We have shown that coactivators and corepressors use similar mechanisms of interaction with distinct NR conformations. Our results begin to shed light on a code underlying NR interactions with functionally distinct coregulators. Sequences flanking both sides of the coactivator L*xx*LL core impart receptor specificity[18,26,27] and dictate that coactivators exclusively recognize the ligand-bound NR conformation. Corepressor CoRNR1 uses a unique sequence on the N terminal side of its I-*x*-*x*-I/V-I core for binding aporeceptors, whereas CoRNR2 interaction with the unliganded NR conformation requires specific C-terminal sequences flanking an L*xx*II core. The precise sequences of the ϕ*xx*ϕϕ cores and flanking sequences are critical determinants of receptor preference. Differences between N-CoR and SMRT may explain why TR preferentially interacts with N-CoR, whereas RXR interacts better with SMRT (Fig. 1d, f). Furthermore, the presence of two CoRNR boxes per corepressor is probably related to the stoichiometry of NR binding[28], and the receptor specificity of CoRNR1 and CoRNR2 may confer specificity for NR homo- and heterodimers[9,10,13]. The ability of short CoRNR polypeptides to selectively block corepressor or coactivator interaction with NR specificity indicates that peptidomimetic compounds may be developed with NR-selective abilities to block repression or activation. These could be of therapeutic benefit in syndromes of thyroid hormone resistance[29] and acute promyelocytic leukaemia[3,4]. □

Methods

Constructs

Deletion constructs and chimaera constructs were made by PCR or ligation of double-stranded oligonucleotides. Point mutations were made using the Quick-change site-directed mutagenesis kit (Stratagene) or double-stranded oligonucleotides. We completely sequenced all relevant regions.

Glutathione *S*-transferase (GST)-pull-down assay

GST-pull-down experiments were done as described[28]. Peptides used in the GST-pull-down experiments were synthesized by Cybersyn or the Protein Chemistry laboratory of the University of Pennsylvania Medical Center (supported by the core grant of the Penn Diabetes Center). Competing peptides were dissolved in DMSO and used at 100 μM unless otherwise indicated. Quantification was performed by phosphorimaging. T3 concentration was 10 μM.

Transient transfection assays

Transient transfections assays of repression, activation and mammalian two-hybrid interactions were performed as described[13] using lipofectamine (GIBCO BRL). All transfections were done in 293T cells. All Gal4 experiments used a (Gal4 × 5)-SV40-Luc reporter and a control β-galactosidase (β-gal) construct. T3 concentration was 1 μM.

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Mitochondrial DNA repairs double-strand breaks in yeast chromosomes

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The endosymbiotic theory for the origin of eukaryotic cells[1] proposes that genetic information can be transferred from mitochondria to the nucleus of a cell, and genes that are probably of mitochondrial origin have been found in nuclear chromosomes[2]. Occasionally, short or rearranged sequences homologous to mitochondrial DNA are seen in the chromosomes of different organisms including yeast, plants and humans[3]. Here we report a mechanism by which fragments of mitochondrial DNA, in single or tandem array, are transferred to yeast chromosomes under natural conditions during the repair of double-strand breaks in haploid mitotic cells. These repair insertions originate from non-contiguous regions of the mitochondrial genome. Our analysis of the *Saccharomyces cerevisiae* mitochondrial genome[4] indicates that the yeast nuclear genome does indeed contain several short sequences of mitochondrial origin which are similar in size and

composition to those that repair double-strand breaks. These sequences are located predominantly in non-coding regions of the chromosomes, frequently in the vicinity of retrotransposon long terminal repeats, and appear as recent integration events. Thus, colonization of the yeast genome by mitochondrial DNA is an ongoing process.

In the yeast *S. cerevisiae*, 90% of the repair of double-strand breaks (DSBs) in chromosomes occurs by homologous recombination[5]. Non-homologous events, which account for the remaining repair events, typically involve the re-ligation of DNA ends by occasional base-pairing[6–7]. Rarely (about 1% of non-homologous events), repair occurs by insertion of a short retrotransposon sequence[8–9].

