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Coordinated Decrease of the Expression of the Mitochondrial and Nuclear Complex I Genes in a Mitochondrial Mutant of Drosophila

Géraldine Farge,[1] Sylvie Touraille,[1] Philippe Lachaume,[1] Roger Debise,[1] Vincent Procaccio,[2] and Serge Alziari[1,3]

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We have studied a mutant strain of Drosophila in which 80% of the mitochondrial DNA molecules have lost over 30% of their coding region through deletion. This deletion affects genes encoding five subunits of complex I of the respiratory chain (NADH:ubiquinone oxidoreductase). The enzymatic activity of complex I in the mutant strain is half that in the wild strain, but ATP synthesis is unaffected. The drop in enzymatic activity of complex I in the mutant strain is associated with a 50% decrease in the quantity of constitutive proteins of the complex. Moreover, in the mutant strain there is a 50% decrease in the steady-state concentration of the transcripts of the mitochondrial genes affected by the deletion. This decrease is also observed for the transcripts of the nuclear genes coding for the subunits of complex I. These results suggest a coordination of the expression of the mitochondrial and nuclear genes coding for mitochondrial proteins.

KEY WORDS: mitochondria; NADH:ubiquinone oxidoreductase; Drosophila; mitochondrial DNA; ferricyanide.

INTRODUCTION

NADH:ubiquinone oxidoreductase (complex I) is composed of numerous subunits: from 13 to 14 in bacteria (Yagi, 1993) to 46 in bovine (Carroll *et al.*, 2003). These subunits are encoded in mitochondrial or nuclear genes and are organized in an L-shaped structure comprising a peripheral and a membrane domain (Hofhaus *et al.*, 1991). The nuclear encoded subunits, synthesized in the cytosol, are imported into the mitochondrial matrix where they assemble with the mitochondrially encoded subunits to form a functional multimeric complex. This assembly was studied in detail in *N. crassa* (Schulte, 2001) where it was shown that the peripheral and membrane domains were formed independently (Tuschen *et al.*, 1990). Moreover, when the synthesis of the subunits encoded by the mitochondrial genome is inhibited by chloramphenicol, subunits encoded by the nuclear genome accumulate as a small form of complex I which has NADH dehydrogenase activity (Friedrich *et al.*, 1989). Conversely, in manganese-limited conditions (Schmidt *et al.*, 1992), or when there is a mutation in a nuclear gene encoding a subunit of the complex (Duarte *et al.*, 2002), the membrane part of complex I accumulates.

The twofold origin of this respiratory complex suggests that regulation of the two genetic systems is coordinated to ensure correct mitochondrial biogenesis. Although the nuclear genome's control of the expression of mitochondrial genes has been intensively studied, little is known about the signals involved in communication between the mitochondrion and nucleus (Garesse and Vallejo, 2001; Poyton and McEwen, 1996). In yeast, studies on cells devoid of mtDNA (rho°) have demonstrated induction of the expression of different nuclear genes, including the *Cit2* gene coding for peroxisomal citrate

[1] Equipe <<Génome Mitochondrial>>, UMR CNRS 6547, Université Blaise-Pascal, Clermont II, Aubière-Cedex, France.
[2] BECP/DRDC, CEA Grenoble, Grenoble-Cedex, France.
[3] To whom correspondence should be addressed; e-mail: serge.alziari@univ-bpclermont.fr.

Abbreviations: MtDNA: mitochondrial DNA; SMP: submitochondrial particles; OXPHOS: oxidative phosphorylations.

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synthase (Liao and Butow, 1993; Parikh *et al.*, 1987). Studies on human cells devoid of mtDNA (Marusich *et al.*, 1997) or on different tissues of patients carrying mutations of mtDNA (Heddi *et al.*, 1993; Heddi *et al.*, 1999; Carrier *et al.*, 1996) have revealed coordinated regulation of the expression of the mitochondrial and nuclear OXPHOS genes. However, the molecular mechanisms by which eukaryotic cells perceive and respond to deficient expression of mtDNA remain largely unknown.

