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A mobile group II intron of a naturally occurring rearranged mitochondrial genome in *Kluyveromyces lactis*

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Summary. Mitochondrial intron content is variable in the yeast *Kluyveromyces lactis*. Strains can be divided into three classes depending on the structure of the cytochrome oxidase subunit 1 (*COX1*) gene: (1) those containing intron Kl cox1.1, (2) those containing Kl cox1.2, 3 and 4 and, (3) those that contain all four introns. In addition, strains belonging to the first class (designated Type B strains), have an altered mitochondrial gene order relative to strains from classes (2) and (3) (Type A, Hardy et al. 1989). Crossing experiments reveal that Kl cox1.1 (a group II intron) transfers at high frequency (89%) to mitochondrial genomes lacking this intron. By contrast, the mobility of the remaining introns (all group I) is of the order of 7%.

Key words: Intron transfer – Mitochondria – *Kluyveromyces* – Rearrangements

Introduction

In *Saccharomyces cerevisiae* some mitochondrial introns have properties of mobile elements. Thus the intron of the large ribosomal RNA subunit (*LSU*) gene, Sc lsu.1, and some introns of the cytochrome oxidase subunit 1 (*COX1*) gene, Sc cox1.1, Sc cox1.2 and Sc cox1.4 (following the nomenclature of Dujon 1989) can be transmitted in crosses to strains lacking them (Zinn and Butow 1985; Wenzlau et al. 1989; Meunier et al. 1990), whereas introns of the apocytochrome b (*COB*) gene are reportedly non-mobile (Meunier et al. 1990).

In *K. lactis* mtDNA the *COB* gene lacks introns (Brunner and Coria 1989) while the *COX1* gene in strain K8 (Hardy et al. 1989) has been found by sequence analysis to contain four introns (Hardy and Clark-Walker, accompanying paper). Of particular interest, the first intron, Kl cox1.1, has 96% base-matching to the Sc cox1.2 intron while exonic sequences show only 88% similarity. The high degree of intronic sequence-concordance suggests that this element is mobile and that it has transferred from one mitochondrial genome to the other since these yeasts diverged from a common ancestor. As survey experiments have shown that some strains of *K. lactis* lack intron 1 (Skelly and Maleszka 1991) we were able to undertake crossing experiments to analyze the transmission of this intron. In this study we show that 89% of zygotic colonies arising from such a cross contain a mitochondrial genome that has acquired Kl cox1.1. On the other hand, zygotic colonies from a cross designed to examine the mobility of introns Kl cox 1.2, 3 and 4 show that less than 10% contain recombinant genomes with these introns.

During the course of this study we needed to map the mitochondrial genome containing the solitary Kl cox1.1 intron. To our surprise we discovered that this mtDNA has a rearranged gene order in comparison to the genome previously examined (Hardy et al. 1989). Moreover, six of the 14 *K. lactis* strains in our possession contain the rearranged mtDNA and each has only the Kl cox1.1 intron.

Materials and methods

Yeast strains. The yeast strains used in this study are listed in Table 1.

DNA preparation, electrophoresis and hybridization. Total cellular DNA isolation, mtDNA isolation, restriction digestion of DNA, electrophoresis, hybridization and washing were carried out as described by Skelly and Clark-Walker (1990).

Probes. A list of probes, including intronic probes from *K. lactis COX1*, is given in Table 2. Probes were labelled by the random priming method (Feinberg and Vogelstein 1983).

