Genetic analysis of temperature-dependent transmission of mitochondrial DNA in *Drosophila*

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In artificially induced mitochondrial DNA (mtDNA) heteroplasmy in *Drosophila*, the effects of chromosome substitution on temperature-dependent selection in mtDNA transmission were investigated. Using two strains of *D. melanogaster*, *bw;e¹¹* and *y;bw;st*, which showed a different temperature dependency in mtDNA transmission, chromosomes were substituted reciprocally, and mtDNA of *D. mauritiana* was introduced into each newly constructed strain. For each heteroplasmy, the transmission of mtDNA was examined at 25°C and 19°C. When either the second or the third chromosome of the *y;bw;st* strain was substituted with that of the *bw;e¹¹* strain, the temperature-dependent selection in mtDNA transmission was altered. The selection was not changed when either the second or the third chromosome of the *bw;e¹¹* strain was substituted with that of the *y;bw;st* strain, or even when both the second and the third chromosomes of the *bw;e¹¹* strain were substituted with those of the *y;bw;st* strain. These results suggest that the temperature-dependent selection in mtDNA transmission is co-operatively regulated by gene products that are encoded by the X, second and third chromosomes.

Keywords: chromosome substitution, *Drosophila*, heteroplasmy, mitochondrial DNA, temperature-dependent selection, transmission.

Introduction

The maintenance and transmission of mitochondrial DNA (mtDNA) involves multiple processes of replication of mtDNA and mitochondria, and the partitioning of mitochondria to daughter cells. These processes are controlled by many genes in the nuclear genome that co-operate with mitochondrial genomes. Some nuclear genes involved in these processes have been reported in *Saccharomyces cerevisiae* (e.g. Foury, 1989; McConnell *et al.*, 1990; Ling *et al.*, 1995; Lockshon *et al.*, 1995). Mitochondrial transcription factor A has recently been shown to be essential in the regulation of the copy number of mtDNA in the mouse (Larsson *et al.*, 1998). However, no other genes that regulate the maintenance and transmission of mtDNA directly have been identified in multicellular organisms.

In higher animals and insects, the transmission of mtDNA has been investigated in heteroplasmy (Solignac *et al.*, 1984; Rand & Harrison, 1986; Laipis *et al.*, 1988; Jenuth *et al.*, 1996). In *Drosophila*, heteroplasmy

can be induced artificially by the transplantation of germ plasm (Matsuura *et al.*, 1989) or egg cytoplasm (de Stordeur *et al.*, 1989). Using these techniques, heteroplasmy has been established with intra- and interspecific combinations between mtDNA and the nuclear genome. In *D. melanogaster* and its closely related species, the transmission of mtDNA in such induced heteroplasmy has been selective, and selection has been shown to be dependent on the temperature at which heteroplasmic strains are maintained (Niki *et al.*, 1989; Matsuura *et al.*, 1991, 1993; Tsujimoto *et al.*, 1991). Similar temperature-dependent selection has also been observed in heteroplasmy constructed with *D. simulans* and *D. mauritiana* (de Stordeur, 1997).

We have reported previously that the temperature-dependent modes of mtDNA transmission in heteroplasmic *D. melanogaster* possessing *D. mauritiana* mtDNA are different when two different strains of *D. melanogaster* are used (Matsuura *et al.*, 1991; Tsujimoto *et al.*, 1991). This difference has been found to be largely determined by the nuclear genome (Matsuura *et al.*, 1997). In the present study, the effect of each major chromosome on temperature-dependent selection

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was examined in order to identify the nuclear genes responsible for the selective transmission of mtDNA.

Materials and methods

Species and strains

Two strains of *D. melanogaster*, *bw;e¹¹* and *y;bw;st*, were used to examine the effect of each chromosome on the selective transmission of mtDNA. In our previous studies on mtDNA transmission in heteroplasmy, mtDNA of *D. mauritiana* (g20) introduced into these two strains was transmitted differently depending on the temperature (Matsuura *et al.*, 1991; Tsujimoto *et al.*, 1991). The entire nuclear genomes of the two strains have been shown to have different effects on temperature-dependent selection in mtDNA transmission (Matsuura *et al.*, 1997). To assess the effect of each chromosome, chromosomes were substituted reciprocally between the two strains. A balancer strain, *SM1/Pm;TM3/Pr*, was used for the substitution. The g20 strain of *D. mauritiana* was used as a germ plasm donor to establish heteroplasmy as described below.

Substitution of chromosomes

Using the balancer strain, chromosome substitution was carried out according to standard procedures (Ashburner, 1989). For the second chromosome substitution, some modification was made to distinguish between the second chromosomes carrying *Pm* and *bw* by using single flies for crosses in the F₂ and F₃ generations. Figure 1 shows the procedure for substituting the second chromosome of the *bw;e¹¹* strain with that of the *y;bw;st* strain. Similar crosses were made for substitutions of the other chromosomes.