We have studied a mutant strain of Drosophila in which 80% of the mitochondrial DNA molecules have lost over 30% of their coding region through deletion (Volz-Lingenhöhl *et al.*, 1992). Genes coding for subunits of complexes I and III of the respiratory chain (*ND1, ND4, ND4L, ND5, ND6,* and *cytochrome b*), as well as four tRNAs (Ser, Thr, Pro, His), are included in the deletion, and the enzymatic activities of complexes I and III in the mutant strain are 50% and 30% lower than in the wild strain, respectively (Debise *et al.*, 1993). Despite this large drop in enzymatic activities, the mutant exhibits ATP synthesis identical to that of the wild strain and has no "pathological" phenotype. To explain this observation, we previously performed experiments of progressive inhibitions of the respiratory complexes (Farge *et al.*, 2002). This work enabled us to propose the existence in the wild strain of an "enzymatic activity pool" of NADH dehydrogenase, which may correspond to the presence of a large population of functional complex I.

To complete this work, we determined in the two strains the concentrations of different constitutive proteins, the kinetic parameters (reduction of ubiquinone), and the NADH-reductase activity of complex I. We also investigated the expression of nuclear and mitochondrial genes coding for complex I subunits. In the mutant strain there was a simultaneous decrease in the concentration of transcripts of the mitochondrial genes affected by the deletion and of the nuclear genes of complex I, suggesting that expression of the two genomes is coordinated.

MATERIALS AND METHODS

Materials

Our study was carried out on flies belonging to the *Drosophila subobscura* strain. The heteroplasmic strain (H) was established from a female collected in the wild (Volz-Lingenhöhl *et al.*, 1992). Wild-type line (W) is homoplasmic standard strain of *Drosophila subobscura*. Wild and mutant strains were raised on standard cornmeal medium as described by Volz-Lingenhöhl *et al.* (1992) at 19°C.

Isolation and Subfractionation of Mitochondria

Mitochondria were isolated by differential centrifugation in isolation buffer (0.22 M sucrose, 0.12 M mannitol, 1 mM EDTA, 40 mM Tricine pH 7.5). Mitochondria suspension (10 mg/mL in isolation buffer) was incubated for 10 min at 4°C with 100 μg/mg digitonin and centrifugated 5 min at 10000 $\times$ g. The pellet (mitoplast fraction) was saved, washed twice with isolation buffer, resuspended in 10 mM tricine, 25 μg/mg Triton X-100 and sonicated for 25 s. After centrifugation at 10000 $\times$ g for 10 min, the supernatant (S10 fraction) was collected and centrifugated 30 min at 150000 $\times$ g in order to separate fractions enriched in the submitochondrial particles (SMP) and the soluble matrix proteins.

Protein concentration was determined by the Bradford's method (Bradford, 1976) using bovine serum albumin as standard protein.

Enzymatic Activities

Assays were performed at 28°C in a final volume of 1 mL.

NADH reductase (complex I) activities were measured by following the oxidation of NADH at 340 nm ($\varepsilon = 6{,}220$ M^{-1} cm^{-1}). NADH:ferricyanide oxidoreductase activity was assayed in a buffer containing 50 mM Tris/HCl pH 7.5, 2 mM KCN, 1 mM potassium ferricyanide, 0.1 mM NADH and 10 μg of mitochondrial proteins (Friedrich *et al.*, 1989). NADH:ubiquinone oxidoreductase activity was determined in an assay buffer containing 35 mM NaH_2PO_4 pH 7.2, 5 mM $MgCl_2$, 2.5 mg/mL BSA, 2 mM KCN, 2 μg/mL antimycin, 97.5 μM ubiquinone-1, 0.13 mM NADH, and 50 μg mitochondrial proteins. Only the rotenone sensitive activity is considered (Hatefi, 1978). Ubiquinone-1 was kindly provided by Eisai Co. Ltd. (Japan).