Intron transmission in crosses. Strains to be crossed were grown overnight at 30 °C in 0.5% yeast extract, 1% bacto peptone and 2% glucose. One ml of each culture was mixed, pelletted, resuspended

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Table 1. *K. lactis* strains

Name	Genotype	mtDNA[a] type	Source
K8	*a*, prototroph	A1234	Hardy et al. 1989
RM1	*a*, *ura*	A1234	uv mutant of strain K8. Maleszka and Clark-Walker 1989
SD11	*a*, *lac4*, *trp1*	A1234	Das and Hollenberg 1982
WM37	*a*, *his*	A234	A. Brunner; Tingle et al. 1968
W231B	*a*, *his*	A234	NRRL Y-11,856
VD1	α, *ura3*, *arg*, *lys*	A234	Bianchi et al. 1987
KA5-11B	α, *ade1(r)*	A234	A. Brunner
K13	α, *ade(w)*, *his*	A234	Segregant from a cross of W231B and W599C
WM12	α, *ade1*, *his4*	A'234	NRRL Y-11,962
W600B	α, *ade1*, *ade2*, *leu1*	B1	A. Brunner
WM52	α, *ade1*, *his7*	B1	NRRL Y-11,961
WM88	α, *lys*	B1	NRRL Y-11,631
KA5-4C	*a*, *ade2(w)*, *leu*	B1	A. Brunner
KZ15-A1	α, *leu1*, *lys1*	B1	A. Brunner
KC17-5D	*a*, *ade2(w)*, *leu1*	B1	A. Brunner

[a] Letters A and B refer to the gene order, A'mtDNA of strain WM12 contains an extra *Hae*III site in the *COX1* gene. Numbers 1–4 record the presence of *COX1* introns

in 0.2 ml sterile water and dropped onto plates solidified with 1.5% w/v agar and containing 2.5% malt extract. After overnight incubation at 25 °C, cells were streaked onto selective minimal medium containing 2% glucose, 0.67% Difco yeast nitrogen base, 1,5% agar, and the plates were incubated at 28 °C for 2–3 days. Total cellular DNA was isolated from prototrophic zygotic colonies chosen at random. Examination of mtDNA was performed an *Hae*III or *Eco*R1 digests of total cell DNA that was electrophoretically separated in 0.8% agarose, transferred to nylon membranes according to Reed and Mann (1985) and hybridized with ^{32}P-radiolabelled probes as described previously (Skelly and Clark-Walker 1990).

Results

Mitochondrial introns in K. lactis strains

Strains of *K. lactis* are polymorphic for *COX1* introns and can be divided into three groups: (1) those lacking Kl cox1.1 yet containing Kl cox1.2, 3 and 4 (1, 2, 3, 7 and 8, Fig. 1), (2) those that contain Kl cox1.1 yet lack Kl cox1.2, 3 and 4 (4, 5 and 6), and (3) those that contain all four introns (9) (Table 1). The mitochondrial map of this last group (designated type A 1234) is given in Fig. 2a and is derived from Hardy et al (1989). The maps of the strains that lack Kl cox1.1 have been constructed by conventional double-digestion techniques and are of two types (A234 and A'234). The map of the A234 mitochondrial genome is identical to that of A1234 with Kl cox1.1 removed (Fig. 2b). Thus the 8.4 and 3.1 kb *Hae*III fragments, that contain Kl cox1.1, are missing in type A234 and are replaced with a 9.0 kb fragment (lanes 8 and 9, Fig. 1). Type A'234 mtDNA contains an additional *Hae*III site compared with A234 that cuts the 7.6 kb fragment into a 6.25 kb and a 1.35 kb fragment (lane 2, Fig. 1; Fig. 2b).

Table 2. DNA probes

(a) *K. lactis COX1* introns

Intron	Name[a]	Description	Source[b]
Kl cox1.1	pKLM1	(196 bp *Hin*PI fragment of intron 1)	1
Kl cox1.2	pKLM2	(1 682 bp *Bst*E2 fragment containing all of intron 2 plus 38 bp flanking exon sequence)	1
Kl cox1.3	pKLM3	(149 bp *Xho*I-*Pst*I fragment of intron 3)	1
Kl cox1.4	pKLM4	(498 bp *Eco*RI fragment of intron 4)	1