When the second and third chromosomes of the *bw;e¹¹* strain were substituted with those of the *y;bw;st* strain, the newly established strains were denoted B-y2 and B-y3 respectively. These strains possessed mtDNA derived from the *bw;e¹¹* strain. For the reciprocal substitutions, the established strains were denoted as Y-b2 and Y-b3, possessing mtDNA derived from the *y;bw;st* strain. Reciprocal substitutions of both the second and the third chromosomes were also carried out. The established strains were similarly denoted as B-y2 + y3 and Y-b2 + b3, possessing mtDNA derived from the *bw;e¹¹* and *y;bw;st* strains respectively. The substitution of both the second and the third chromosomes resulted in the substitution of the X chromosome. That is, the X chromosome of the *bw;e¹¹* strain was substituted with that of the *y;bw;st* strain in the Y-b2 + b3 strain, and vice versa.

The compositions of chromosomes and mtDNA in these constructed strains are shown in Fig. 2. The Y chromosome in each strain was derived from the strain used as male for a cross in the F₂ generation. The fourth chromosome was mixed among the three strains, including the balancer. These strains possessed only one type of mtDNA on the basis of Southern hybridization (data not shown).

Establishment and maintenance of the heteroplasmic line

Using the experimental strains in which chromosomes were substituted as recipients and the g20 strain of *D. mauritiana* as a germ plasm donor, heteroplasmy was constructed by germ plasm transplantation, as described previously (Matsuura *et al.*, 1989). Heteroplasmic lines were maintained at 25°C and 19°C, and changes in the proportions of the two types of mtDNA were examined for 8–15 generations according to procedures described previously (Matsuura *et al.*, 1989).

Results

The second chromosome

Using the B-y2 strain as a recipient, four heteroplasmic lines were established. Changes in the proportions of g20 mtDNA were examined at 25°C and 19°C for 12 and 13 generations respectively (Fig. 3a). The proportions of g20 mtDNA clearly increased in the four lines at 25°C but decreased at 19°C, and g20 mtDNA was lost by the 13th generation.

In the Y-b2 strain established by the reciprocal substitution, four heteroplasmic lines were examined for changes in the proportions of g20 mtDNA (Fig. 3b). At 25°C, the proportions of g20 mtDNA increased by the 10th generation. At 19°C, however, the proportions slowly decreased. To determine whether this decrease was caused by the initial proportions of g20 mtDNA being relatively low, sublines were re-established from single females of one of the heteroplasmic lines at the sixth generation at 25°C, in which the initial proportions of g20 mtDNA were 40–79%. These sublines were maintained for five generations at 19°C, and the proportions of g20 mtDNA clearly decreased (data not shown).

The third chromosome

Using the B-y3 and Y-b3 strains as recipients, three and four heteroplasmic lines were established respectively. Changes in the proportions of g20 mtDNA were examined at 25°C for 10 generations and at 19°C for eight generations. The results were essentially the same

Fig. 1 Mating scheme for the substitution of the second chromosome of the *bw;e¹¹* strain with that of the *y;bw;st* strain in *Drosophila melanogaster*. Black, dotted and hatched bars represent chromosomes derived from the *bw;e¹¹* strain, the *y;bw;st* strain and the balancer strain respectively.

as those for the second chromosome (data not shown). The proportions of g20 mtDNA increased at 25°C in two of the three lines for the B-y3 strain and in all four lines for the Y-b3 strain. At 19°C, the proportions clearly decreased in all lines examined.

The second and third chromosomes

Using the Y-b2 + b3 and B-y2 + y3 strains as recipients, three and four heteroplasmic lines were established respectively. Changes in the proportions of g20 mtDNA were examined for 15 generations at both temperatures. The results were similar to those described above (data not shown). The proportions of g20 mtDNA increased at 25°C and decreased at 19°C in all lines examined.

Discussion

In our previous study, the nuclear genomes of the *bw;e¹¹* and *y;bw;st* strains showed different effects on selection in mtDNA transmission in heteroplasmy (Matsuura *et al.*, 1997). With the nuclear genome of the *bw;e¹¹* strain, the proportions of *D. mauritiana* (g20) mtDNA increased at 25°C and decreased at 19°C. But with the nuclear genome of the *y;bw;st* strain, the proportions increased at both temperatures at different rates.

To determine which chromosome has the major effect on the selection, six kinds of chromosome constitution were constructed in the present study. As the effects of the nuclear genome on the selective transmission of mtDNA are large compared with the effects of the

	mtDNA (<i>bw</i> ;e ¹¹ + g20)	mtDNA (<i>y</i> ;bw; <i>st</i> + g20)
The second chromosome substitution	B-y2	Y-b2
The third chromosome substitution	B-y3	Y-b3
The second and third chromosomes substitution	B-y2+y3	Y-b2+b3

Fig. 2 Composition of the chromosomes for each strain of *Drosophila melanogaster* used as a recipient of germ plasm transplantation. Black and dotted bars represent chromosomes derived from the *bw*;e¹¹ strain and the *y*;bw;*st* strain respectively.