Cytochrome *c* oxidase (complex IV) activity was determined by following the oxidation of cytochrome *c* at 550 nm ($\varepsilon = 18{,}500$ M^{-1} cm^{-1}) in a buffer composed of 30 mM KH_2PO_4 pH 7.4, 1 mM EDTA, 56 μM cytochrome *c*, and 5 μg mitochondrial proteins (Errede *et al.*, 1978).

Apparent K_m and V_m Values

NADH:ubiquinone oxidoreductase activity of isolated mitochondria was assayed with various concentrations (2–100 μM) of ubiquinone-1. A double reciprocal

Table I. Primers and Reaction Conditions Used for PCR

Gene name	Gene symbol	Left primer (5′ → 3′)	Right primer (5′ → 3′)	AT	size
mt NADH:ubiquinone reductase 3	*mt:ND3*	TCACCATTTGAATGTGGATTT	TGGTAAAATTAAAGCAATTTCTACATC	52°C	120 pb
NADH:ubiquinone reductase 23kD	*ND23*	CGCAGTAGATGCACTTGGTC	GAGGTTCCCAAGGGCTATGT	55°C	370 pb
NADH:ubiquinone reductase 49kD	*ND49*	TAAAGCTGCCAAGTGAGCAA	GACGAAGTGGAGGATGTGT	54°C	543 pb
NADH:ubiquinone reductase 24kD	*ND24*	GTGATACTTGCCAGTGGGCT	AACATCCCGTTCGAGTTCAC	49°C	246 pb

Note. AT: annealing temperature.

plot was used to determine the apparent K_m and V_m values.

Western Blot

Five μg (mitoplasts suspension) or 15 μg (matrix) of proteins were separated by SDS-PAGE on a 12% polyacrylamide separating gel and transferred to a cellulose nitrate membrane (BioBond-NC Whatman). The membrane was blocked with 10% dried milk, 0.2% Tween in TBS buffer (Tris Buffer Saline) for 1 h and incubated for 2 h in TBS with rabbit antisera against the 23, 49, and 24 kDa subunits of complex I. The membrane was rinsed twice in TBS and saturated for additional 30 min. After incubation for 1 h with a peroxidase conjugated antirabbit antibody (Sigma) in 1%BSA/TBS, the immunoblot was revealed by chemiluminescence (ECL, Amersham Pharmacia). Signal intensity was quantified densitometrically and analyzed using image analysis software (Quantity One, Biorad).

Antibodies were kindly provided by J. Lunardi, CEA Grenoble, France (anti-23 and anti-49 kDa) and A. Videira, Instituto de Biologia Molecular e Celular, Porto, Portugal (anti-24 kDa).

Northern Blot

Extraction of Total RNA

Total RNA was isolated from 50 to 60 flies by a modified guanidinium thiocyanate-phenol chloroform extraction method adapted from Chomczynski and Sacchi (1987) using RNA-PLUS^{TM} (Qbiogene).

Probes

The probes corresponding to *mt:ND4* and ribosomal 18S and 12S RNA are DNAs cloned from *Drosophila melanogaster* as described previously (Béziat *et al.*, 1993; Morel *et al.*, 1995).

Complex I subunits probes were synthesized by Polymerase Chain Reaction, using total DNA isolated from *Drosophila subobscura* flies. PCR primer pairs (Table I) were constructed from *Drosophila melanogaster* specific sequence data (Flybase: http://flybase.bio.indiana.edu/genes). PCR products were confirmed by agarose gel electrophoresis and automatic sequencing (CEQ2000, Beckman).

Estimation of the RNA Content

RNA content was estimated by the Northern blot technique, using 18S (for nuclear genes) or 12S RNA (for mitochondrial genes) as internal control. The signals were analyzed by densitometry with a phosphoimager using image analysis software (Quantity One, Biorad).

RESULTS

Kinetic Analysis of Complex I

The value of V_m in the wild strain was twice that in the mutant, however, the apparent K_m of the enzyme for ubiquinone was identical in the two strains (Table II).