(b) Probes containing specific mtDNA fragments

Gene[c]	Name	Description	Source
LSU	pCH20	3.8 kb *Hin*dIII fragment of K8 mtDNA containing 3' end *LSU*	C. Hardy
SSU	pSEM5	~1.8 kb *Kpn*I fragment containing entire *SSU* gene from *S. exiguus* mtDNA	G. D. Clark-Walker
COX1 (exons 1–2)	DS6/A401	6.1 kb fragment of *S. cerevisiae* mtDNA	2
COX2	pCH14	952 bp *Fnu*DII fragment containing entire *COX2* gene	3
A9	DS400/A3	1.8 kb fragment of *S. cerevisiae* mtDNA	4
COB (exon 3)	DS400M11	3.36 kb fragment of *S. cerevisiae* mtDNA	5

[a] All DNA fragments have been cloned in pTZ18

[b] References: 1, Hardy and Clark-Walker 1991; 2, Bonitz et al. 1980; 3, Hardy and Clark-Walker 1990; 4, Macino and Tzagoloff 1979; 5, Nobrega and Tzagoloff 1980

[c] Gene abbreviations: *LSU*, large subunit rRNA; *SSU*, small subunit rRNA; *COX1*, *COX2* and *COX3*, subunits 1, 2 and 3 of cytochrome oxidase; *A6*, *A8* and *A9*, subunit 6, 8 and 9 of ATPase respectively; *COB*, apocytochrome b

A rearranged mitochondrial genome

Strains (type B1), that contain only the Kl cox1.1 intron, have mtDNA with a radically different *Hae*III digestion profile compared to mtDNA from type A strains (Fig. 1). Since the difference between A and B mitochondrial genomes cannot be explained by intron content, mtDNA from a type B strain (W600B) was mapped (Fig. 2c). The size of W600B mtDNA is 34.9 kb whereas that of K8 is 39.5 kb (Hardy et al. 1989), however, the most striking difference between type A and B mitochondrial genomes is that a rearrangement exists. Thus, type B mtDNA has approximately 10 kb of sequence, containing genes for *A9*, *COX3* and *COX2*, interposed between *COB* and *SSU*, whereas in type A mtDNA this block is bounded by *LSU* and *COX1*. This arrangement of genes results in three novel junctions in type B relative to type A (Fig. 3). The first novel junction is demonstrated by hybridization of probes for *LSU* and *COX1* to an 8.0 kb *Sal*1-*Eco*R1 fragment, while the flanking 5.0 kb *Sal*1 fragment and