We have investigated non-homologous repair events in haploid *S. cerevisiae* cells after induction of a DSB, mediated by the I-*Sce*I nuclease. A *URA3* cassette, between two I-*Sce*I sites in opposite orientation, was introduced into chromosome XI in place of either *YKL222c* (D. Alexandraki, unpublished data) or *YKR098c* (ref. 10). Haploid cells were then transformed with the plasmid pPEX7, which bears the *LEU2* marker gene and which encodes the rare cutter restriction endonuclease I-*Sce*I under the control of a galactose-inducible promoter[11]. Transformed cells were plated onto a medium selective for leucine and containing galactose as the sole carbon source. Expression of I-*Sce*I resulted in the removal of the *URA3* gene (Fig. 1). Under these conditions, repair by homologous recombination was not possible and the great majority of cells did not form colonies. Approximately 1% of the total population formed colonies, compared with control cells (grown on the same medium but containing glucose, and thus not expressing I-*Sce*I). Polymerase chain reaction (PCR) fragments of 1,248 and 894 base pairs (bp) were expected if re-ligation of the extremities occurs by non-homologous end-joining (NHEJ) at the *YKL222c* and the *YKR098c* loci, respectively (Fig. 1). Out of a total of 265 randomly isolated survivors analysed, 1 (0.4%) resulted in a shorter and 5 (1.9%) in a longer DNA fragment, and the remaining clones either gave PCR fragments of the expected length or did not amplify (see Table 1, experiment 1). Sequencing of seven randomly chosen DNA fragments of the expected length showed that re-ligation of the extremities by NHEJ had occurred, as previously described[7]. The shorter DNA fragment had a deletion of 144 bp (28 and 116 bp from each extremity, respectively).

The five longer DNA fragments all contained an insertion which corresponded to mitochondrial DNA sequences (Fig. 2). In survivors 34pAS7, 34pAS16 and 34pAT9 (all corresponding to repair at the *YKR098c* locus), single insertions of 47, 77 and 97 bp fragments of mitochondrial DNA, respectively, had occurred. These insertions exhibited sequences identical to three distinct regions of the mitochondrial map (Fig. 3b), and all showed a concomitant loss of a single A residue at one of the two original DNA extremities. The two remaining survivors analysed, 622pBS8 (repair at the *YKL222c* locus) and 34pAS15 (repair at the *YKR098c* locus), were more complex because, in both cases, two non-contiguous mitochondrial sequences were inserted in a tandem array at each locus (Fig. 2). Survivor 622pBS8 contained two DNA fragments of 101 and 52 bp, with 100% identity to mitochondrial regions 19870–19770 and 30114–30063, respectively. Both the proximal and the distal nuclear chromosomal moieties were deleted by one nucleotide (Fig. 2). Survivor 34pAS15 contained two DNA fragments of 189 and 49 bp with 100% identity to mitochondrial regions 74600–74412 and 34946–34994, respectively. This insertion resulted in an intact chromosomal sequence proximally and a 4-nucleotide chromosomal deletion distally. A second anomaly of this survivor was the presence of one G residue downstream of the double insertion (Fig. 2). This residue could originate either from a single base addition concomitant to the repair process or from a point mutation. Sequencing or PCR artifacts were excluded as this residue was detected in three independent experiments and was absent from two independent mitochondrial DNA sequencing studies[4,12].

The inserted mitochondrial sequences described corresponded to coding and non-coding mitochondrial DNA, and to both orientations with respect to the major transcribed strand of the mitochondrial genome[4] (see Fig. 3). One of the two mitochondrial sequences inserted in 34pAS15 is part of a G-C cluster. All other insertions have the typical A-T-rich pattern of yeast mitochondrial DNA. In all of the five cases in experiment 1, deletion of one residue (four