mitochondrial genome that coexists with g20 mtDNA (Matsuura *et al.*, 1997), the mitochondrial genome derived from either the *bw*;e¹¹ strain or the *y*;bw;*st* strain was used for each chromosome constitution according to the substitution procedure. An X chromo-

somes substitution was also carried out by a similar procedure using a strain in which the entire nuclear genome of *bw*;e¹¹ was substituted with that of *y*;bw;*st* (Matsuura *et al.*, 1997), and heteroplasmy possessing *D. mauritiana* (g20) mtDNA was established. When

Fig. 3 Changes in the proportions of *Drosophila mauritiana* (g20) mtDNA introduced into the strains of *D. melanogaster* in which the second chromosome was substituted. (a) The B-y2 strain was used for the nuclear genome. (b) The Y-b2 strain was used for the nuclear genome.

almost the same constitutions of chromosomes were compared with two different combinations of mtDNA, the results from the heteroplasmy of *bw;e¹¹* and g20 mtDNA were essentially the same as those from the heteroplasmy of *y;bw;st* and g20 mtDNA (data not shown).

For the nuclear genomes in which major chromosomes were substituted, the proportions of g20 mtDNA increased at 25°C but decreased at 19°C when the chromosomes of the *y;bw;st* strain were substituted with those of the *bw;e¹¹* strain. However, the reciprocal substitutions did not change the direction of the selection at 19°C. These results indicate that each of the three chromosomes of the *bw;e¹¹* strain can decrease the proportions of g20 mtDNA at 19°C. From our previous results, the entire nuclear genome of the *y;bw;st* strain could function to increase the proportions of g20 mtDNA at 19°C (Matsuura *et al.*, 1997). This suggests that each of the three major chromosomes was involved in the temperature-dependent selection in mtDNA transmission, and that at least three genes co-operatively regulate the temperature-dependent process in mtDNA transmission.

To assess the effect of each chromosome substitution, the intensity of selection was estimated according to the method based on a model similar to genic selection in haploid organisms (Matsuura *et al.*, 1993). Table 1 shows the averages of the selection coefficients for *D. mauritiana* mtDNA at 25°C and 19°C for each heteroplasmy. Comparing the values obtained in the present study with those for the *bw;e¹¹* and *y;bw;st* strains, possible differences in the effect of each chromosome substitution were suggested at 25°C as well as at 19°C. It also appeared that the effects were larger when the X chromosomes were changed (B-y2 + y3 and Y-b2 + b3). The tendency observed for the B-y2 + y3 strain was confirmed in a different set of heteroplasmic

strains established from another female (data not shown). These results suggest that the contribution of each chromosome to the temperature-dependent selection is not equal.

At present, the mechanism of the temperature-dependent transmission of mtDNA has not yet been clarified in *Drosophila*. From the present findings, two mechanisms could be proposed. First, a protein molecule composed of multiple subunits is critical for the temperature-dependent process. Substitution of any one of such subunits may affect the efficiency of the process. In this case, such a protein complex may be directly involved in the maintenance of mitochondrial genomes. For example, protein factors composed in a subunit structure are known to be necessary for the replication and/or transcription process of mtDNA (Shadel & Clayton, 1997). Although the temperature dependency of such proteins has not been noted, one of these may show temperature-dependent activity. In *D. melanogaster*, DNA polymerase γ is composed of two subunits (Olson *et al.*, 1995). However, both have been found to be encoded on the second chromosome (Lewis *et al.*, 1996; Wang *et al.*, 1997). Secondly, multiple steps are involved in the temperature-dependent process, and many genes are responsible for the selection. Even for the normal activity of mitochondria, including both metabolic function and biogenesis, many proteins that are encoded in the nuclear genome are required. Some of these proteins may show temperature-dependent activity and may finally lead to the biased transmission of two types of mitochondria under certain conditions. In a recent report on mtDNA transmission in *D. melanogaster*, temperature-dependent selection was not detected in naturally occurring heteroplasmy for size variation in the A + T-rich region that contains the replication origin of mtDNA (Kann *et al.*, 1998). The absence of temperature dependency in this case may possibly be explained by the two types of mtDNA being identical for the coding region, although the variable portion within the A + T-rich region was not identified.

In either case, the temperature-dependent selection should provide an opportunity for elucidating the molecular mechanisms of mtDNA transmission in multicellular organisms. Temperature-dependent selection controlled by the nuclear genome has not been reported in any organisms other than *Drosophila*. Similar phenomena, however, may be observed for other animal mtDNA when two types of mtDNA with different sequences can coexist within a cell. Genetic and molecular tools in *Drosophila* are useful for the future identification of nuclear genes involved in mtDNA transmission. Clarification of the selection in mtDNA transmission is essential in understanding the evolution of communication between the nuclear and mitochondrial genomes.

Table 1 Averages of selection coefficients for *Drosophila mauritiana* mtDNA in heteroplasmy

Strain used as a recipient	Selection coefficient	
	25°C	19°C
<i>bw;e¹¹†</i>	0.329	-0.334
<i>y;bw;st†</i>	0.476	0.275
B-y2	0.182	-0.318
Y-b2	0.250	-0.237
B-y3	0.283	-0.211
Y-b3	0.330	-0.266
B-y2 + y3	0.596	-0.401
Y-b2 + b3	0.456	-0.169

†Matsuura *et al.* (1993).

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