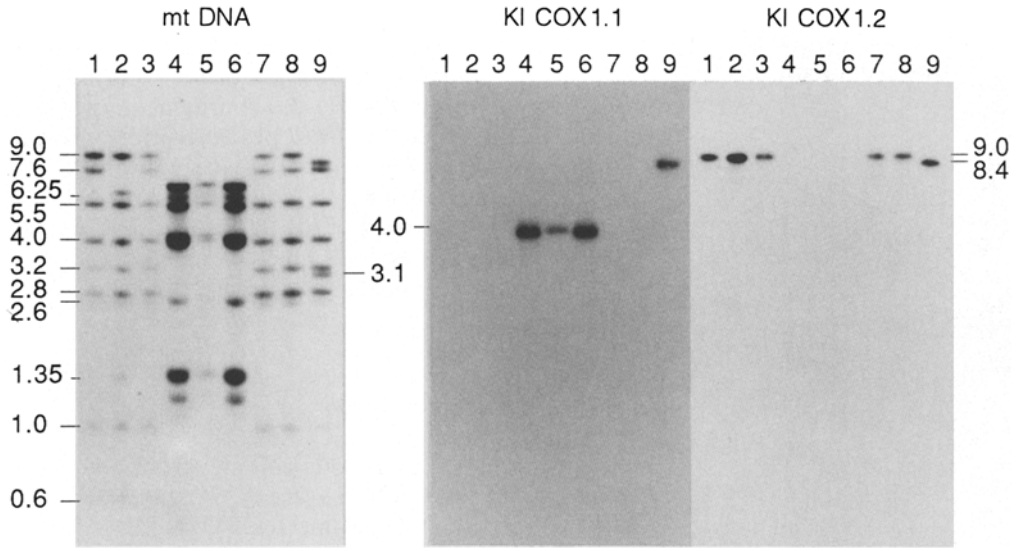

Fig. 1a–c. Autoradiograms showing hybridization of ^{32}P-labelled *K. lactis* (strain K8) mtDNA (**a**), KI cox1.1 (**b**) and KI cox1.2 (**c**) to *Hae*III digests of nine *K. lactis* strains. *1*, KA5-11B; *2*, WM12; *3*, K13; *4*, KZ15-A1; *5*, WM52; *6*, W600B; *7*, VD1; *8*, WM37; *9*, RM1. Fragment sizes are indicated (kb). Hybridization of introns KI cox1.3 and 1.4, that are identical to KI cox1.2, are not shown