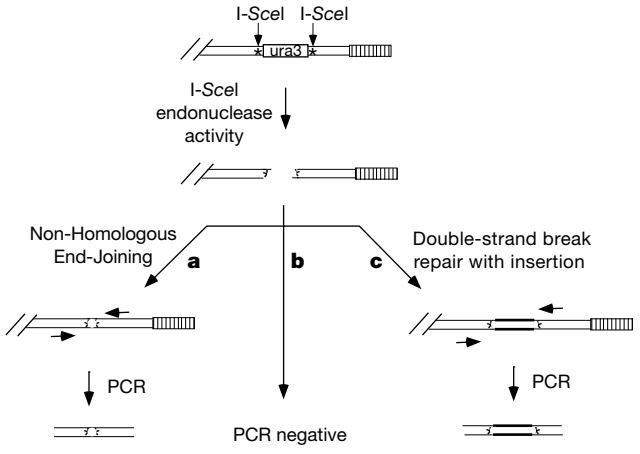


Figure 1 Flow-diagram of the experimental design. An artificial *URA3* cassette between two I-*Sce*I sites is inserted in yeast chromosome XI replacing either *YKL222c* or *YKR098c*. The scheme represents part of the right arm of chromosome XI (where the deleted ORF *YKR098c* was located; a mirror symmetry has to be used for the deleted ORF *YKR222c* which is located on the left arm of the chromosome). Striped rectangles indicate the telomere. Asterisk indicates an intact I-*Sce*I site. After action of I-*Sce*I, the double-strand break (DSB) can be repaired by either non-homologous end-joining (**a**) or by insertion of a foreign sequence (**c**). The two mechanisms are distinguished by PCR amplification from oligonucleotides located in flanking sequences (arrows). PCR negative cases (**b**) are not considered in this work.

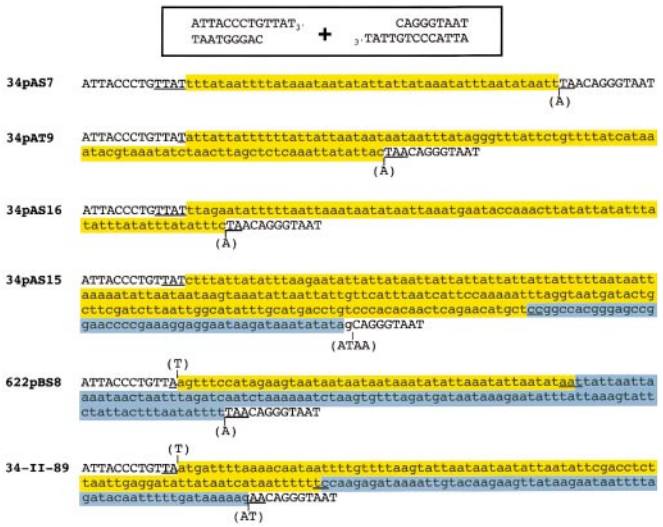


Figure 2 Sequencing of repaired double-strand breaks. The box at the top of the figure indicates expected chromosome ends resulting from I-*Sce*I cleavage (the two I-*Sce*I sites were placed in opposite orientation). Chromosomal sequences are indicated by capital letters (deleted nucleotides are shown in parentheses) and inserted sequences by lower-case letters. Coloured sequences are identical to mitochondrial DNA. Non-contiguous mitochondrial sequences are represented by different colours. Underlined residues indicate sequence homology at the ends of fragments either between the insertion and the chromosomal moiety or, in the case of double insertions, between the two inserted mitochondrial DNA fragments. All sequences are shown oriented as in the Watson strand of chromosome XI.

Table 1 PCR analysis of survivors after DSB induction

Experiment	Relevant nuclear genotype	Mitochondrial genotype	Total number of tested clones	Longer PCR DNA fragments
1	Δykl222c::URA3-I-SceI	ρ^+	92	1
1	Δykr098c::URA3-I-SceI	ρ^+	173	4
2	Δykr098c::URA3-I-SceI	ρ^+	166	1
2	Δykr098c::URA3-I-SceI	ρ^-	177	0
2	Δykr098c::URA3-I-SceI	ρ^0	173	0
2	Δykr098c::URA3-I-SceI	ρ^0	47	0
2	Δykr098c::URA3-I-SceI	ρ^0	165	0
2	Δykr098c::URA3-I-SceI, Δyme1::KANMX	ρ^+	240	0

All strains are FYBL2-5D derivatives transformed with the I-*Sce* I expression vector pPEX7. Complete genotypes are given in the Methods; relevant aspects are indicated in column 2 or 3 for the nuclear or mitochondrial genomes, respectively.

residues in 34pAS15) at the distal end of the chromosomal DSB suggests that a specific excision activity accompanies the mitochondrial DNA insertion. Based on the complete mitochondrial sequence[4], the two fragments in the double insertion events share a terminal homology of 2–3 nucleotides (underlined in Fig. 2). A homology of 1–4 nucleotides is also present between the inserted sequences and the expected DSB ends. The presence of such short homologies is typical of non-homologous junctions in mammalian[13] and yeast cells[6].

