the lowest and highest complex I activity, respectively; ρ^0, ρ^0 HeLa cells.

or overall complex activities beyond the normal variation limits, suggesting the complete functional integrity of platelet mtDNAs from PD patients, contrary to the previous reports of Swerdlow *et al.* (11), Gu *et al.* (30), and Trimmer *et al.* (31).

A controversial issue with respect to the complex I activity of PD patients is that some reports have suggested deficiency in various tissues of the patients (19–21) and others have suggested no difference between tissues from patients and age-matched controls (27–29). Recently, cytoplasmic transmission of 20% reduction of complex I activity has been reported in all cybrid clones with imported mtDNA exclusively from PD patients (11, 31). These reports proposed that mtDNA mutations together with environmental mitochondrial toxins, such as 1-methyl-4-phenylpyridinium ion (MPP+), induced reduction of complex I activity and resultant increase in production of reactive oxygen species, leading to apoptotic cell death and onset of PD. In another study, cybrids with mtDNA from platelets of PD patients with lower complex I activity were selected for further examination, and the mtDNA was reported to be responsible for the complex I deficiency in a subgroup of PD patients (30). However, this concept is based on circumstantial evidence without providing evidence of pathogenic mtDNA mutations in platelet of PD patients and their maternal transmission. Our study showed that PD patients do not possess sufficient pathogenic mtDNA mutations to induce complex I deficiency. We found large variations in respiratory enzyme activities in cybrid clones with mtDNA from normal subjects, contrary to the previous observations (11), and some of them showed 20% reduction of the average activities. Moreover, cybrid clones with mtDNA from PD patients restored mitochondrial respiration activity to normal levels, and their range of variation in enzyme activities was comparable to those of the cybrid clones with mtDNA from normal subjects. Therefore, our results argue against the generalization of the concept that platelets in PD patients consistently possess mtDNA lesions causing 20% reduction of complex I activity, resulting in onset of the disease phenotypes (11, 30, 31).

There is more evidence that does not support the idea that PD patients possess sufficient amounts of pathogenic mtDNA mutations for reducing complex I activity. First, no pathogenic mtDNA mutations that are responsible for complex I deficiency had been identified even in recent reports (30, 31), even though their presence was suggested more than 4 years ago (11). Other groups carried out mtDNA sequence analysis of PD patients, but could not determine specific mutations for PD (37–39). Although some mtDNA mutations have been found in tRNA genes or genes encoding subunits of complex I at greater frequency in PD patients than in control subjects (38–40), no mutations were shown to be pathogenic and cause decline in complex I activity.

Second is the absence of maternal inheritance of PD. If the consistent 20% reduction of complex I activity in platelets of PD patients was involved in the pathogenesis of PD, and transmitted cytoplasmically to the cy-

brids (11, 31), most PD should also be transmitted maternally to the progenies, or at least there should be some bias to maternal inheritance as in the case in Leber's hereditary optic neuropathy (LHON) expressing complex I deficiency (2, 9), but this was not the case in PD (40). Usually, both disease phenotypes and their pathogenic mtDNA mutations are cotransmitted from the mother in mtDNA-based mitochondrial diseases. Apparent absence of maternal transmission in Kearns-Sayre syndromes would be due to lethality of their pathogenic mutant mtDNAs with large-scale deletion (2, 9).

Third, if the defect of complex I activity could be a trigger or one of the risk factors for the onset of PD, patients with mitochondrial diseases expressing complex I deficiency, such as LHON (2, 9) CM (35) and DM (36), must show onset of PD much more frequently than normal subjects. However, there have been no reports suggesting association of these mitochondrial diseases with PD. In fact, no members of families with CM (35) and DM (36) having only complex I deficiency showed onset of PD.

Finally, even if the putative mutant mtDNAs were not transmitted from the mother, but newly formed by acquired mutations during aging in PD patients, it is unlikely that platelets possess these mutations sufficiently to induce mitochondrial dysfunction, because mitotic tissues do not accumulate mtDNA with pathogenic mutations due to mitotic segregation (15, 41). Considering the random nature of somatic mutations, it is also unlikely that brain tissues and platelets in PD patients simultaneously accumulate mtDNAS with specific somatic mutations that exclusively reduce complex I activity. All these facts warrant careful reexamination of the previous claim that mtDNA mutations are responsible for 20% reduction of complex I activity observed in platelets of PD patients in the absence of identification of the mutations in complex I genes of mtDNA (11, 30, 31, 42).

A similar controversial issue with respect to mitochondrial dysfunction was raised on the pathogenesis of AD. Using cybrids isolated by fusion of platelets from AD patients and ρ^0 human cells, Davis *et al.* (12) found that specific mtDNA mutations in complex IV genes are responsible for the complex IV deficiency observed in platelets and the brain of AD patients. These complex IV mutations proposed to cause AD (12) were subsequently concluded not to be derived from mtDNA, but from mtDNA-like sequences located in nuclear DNA-coded pseudogenes because of the presence of these sequences even in ρ^0 human cells (43, 44). However, the results did not completely exclude the possibility of involvement of other mtDNA mutations in expression of COX deficiency, since cybrid clones with platelet mtDNA from all AD patients examined consistently showed reduced COX activity (12, 13). Nonetheless, it is unlikely that all AD patients possess sufficient pathogenic mtDNA mutations for inducing complex IV deficiency, since our previous observations suggested that platelet mtDNAs from AD patients as well as from normal aged subjects did not contain sufficient mtDNAs with pathogenic mutations to reduce mitochondrial respiratory function (18).

In contrast to mitotic tissues, post-mitotic tissues require much higher energy producing activities, and the resultant progressive oxidative stress in mitochondria is proposed to be responsible for preferential accumulation of somatic mtDNA mutations leading to mitochondrial disorders in brain tissues of aged subjects and those with PD or other neurodegenerative diseases (1–3, 5, 6). For testing this hypothesis, mtDNA in human brain tissues must be transferred to ρ^0 human cells and the resultant cybrid clones must be examined for cotransmission of reduced respiratory activities. We recently showed that even more than one month after the sacrifice of mice, the mtDNA in their brain tissues survived and could be rescued in ρ^0 mouse cells (45) without change in its functional properties (46), and applied this procedure to AD patients (18). This kind of experiment has to be carried out to extend our conclusion to PD patients, but could not done in this study due to difficulty in obtaining the informed consent. Recently, we generated mice expressing mitochondrial diseases by introduction of somatic mutant mtDNA with a 4696 deletion mutation into mouse zygotes (47), which would be useful for completely resolving the issue of whether mtDNA mutations in brain tissues are involved in the pathogenesis of neurodegenerative diseases.

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