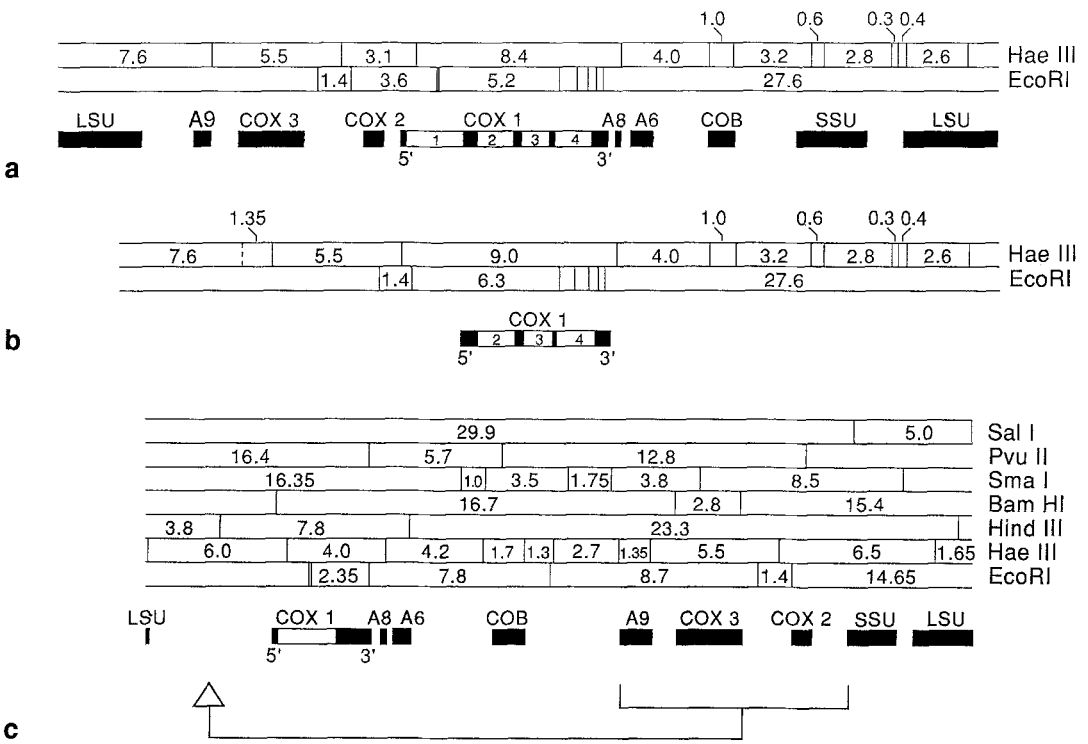

Fig. 2a–c. Maps of mtDNAs from *K. lactis* strains K8, A1234 (**a**) WM37, A234 (**b**) and W600B, B1 (**c**). Maps have been linearized at the *Sal*1 site in the large subunit of the rRNA gene (*LSU*). Other symbols represent; *COX1*, *2* and *3*, subunits 1, 2 and 3 of cytochrome oxidase; *COB*, apocytochrome b; *A6*, *8* and *9*, subunits 6, 8 and 9 of the ATPase complex and *SSU*, small subunit of rRNA. The *Hae*III 1.35 kb fragment, that is characteristic of genome A′234, is shown in **b** as a *dotted line*. The small *Eco*R1 fragments at the 3′ end of the *COX1* gene in types A1234 and A′234, (**a** and **b**), have sizes of 550, 500, 330 and 230 bp. The arrangement of exons and introns in the *COX1* gene are indicated by *filled* and *open boxed* regions. Intron 1 contains two juxtaposed *Eco*R1 sites 90 bp apart. The *bracketed region* in type B1 mtDNA (**c**) is the segment that has been translocated to form the type A genome or vice versa. The positions of the *COX2*, *COX1* and *A8* genes have been located by sequence analysis; locations of other genes are not known so accurately

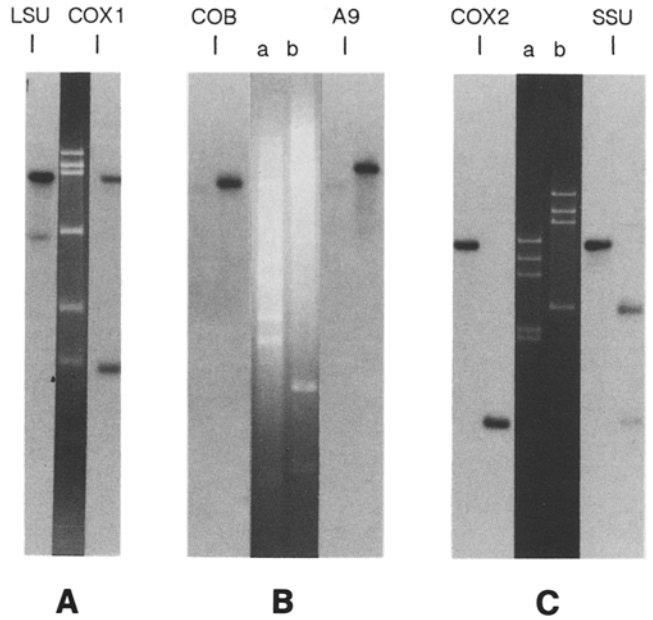

Fig. 3A–C. Autoradiograms showing hybridization of ^{32}P-labelled mtDNA probes to digests of *K. lactis* W600B mtDNA. Digests to demonstrate the three novel junctions 1–3, have been generated with A, *Eco*R1/*Sal*1; Ba, *Bam*H1/*Puv*II; Bb, *Eco*R1; Ca, *Hae*III; Cb, *Eco*R1/*Sal*1. Probes for hybridization, described in Table 2, are *LSU*, pCH 20; *COX1*, DS6/A401; *COB*, M11; *A9*, DS400/A3; *SSU*, pSEM5 and *COX2*, pCH14

the 2.35 kb *Eco*R1 fragment hybridize to the *LSU* and *COX1* probes respectively. The second novel junction is revealed by common hybridization of the *COB* and *A9* probes to an 8.0 kb *Bam*H1-*Puv*II fragment, whereas with the *Eco*R1 digest the *COB* probe hybridizes to the 7.8 kb fragment and the *A9* probe reacts with the 8.7 kb band. Demonstration of the third novel junction is shown by common hybridization of the *COX2* and *SSU* probes to the 6.5 kb *Hae*III and 3.1 kb *Sal*1-*Eco*R1 fragments. In addition, the *SSU* probe hybridizes to the 5.0 kb *Sal*1 band.