The insertion of 'filler' DNA, usually less than 40 bp, is common at illegitimate junctions in mammalian and plant cells, where it is accompanied by rearrangements at the junction site[14–16]. In contrast, our analysis shows that the integration of mitochondrial sequences into the *S. cerevisiae* chromosome is not accompanied by modifications at the junction site, except for the loss or gain of 1–4 nucleotides (Fig. 2). This was also the case for the insertion of retrotransposon Ty1 DNA fragments in yeast[9]. This suggests that the mechanism of integration of exogenous sequences into yeast chromosomes differs from that of filler DNA in higher eukaryotes.

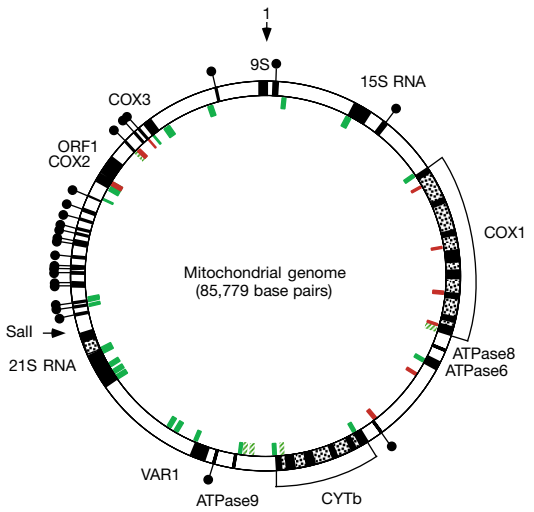
Transfer of mitochondrial sequences to the nucleus has been previously reported in yeast after transformation[17] or after microprojectile bombardment[18]. In the latter case, artificial ρ^- mutants, which did not contain endogenous mitochondrial DNA but carried a plasmid bearing a mitochondrial and a nuclear marker gene, increased the transfer efficiency by ~30 times compared with normal ρ^+ cells (containing a wild-type mitochondrial genome)[19]. Similarly, the nuclear mutation *yme1* increased transfer in ρ^+ cells[19].

To determine whether ρ^- and *yme1* mutants would increase the frequency of DSB repair by mitochondrial DNA, we constructed a ρ^- derivative and *Δyme1* mutant of the *Δykr098c* strain and repeated DSB induction and analysis of the surviving clones (Table 1, experiment 2). Our results show that no single case of transfer was observed for the ρ^- mutant (0/177 survivors) or for *Δyme1* (0/240 survivors). Additional experiments, performed with three different ρ^0 mutants, whose mitochondria do not contain DNA, did not show any mitochondrial insertion (0/385 survivors). As a control, when the experiment was repeated with the original ρ^+*YME1* (*Δykr098c*) strain, another case of transfer was found (1/166 survivors). This survivor, designated 34-II-89, contained a double insertion of 92 and 65 bp having 100% identity to mitochondrial regions 23396–23487 and 14804–14868, respectively (Figs 2 and 3). These results demonstrate that some mitochondrial DNA fragments are transferred to the nucleus of normal cells, at least transiently. In contrast to a previous report[19], this transfer appears to be independent of mitochondrial genomic alterations and of the *Δyme1* mutation.

The mechanisms by which mitochondrial DNA molecules escape the organelle and reach the nucleus remain to be elucidated. Membrane fusion, lysis of mitochondria, and illegitimate use of the nuclear import machinery have all been proposed (see ref. 3). Given that in yeast the nuclear membrane remains intact during mitosis[20], the passive incorporation of exogenous nucleic acids into the nucleus during this phase is unlikely.

The frequency of the integration events observed here (~1.5% of survivors) suggests that this is not an exceptional event. We therefore re-examined the presence of mitochondrial sequences in the nuclear genome by comparing the sequence of the sixteen yeast chromosomes with the recently sequenced mitochondrial genome[4] (both sequences are from the same strain). Table 2 lists the 30 mitochondrial sequences (22–230 bp in length) that were found in 13 out of 16 yeast chromosomes, with sequence identity varying from 86–100%; a few of these sequences were previously described[21–23], but 22 are new. Among these, a 123-bp mitochondrial intergenic segment in chromosome IV, a 104-bp segment of