Enzymatic Activities

The activities of NADH:ferricyanide oxidoreductase, NADH:ubiquinone oxidoreductase and cytochrome *c*

Table II. Kinetic Analysis of Complex I (Ubiquinone Reduction) in the Wild (W) and Mutant (H) Strains

Strain	K_m (μM)	V_m (nmol · min^{-1} · mg^{-1})
W	15,7 ± 2,5	575 ± 131*
H	15,3 ± 2,6	303 ± 46

Note. NADH:ubiquinone oxidoreductase activities were measured with different concentrations of ubiquinone as described in Materials and Methods. Data represent mean ± SE for five determinations. Statistical significances were calculated using Student's *t*-test.
* $p < 0.05$.

oxidase were determined for the two strains in the different mitochondrial subfractions: S10 (supernatant obtained after treatment of the mitoplasts with Triton X-100, sonication, and centrifugation at 10000*g*), submitochondrial particles, and matrix (Fig. 1). While the reduction of ubiquinone requires a fully functional complex I, the reduction of ferricyanide can be catalyzed by the "NADH dehydrogenase module" of complex I which contains the 24, 51, and 75 kDa subunits (Friedrich and Weiss, 1997).

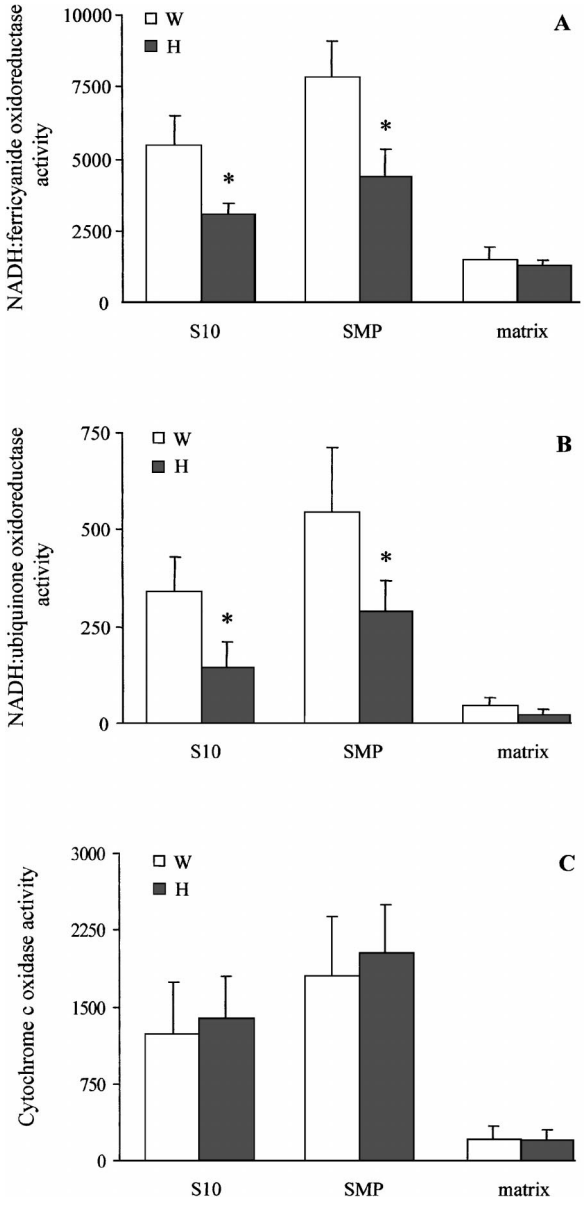

Fig. 1. Comparison of enzymatic activities in the different mitochondrial fractions in the wild (W) and mutant (H) strains. NADH:ferricyanide oxidoreductase activity (A), NADH:ubiquinone oxidoreductase activity (B), and cytochrome *c* oxidase activity (C) were assayed in S10 fraction (see Materials and Methods), submitochondrial particles (SMP), and mitochondrial matrix. Data are expressed in nmol · min^{-1} · mg^{-1} and represent mean ± SE (bars) for 10 experiments. Statistical significances were calculated using Student's *t*-test: $^{*}p < 0.05$.