Intron transmission in crosses

Intron transmission in zygotic colonies from a cross of W600B (type B1) to WM37 (type A234) were examined by hybridization with total mtDNA (Fig. 4a) or intron K1 cox1.1 (Fig. 4b). Most progeny (89% Table 3a), contain a recombinant genome characterized by the presence of two novel *Eco*R1 bands, of 5.2 and 3.6 kb, that are not found in either parent (Fig. 4a). All recombinant mtDNAs contain intron K1 cox1.1 (Fig. 4b) to yield a mitochondrial genome which is identical to that of strain RM1 (Fig. 2a). In other words, the A234 type mtDNA has gained the *COX1* intron from type B1 mtDNA to become A1234. In addition, most zygotic colonies also con-

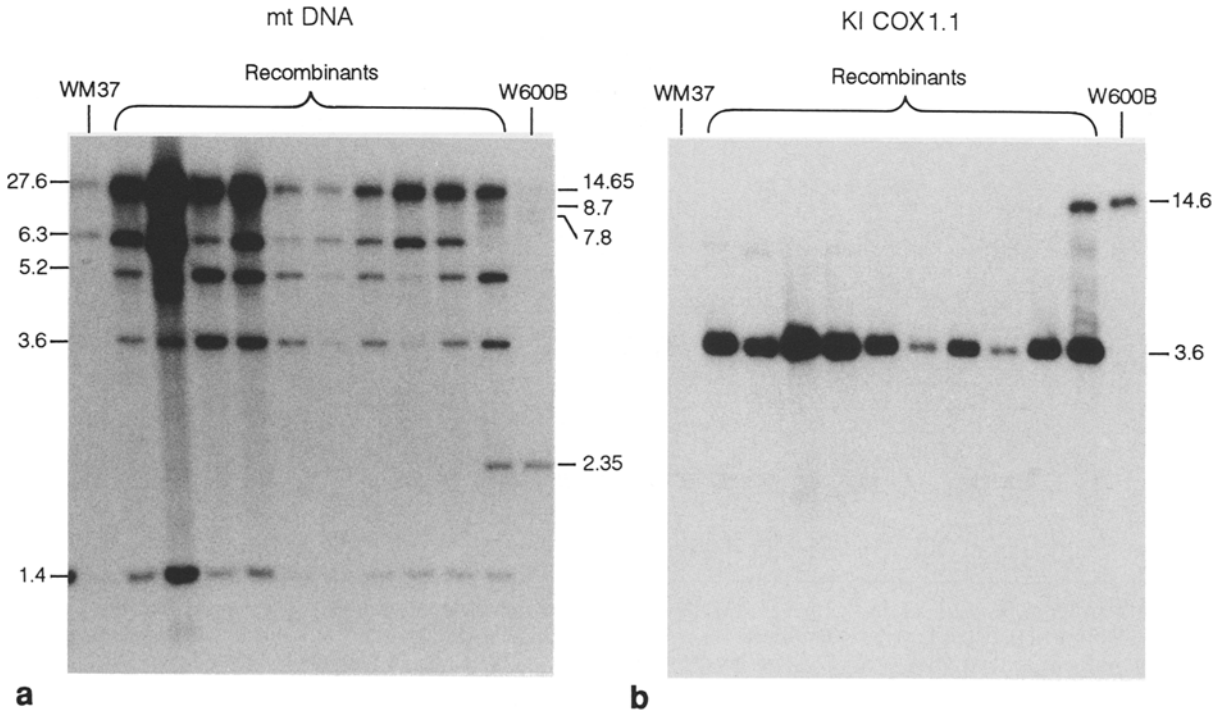

Fig. 4a, b. Autoradiograms showing hybridization of ^{32}P-labelled strain K8 mtDNA (**a**) and K1 cox1.1 (**b**) to *Eco*RI digests of total cellular DNA from zygotic colonies arising from a cross of strain WM37 with W600B. Recombinants are indicated by the presence of two novel bands of 5.2 and 3.6 kb. A small amount of W600B mtDNA is present in the last zygotic colony while the remaining zygotic colonies contain a mixture of the recombinant genome and WM37 mtDNA