a



b

Survivor	Insertion length (nucleotides)	Position of fragment on the mitochondrial DNA sequence	Characteristics of the original mitochondrial sequence	Orientation
34-II-89 (b)	65	14,804–14,868	COX1(intronic ORF ai1)	parallel
622pBS8 (a)	101	19,870–19,770	COX1(intronic ORF ai3 encoding I-SceII)	antiparallel
34-II-89 (a)	92	23,396–23,487	Intergenic region	parallel
34pAT9	97	25,795–25,699	COX1(intron ai5 γ)	antiparallel
622pBS8 (b)	52	30,114–30,063	Intergenic region (between ori7 and ORF5)	antiparallel
34pAS15 (b)	49	34,946–34,994	Intergenic region (between ori2 and tRNAglu). G–C cluster	parallel
34pAS15 (a)	189	74,600–74,412	COX2 and ORF1	antiparallel
34pAS16	77	77,789–77,713	Intergenic region (between tRNAphe and tRNAthr1)	antiparallel
34pAS7	47	79,020–79,066	Intergenic region (upstream of COX3)	parallel

Figure 3 Description of mitochondrial DNA found in the nucleus. **a**, Mitochondrial map. The map of *Saccharomyces cerevisiae* mitochondrial DNA is deduced from its complete sequence[4]. Filled boxes indicate coding exons, dotted boxes indicate introns. Only major mitochondrial genes are labelled; transfer RNA gene locations are indicated by sticks with circles (see ref. 4 for complete annotation). Segments of mitochondrial DNA sequences found in the nuclear yeast sequences are indicated by green boxes (see Table 2 for complete list); those found more than once are striped. Segments of mitochondrial DNA transferred to the nucleus after DSB repair are indicated as red boxes. **b**, Description of the sequences inserted at the DSB. The orientations 'parallel' (or 'antiparallel') indicate that the sequence of the Watson strand of chromosome XI (Fig. 2) corresponds (or does not correspond) to the non-transcribed strand of mitochondrial DNA.