In fraction S10, which contains the inner mitochondrial membranes and the soluble matrix proteins, the activities of NADH:ferricyanide and NADH:ubiquinone oxidoreductase in the wild strain were twice those in the mutant strain. Similar results were recorded in the subparticles: the NADH:ferricyanide and NADH:ubiquinone oxidoreductase activities in the mutant strain were half those in the wild strain. These NADH-reductase activities reflect the functional complexes I present in the inner mitochondrial membrane.

In the matrix compartment, the activity of NADH:ubiquinone oxidoreductase was weak in both strains as it represented only 3 and 7% of the total activity in the wild and mutant strains, respectively. This activity is probably due to contamination of the inner mitochondrial membranes. In contrast to the other fractions, the NADH:ferricyanide oxidoreductase activity measured in the matrix was identical in the two strains and represented a nonnegligible percentage of the total activity (9% for the wild strain and 15% for the mutant).

Lastly, the activity of complex IV (unaffected by the deletion) was identical in the two strains, irrespective of the fraction considered (Fig. 1(C)).

Complex I Subunit Content

We investigated whether the decrease in the enzymatic activity of complex I (50%) in the mutant strain was associated with a decrease in the quantity of complex I. We performed Western blotting with specific antibodies against three nuclear encoded complex I subunits: 24 kDa, 23 kDa (or TYKY), and 49 kDa. The 24 kDa subunit belongs to the "NADH dehydrogenase module" of complex I (Friedrich and Weiss, 1997), while the 49 kDa has been recently localized at the extremity of the peripheral domain of complex I (Zickermann *et al.*, 2003). The 23 kDa subunit is located at the vicinity of the membrane where it could serve as a connector between the peripheral and the membrane domains (Yano and Yagi, 1999).

In the mitoplasts (Fig. 2(A)), these three proteins with nuclear coding were twice as abundant in the wild strain as in the mutant strain. Complex I was therefore present in lesser amounts in the mutant strain. These three proteins were also detected in the matrix fraction (Fig. 2(B)): a 50% decrease was seen in the quantity of 23 kDa and 49 kDa subunits in the mutant strain; the quantity of 24 kDa protein, on the other hand, was identical in the two strains.

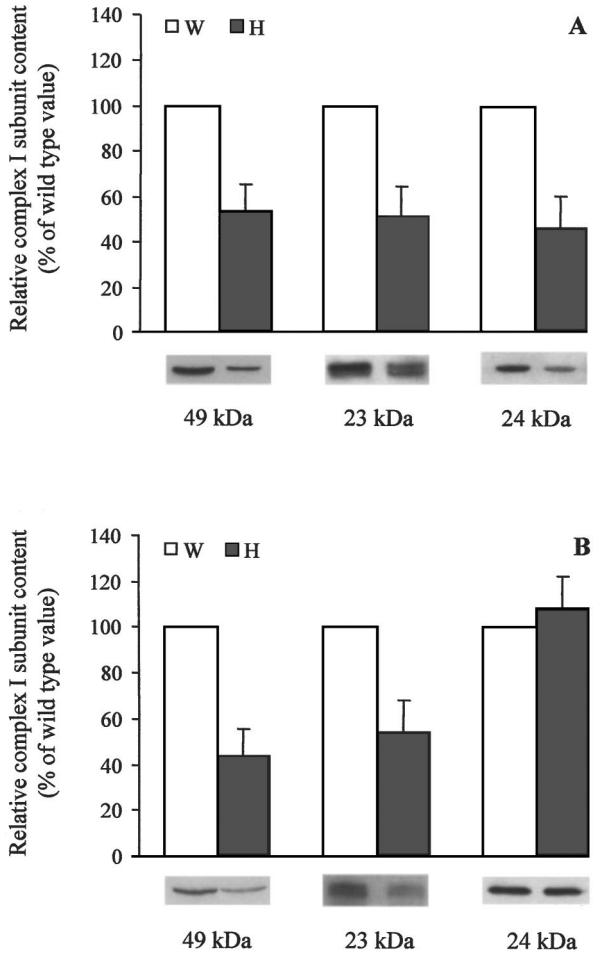

Fig. 2. Quantification of the complex I subunits by Western blot analysis in mitoplasts (A) and mitochondrial matrix (B). Comparison between the wild (W) and mutant (H) strains. Data are mean ± SE (bars) values of at least five experiments.