Table 3. (a) mtDNA type in progeny from a cross of strain W600B (mtDNA type B1) with WM37 (A234), $n=46$

mtDNA type % (*n*)			
W600B	WM37	Recombinant[a]	Mixed[b]
4 (2)	4 (2)	89 (41)	2 (1)

Table 3. (b) mtDNA type in progeny from a cross of strain RM1 (A1234) with WM52 (B1), ($n=124$)

mtDNA type % (*n*)			
RM1	WM52	Recombinant	Mixed[b]
14 (17)	31 (38)	7 (9)	48 (60)

[a] Most recombinants (37/41) also contained the parental WM37 genome; the remaining recombinants contained the W600B genome
[b] Indicates colonies containing both parental genomes

tain a WM37 mitochondrial genome characterized by the 6.3 kb *Eco*R1 fragment or, less frequently, W600B mtDNA (last recombinant colony Fig. 4).

To examine transmission of the remaining *COX1* introns, mtDNAs of a cross involving RM1 (type A1234) and WM52 (type B1) were analysed (Table 3b). In this case only 7% of the progeny contain recombinant mitochondrial genomes, that are type B, with all four introns (data not shown). In this experiment, a large proportion of colonies were found to contain both parental genomes (60/124).

Discussion

The two notable observations from this study are that some *K. lactis* strains contain a rearranged mitochondrial genome and that in crosses the Kl cox1.1 intron is mobile.

Mitochondrial genomes from different species of yeasts and filamentous fungi are known to vary in both length and gene order (Clark-Walker 1989, 1991). Even within a single yeast genus, mitochondrial genomes have been found to vary in size from 28–101 kb and to have several rearrangements (Hoeben and Clark-Walker 1986). In *S. cerevisiae*, rearranged mitochondrial genomes have been generated in the laboratory (Clark-Walker 1989). However, to our knowledge, the demonstration that mtDNA from strain W600B is rearranged, relative to strain K8, is the first report of two topological forms of mtDNA being found in naturally occurring members of a single species. This unusual result calls into question whether the various strains harbouring the two types of mtDNA have been misclassified. Other data would argue against this notion. First, we find that opposite mating strains, containing type A or type B mtDNAs, can be crossed (this study and unpublished observations); second, meiosis and sporulation of the resulting diploids appears normal and third, ascus dissection gives four viable ascospores (Galeotti 1981). Furthermore, electrophoretic karyotyping by pulsed field gel electrophoresis reveals that strains with type A or type B mtDNAs have the same number of chromosomes (Maleszka and Clark-Walker 1989; Maleszka, unpublished observations). Hence it appears that, even within interbreeding strains, rearrangement of mtDNA can occur with the consequence that gene order constancy is not necessarily a useful taxonomic yardstick.

As noted, the remarkable sequence similarity between the *S. cerevisiae* cox1.2 and *K. lactis* cox1.1 introns (96% base matching, Hardy and Clark-Walker 1991) strongly suggests that horizontal mobility of this group II intron is possible. Supporting this idea is the observation that in crosses of *K. lactis* strains, the Kl cox1.1 intron is transferred from the B1 to the A234 mtDNA. There are two possible explanations for this result. First, intron transfer may have taken place by a site-specific recombination between the two mitochondrial genomes. Results in accord with this mechanism come from crosses of *S. cerevisiae* strains with mtDNAs differing in the presence or absence of large segments of non-template, intergenic, DNA up to 5 kb in size (Skelly and Clark-Walker 1990). In these studies, the preferential recovery of larger mtDNA molecules in zygotic colonies is best explained by conversion of the smaller genome in a process analogous to gene conversion at genetic loci. Thus, it is possible that formation of recombinant genomes containing the four *COX1* introns could have taken place by an analogous process.