Table 2 Mitochondrial sequences present in *S. cerevisiae* chromosomes

Mitochondrial segment							Nuclear segment			
Coordinates		Gene/intergene	Orientation	Size (bp)	Percentage identity	BLAST2 score	Chromosome number	Chromosome coordinates (reference)	Chromosome region	
41088-41317	*	CYTB-i4	<	230	100	1.9×10^{-89}	IX	I 7245-7016 (ref. 22)	YIL177c <>YIL176c	
41088-41317	*	CYTB-i4	<	230	100	1.9×10^{-89}	X	J 7228-6999 (ref. 22)	YJL225c <>YJL223c	
1367-1489		tRNApro <> ORF6	<	123	99	3.3×10^{-44}	IV	D 544798-544676	YDR043c <> YDR044w	‖
61906-62037		21SRNA <> tRNA thr2	<	132	86	2.1×10^{-16}	II	B 363938-363807 (ref. 23)	YBR060c <> YBR061c	
6836-6889		15S RNA	>	54	100	2.9×10^{-16}	XII	L 682556-682609	YLR270w <> YLR271w	‖
58961-59016		21S RNA	<	56	98	6.3×10^{-16}	XI	K 16248-16193 (ref. 23)	YKL219w <> YKL218c	
42749-42800	*	CYTB <> ori6	>	52	98	1.1×10^{-14}	I	A 189215-189266 (ref. 23)	YAR033w <> LTR-YARdelta7	†
42749-42800	*	CYTB <> ori6	>	52	96	6.3×10^{-14}	VII	G 404800-404851	YGL051w <> LTR-YGLWdelta8	‡
36933-37032		CYTB (bl2 + non-coding)	<	104	92	1.3×10^{-13}	X	J 416522-416419	LTR-YJLWdelta8 <> LTR-YJLWdelta9	
74289-74349		COX2	<	61	86	3.5×10^{-13}	IX	I 429527-429467 (ref. 23)	YIR038c <> YIR039c	
42828-42909	*	CYTB <> ori6	>	83	98	6.3×10^{-13}	VII	G 404849-404931	YGL051w <> LTR-YGLWdelta8	‡
42828-42909	*	CYTB <> ori6	>	83	98	6.3×10^{-13}	I	A 189264-189346 (ref. 23)	YAR033w <> LTR-YARdelta7	†
43605-43648		CYTB <> ori6	>	44	98	2.4×10^{-11}	XIII	M 183458-183501 (ref. 21)	YML046w <> tRNA Gly	§
49454-49530		VAR1	>	78	95	1.4×10^{-10}	XIII	M 183523-183600 (ref. 21)	YML046w <> tRNA Gly	§
73712-73792		tRNA met <> COX2	>	78	92	6.7×10^{-10}	IX	I 246046-246123 (ref. 23)	YIL061c <> LTR-YILCdelta2	‖
29100-29169		ATPase6	>	70	95	1.9×10^{-09}	XII	L 412638-412707)	YLR134w <> YLR135w	‖
58341-58384		21S RNA	<	44	90	4.2×10^{-09}	IV	D 159237-159194	YDL169c <> YDL168w	
26324-26359	*	COX1	<	36	100	9.0×10^{-09}	VII	G 404972-404937	YGL051w <> LTR-YGLWdelta8	‡
64412-64494		includes tRNAcys	<	83	88	1.4×10^{-08}	VII	G 2350-2268	LTR-YGLomega1 <> YGL263w	
58841-58922		21S RNA	<	92	86	8.0×10^{-08}	VII	G 567910-567819	LTR-YGRCdelta16 <> LTR-YGRCdelta17	‖
6556-6593		15S RNA	<	38	92	2.3×10^{-07}	XIII	M 57783-57746	YML106w <> YML105c	‖
26330-26359	*	COX1	<	30	100	2.8×10^{-06}	I	A 189382-189353 (ref. 23)	YAR033w <> LTR-YARdelta7	†
45799-45835		ori6 <> ATPase9	>	37	89	3.4×10^{-06}	VII	G 897226-897262	YGR198w <> YGR199w	
79268-79326		COX3	<	59	88	2.4×10^{-05}	III	C 272219-272161 (ref. 23)	YCR090c <> YCR091w	‖
60312-60338		21S RNA	<	27	100	5.0×10^{-05}	XV	O 462258-462232	YOR072w <> YOR073w	‖
13770-13799		ori8 <> COX1	>	30	93	8.8×10^{-05}	XV	O 1037531-1037560	within YOR373w	‖
64614-64643		tRNAhis	<	30	93	8.8×10^{-05}	VII	G 2219-2190	LTR-YGLomega1 <> YGL263w	
52679-52702		ORF10 <> ori3	>	24	100	8.8×10^{-04}	XVI	P 939132-939155	YPR199c <> YPR200c	
51903-51927		ORF10 <> ori3	<	25	96	1.9×10^{-03}	XI	K 428468-428444	YKL008c <> YKL007w	‖
64603-64627		tRNAhis	<	25	96	1.9×10^{-03}	XVI	P 52950-52926	YPL259c <> YPL258c	
84295-84319		ori5 <> tRNAf-met	<	25	96	1.9×10^{-03}	VIII	H 197263-197239	YHR045w <> YHR046c	
80378-80400		COX3 <> ori5	<	23	100	2.3×10^{-03}	VII	G 450114-450092	within YGL024w	
77611-77634		tRNAphe <> tRNAthr1	<	24	95	4.9×10^{-03}	XVI	P 777107-777084	LTR-YPRCdelta15 <> LTR-YPRWdelta16	
13723-13744		ori8 <> COX1	>	22	100	6.0×10^{-03}	XII	L 35427-35448	YLL054c <> YLL053c	

Results are classified by decreasing order of probabilities compared with random occurrence of similar sequences. Mitochondrial sequences that have undergone substantial divergence or which have a short size are not reliably detectable and were therefore excluded from this analysis. The mitochondrial and nuclear chromosomal coordinates of the homologous sequences are given, together with the positions relative to genes on the mitochondrial and chromosomal maps, where the symbol <> indicates genomic objects flanking the analysed sequence. Nomenclature of mitochondrial genetic objects, such as ori, ORF, cox, is as defined in ref. 4. Sequences previously observed are indicated with a reference number on the right of the chromosome coordinates. The size of the nuclear sequence is indicated, as is its percentage of identity with the corresponding mitochondrial sequence. The orientation of the mitochondrial segment is given, where > indicates that the non-transcribed strand of the mitochondrial genome is represented on the Watson strand of the nuclear yeast sequence, and < the Crick strand. Mitochondrial sequences that are present more than once in yeast chromosomes are indicated with an asterisk.

†, ‡, § Multiple insertions of mitochondrial sequences at the same chromosomal site are indicated with identical symbols.