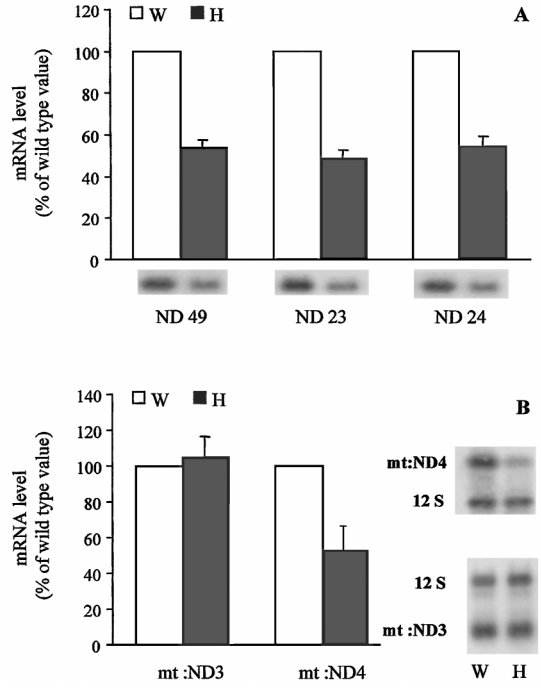

Fig. 3. Quantification of the nuclear (A) and mitochondrial (B) complex I gene mRNAs by Northern blot analysis. Comparison between the wild (W) and mutant (H) strains. Data are mean ± SE (bars) values of five experiments.

Complex I Subunit Transcript Levels

To determine whether the decrease in constitutive proteins of complex I observed in the mutant strain was due to a transcriptional regulation, the expression of the nuclear genes coding for the 23, 24, and 49 kDa subunits was studied by Northern blotting. Specific probes were synthesized and the steady-state concentration of the different mRNAs was estimated by comparison with the concentration of the 18S transcript (Fig. 3(A)). The concentration of the *ND23, ND24,* and *ND49* gene transcripts in the mutant strain was about half that in the wild strain. The decrease in the 23, 24, and 49 kDa proteins was therefore associated with a decrease in concentration of the corresponding gene transcripts. This decrease could be due to a drop in the gene transcription rate or from reduced mRNA stability.

The concentrations of the transcripts of two mitochondrial genes of complex I was determined (Fig. 3(B)) by comparison with the concentration of 12S rRNA. The transcript concentration for the *mt:ND3* gene, which is not included in the deletion, was identical in the two strains, whereas the transcript concentration for the *mt:ND4* gene, which is affected by the deletion, was reduced by about 50% in the mutant strain compared with the wild strain, as shown before (Béziat *et al.*, 1993).

DISCUSSION

Complex I Is Quantitatively Different in the Two Strains

Determination of the K_m of complex I for its substrate, ubiquinone, revealed that kinetic properties of complex I are identical in the wild and mutant strains (Table II). The maximum velocity (V_m) of the reaction catalyzed by complex I in the mutant strain is half that in the wild strain. The activity of complex I was measured independently of the activities of the other respiratory complexes (use of specific inhibitors). Moreover, a decrease of 30%

of the complex III activity does not affect ATP synthesis nor energetic equilibrium in the mutant strain (Beziat *et al.*, 1997). These observations indicate that complex III could maintain electron transfer between complex I and complex IV and does not disrupt electrochemical potential across the inner mitochondrial membrane. Thus, in spite of the major drop of most mitochondrial genes coding for a complex III subunit (cytochrome b) in the mutant strain, the respiratory capacities are preserved and this mutation does not interfere with our results.