Alternatively, intron transfer may have occurred by a reversal of the intron-splicing reaction. Recently, it has been demonstrated that a group II intron RNA of the *S. cerevisiae* mitochondrial genome can reintegrate into an RNA substrate in vitro (Augustin et al. 1990; Morl and Schmelzer 1990a, b). As group II introns contain open reading frames coding for polypeptides that have sequence similarities to reverse transcriptases (Michel and Lang 1985; Xiong and Eickbush 1990), it is conceivable that intron transfer could occur by recombination of a single-stranded or double-stranded DNA produced from a recombinant RNA formed by splicing reversal (Lambowitz 1989; Augustin et al. 1990; Grivell 1990; Morl and Schmelzer 1990a). Evidence from our results in support of intron transfer by this mechanism is that, in the cross between strains containing type B1 and A234 mitochondrial genomes, we only find type A1234 and not the reciprocal B1234 mtDNAs. This result, together with the low transmission (approximately 10%) of the group I introns 2, 3 and 4 in the cross between types B1 and A1234, suggests that conversion, if it occurs, can only account for a small proportion of the Kl cox1.1 intron transmission.

During the course of our studies, analogous observations have been reported for intron transmission in *S. cerevisiae*. In this yeast the group II introns Sc cox1.1 and 1.2 were found to transpose at frequencies similar to that reported here for Kl cox1.1 (Meunier et al. 1990).

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References

Augustin S, Muller MW, Schweyen RJ (1990) Nature 343:383–386
Bianchi MM; Falcone C, Chen XJ, Wesolowski-Louvel M, Frontali L, Fukuhara H (1987) Curr Genet 12:185–192

Bonitz SG, Coruzzi G, Thalenfeld BE, Tzagoloff A, Macino G (1980) J Biol Chem 255:11927–11941
Brunner A, Coria R (1989) Yeast 5:209–218
Clark-Walker GD (1989) Proc Natl Acad Sci USA 86:8847–8852
Clark-Walker GD (1991) Int Rev Cytol (in press)
Das S, Hollenberg CP (1982) Curr Genet 6:123–128
Dujon B (1989) Gene 82:91–114
Feinberg AP, Vogelstein B (1983) Anal Biochem 132:6–13
Galeotti CL, (1981) PhD Thesis. Australian National University
Grivell LA, (1990) Nature 344:110–111
Hardy CM, Clark-Walker GD (1990) Yeast 6:403–410
Hardy CM, Clark-Walker GD (1991). Curr Genet
Hardy CM, Galeotti CL, Clark-Walker GD (1989) Curr Genet 16:419–427
Hoeben P, Clark-Walker GD (1986) Curr Genet 10:371–379
Lambowitz AM (1989) Cell 56:323–326
Macino C, Tzagoloff A (1979) J Biol Chem 254:4617–4623
Maleszka R, Clark-Walker GD (1989) Curr Genet 16:429–432
Meunier B, Tian GL, Macadre C, Slonimski PP, Lazowska J (1990) In: Quagliariello E, Papa S, Palmieri F, Saccone C (eds), Structure, function and biogenesis of energy transfer systems. Elsevier, Amsterdam, pp 169–174
Michel F, Lang BF (1985) Nature 316:641–643
Morl M, Schmelzer C (1990) Cell 60:629–636
Morl M, Schmelzer C (1990) Nucleic Acids Res 18:6545–6551
Nobrega FG, Tzagoloff A (1980) J Biol Chem 255:9828–9837
Reed KC, Mann DA (1985) Nucleic Acids Res 13:7207–7221
Skelly PJ, Clark-Walker GD (1990) Mol Cell Biol 10:1530–1537
Skelly PJ, Maleszka R (1991) Curr Genet 19:89–94
Tingle M, Herman A, Halvorson HO (1968) Genetics 58:361–371
Wenzlau JM, Saldanha RJ, Butow RA, Perlman PS (1989) Cell 56:421–430
Xiong Y, Eickbush TH (1990) EMBO J 9:3353–3362
Zinn AR, Butow RA (1985) Cell 40:887–895

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