Immunoblotting of constitutive proteins of complex I shows that the decrease in complex I activity is associated with a 50% decrease in the quantity of the 24, 23, and 49 kDa subunits in the mutant strain compared with the wild strain (Fig. 2). The loss of 80% of the genes coding for four tRNAs (Ser, Pro, Tyr, and His), essential for mitochondrial protein synthesis, could not account for the decrease in the quantity of these complex I subunits. Indeed, previous studies by cytochemistry and in situ hybridization (Lecher *et al.*, 1994, 1996) show that heteroplasmy is intramitochondrial and that "functional complementation" could occur between wild and deleted genomes, thus allowing efficient synthesis of mitochondrial proteins.

These results seem to confirm that the difference observed between the two strains in experiments on complex I inhibition (Farge *et al.*, 2002) stems from a decrease in the quantity of functional complex I in the mutant strain. The "enzymatic activity pool" of NADH dehydrogenase in the wild strain is due to the presence of large amount of complex I in the inner membrane of the mitochondria. In the mutant strain, the deletion of a large part of the regions coding for certain subunits of complex I results in an important decrease in the number of functional complexes (50%), leading to a very limited reserve of activity.

Preassembly of the "NADH Dehydrogenase Module" of Complex I

The "NADH dehydrogenase module" of complex I, which comprises the 24, 51, and 75 kDa subunits, can catalyze the oxidation of NADH by artificial electron acceptors like ferricyanide or hexammine-ruthenium III (Friedrich and Weiss, 1997). In the matrix fraction, we found that the mutant strain has an NADH:ferricyanide oxidoreductase activity identical to that of the wild strain (Fig. 1(A)). Moreover, the two strains contained equivalent quantities of the 24 kDa subunit in the mitochondrial matrix (Fig. 2(B)). These observations suggest that the similar activity of NADH:ferricyanide oxidoreductase in the two strains is the consequence of the presence in the matrix of an identical quantity of the assembled "NADH dehydrogenase module" of complex I (component of the peripheral part of complex I).

This accumulation of a peripheral part of complex I possessing NADH-reductase activity in the absence of the mitochondrial subunits has already been observed. For example, in *C. reinhardtii*, mutants for the mitochondrially encoded subunits of the membrane part of complex I lose the capacity to form a functional complex, but there is an accumulation in the soluble fraction of the peripheral part with nuclear coding of the complex, which is able to catalyze the oxidation of NADH by ferricyanide (Cardol *et al.*, 2002).

Complex I is located in the inner membrane of the mitochondria, yet subunits of complex I are found in the mitochondrial matrix. These subunits could be proteins not assembled into functional complex I. The 24 kDa subunit is present in equivalent amounts in the mitochondrial matrix of the two strains, whereas the 23 and 49 kDa subunits are less abundant in the mutant strain. Despite the decrease in the concentration of the *ND24* gene transcript (Fig. 3), there is thus an accumulation of the 24 kDa protein in the mitochondrial matrix of the mutant strain. This could be explained by the fact that the 24 kDa protein appears to complex with other proteins to form the "NADH dehydrogenase module" of complex I, thus increasing its stability compared with the 23 and 49 kDa proteins which may not find partners in the mitochondrial matrix and would therefore be degraded more rapidly.

Nuclear-Mitochondrial Coordination

The responses of the nuclear genome to mitochondrial dysfunction have been studied principally in cells devoid of mtDNA (rho$^\circ$) (Liao and Butow, 1993; Marusich *et al.*, 1997; Parikh *et al.*, 1987; Wang and Morais, 1997). However, the total depletion of mtDNA constitutes an extreme situation for the cell. Studies in patients carrying mtDNA mutations allow investigation of this phenomenon under less deleterious conditions (Carrier *et al.*, 1996; Heddi *et al.*, 1993, 1999). Our Drosophila model approximates in molecular terms to cases of Kearns–Sayre syndrome. The mutant strain has in fact undergone a large deletion of mtDNA in the heteroplasmic state (80%). However, it seems that this large loss of genes does not handicap the mutant, since its different stages of development and lifespan are identical to those of the wild strain (Petit *et al.*, 1998).

Analysis of the expression of the *mt:ND3* gene, which is not included in the deletion, shows that the steady-state concentration of this transcript is identical in the mutant and wild strains (Fig. 3(B)). Likewise, the concentrations

of the transcripts of the *COI* and *COIII* genes, which are located outside the deletion in the mutant strain, are similar in the two strains (Béziat *et al.*, 1993). In Drosophila, ND3 is part of a putative transcription unit which includes the genes *ND2*, *COI*, *COII*, *COIII*, and *ATPase 6* and *8* (Berthier *et al.*, 1986). It therefore seems that the transcription of this block is equivalent in the two strains. In the mutant strain, ND3 mRNA then seems to be more abundant (50%) than the mRNAs of the other mitochondrial and nuclear subunits of complex I ((Béziat *et al.*, 1993) and Fig. 3).

As for the mitochondrial genes affected by the deletion, there is an approximately 50% decrease in the steady-state concentration of the *ND1*, *ND4*, *ND4L*, and *ND5* gene transcripts in the mutant strain compared with the wild strain (Béziat *et al.*, 1993) and Fig. 3(B)). The 50% decrease in enzymatic activity of complex I in the mutant strain (Table II) is associated with a 50% drop in the quantity of constitutive proteins of complex I (Fig. 2(A)), and this drop is itself associated with a diminution in the quantity of transcripts of the corresponding nuclear genes (Fig. 3(A)). Our study therefore reveals a concomitant decrease in the concentration of the transcripts of the mitochondrial genes included in the deletion and the transcripts of the nuclear genes of complex I (Fig. 3). This suggests there is coordination of the expression of the two genomes. These results differ from those recorded in analyses of patients carrying mutations of mtDNA where there is an increase in the quantity of transcripts of different mitochondrial and nuclear genes involved in energy metabolism (Carrier *et al.*, 1996; Heddi *et al.*, 1993, 1999; Shoubridge *et al.*, 1990). However, our results agree with those of Heddi (Heddi *et al.*, 1993), who has shown that in Kearns–Sayre syndrome the expression of mitochondrial genes outside the deletion is greater than or similar to that of controls, and that there is a simultaneous decrease in transcripts of the mitochondrial genes affected by the deletion and of the nuclear genes.

In our study, the concomitant decrease in the mutant strain in the quantity of transcripts of three different nuclear subunits of complex I suggests a possible coregulation of the corresponding genes. A computer analysis of the promoter regions of the *ND23*, *ND24*, and *ND49* genes of Drosophila allowed us to identify numerous putative transcription factor binding sites, including at least one binding site for the buttonhead and pleiohomeotic transcription factors (homologues of the mammalian transcription factors Sp1 and YY1, respectively) on each of these three promoters. Sp1 and YY1 are involved in the regulation of numerous OXPHOS genes (Scarpulla, 2002) and binding sites for Sp1 were identified on the promoters of human genes coding for proteins of complex I (Hattori *et al.*, 1995; Lescuyer *et al.*, 2002).

Note finally that most studies suggesting coordination of the two genomes do not elucidate the question of the signaling pathways or of the molecules involved in this communication. Different mechanisms have been suggested, such as oxygen tension, reactive oxygen species (Poyton and McEwen, 1996), and alterations in cellular energy levels (Biswas *et al.*, 1999). It has been shown in mouse myocytes with a low mtDNA content that the decreases in ATP synthesis and in mitochondrial membrane potential lead to an increase in cytosolic Ca^{2+} levels, which may underlie a complex cellular response (Biswas *et al.*, 1999). We are currently studying the mitochondrial membrane potential which, if it is altered in the mutant strain, could constitute a signal in the communication pathway to the nucleus.

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