‖ Chromosomal regions that are part of ancestral duplications[28].

the *CYTB* gene (containing both coding and non-coding regions) in chromosome X, a fragment of 92 bp of the mitochondrial 21S RNA gene and a region of 83 bp which includes mitochondrial tRNAcys gene both in chromosome VII and a fragment of 70 bp of *ATPase6* in chromosome XII. The intergenic region of 83 bp, previously seen only in chromosome I (ref. 23), is also present in chromosome VII. In most cases, a given mitochondrial fragment is found only once in the nuclear genome. However, four sequences are present twice, and are located on different chromosomes (asterisks in Table 2). These have homologous or identical flanking sequences for several thousand bp[22,24,25], suggesting that they result from duplication of chromosomal regions that occurred after the mitochondrial DNA insertion.

In a few cases (see chromosomes I, VII, and XIII, Table 2) multiple insertions of non-contiguous mitochondrial fragments are present at the same site on the chromosome, as in the cases of the double insertions found in our DSB repair experiments. In a previous study[21], the authors postulated that such non-contiguous sequences may have originated from ρ^- rearranged mitochondrial DNA transferred to the nucleus. However, the survivors described in our experiments were ρ^+ (data not shown) and the ρ^- mutant failed to repair DSBs with its mitochondrial DNA (experiment 2). Although we cannot exclude the possibility that rare ρ^- mitochondria, present in wild-type ρ^+ cells, could be a source of deleted and/or rearranged DNA, we think that the joining of two mitochondrial sequences observed here occurred during the repair of DSBs.

A total of 34 distinct insertion sites, including the double events, was observed. Ninety-four per cent (32/34) of the mitochondrial sequences are inserted within intergenic regions of the yeast genome and, interestingly, 44% (14/32) of these are in the immediate vicinity of retrotransposon long terminal repeats (LTRs) or transfer RNA (tRNA) genes (Table 2). Because intergenic regions represent only 28% of the *S. cerevisiae* nuclear genome[26], traces of mitochondrial DNA sequences are 41 times more frequent in the non-coding areas than in the coding ones, consistent with the notion that integration of mitochondrial DNA into a gene would be deleterious. Alternatively, mitochondrial DNA sequences may preferentially insert into non-coding regions after, for example, transient DSBs occurring during meiosis. However, the insertion in Table 2 and other shorter sequences described in the Methods do not correlate with the mapped 'hotspots' of meiotic recombination in chromosome III (ref. 27). Table 2 and Fig. 3a show that the transferred sequences originate from all regions of the mitochondrial DNA map. They correspond to coding (38%) and non-coding areas (62%) in proportions close to the relative proportions of these regions in the mitochondrial genome, indicating an absence of bias for the origin of the colonizing DNA.

The complete or near-complete identity of the chromosomal sequences with mitochondrial DNA of the same strain, and the fact that when paralogous chromosome regions (resulting from an ancestral duplication event) are present[28] mitochondrial sequences are found in only one of them, indicate that these events correspond to recent transfers.

Thus, DSB repair offers a plausible mechanism to explain mitochondrial insertions in nuclear chromosomes and to account for the presence of mitochondrial DNA fragments and even genes in nuclear genomes. Mitochondrial transfer can take place under laboratory conditions, but it has certainly occurred naturally. From our experiments and from the analysis of the yeast genome sequences, we conclude that a constant flow of genetic information takes place from mitochondria to the nucleus. The precise mechanisms of mitochondrial insertions, which affects the evolution of the nuclear genome, need to be further elucidated. Unresolved

questions concern the mechanism of transfer, the frequent co-insertion of non-contiguous mitochondrial inserts and the presence of mitochondrial insertions in the vicinity of LTRs and tRNA genes. □

Methods

Experimental methods

The two haploid strains used are derivatives of FYBL2-5D (*MAT*-α, *ura3Δ851*, *leu2Δ1*, *trp1Δ63*), in which *YKL222c* (position 3507–5621) or *YKR098c* (position 632659–634809) have been replaced by the *URA3* I-*Sce*I cassette. Both strains are isogenic to the FY1679 (S288C) strain whose nuclear genome has been sequenced[26], except for the markers indicated. Both mutants retained the wild-type phenotype of the haploid cell. Yeast cells were transformed with the replicative I-*Sce*I expression plasmid, pPEX7, by the lithium acetate method. Analysis of repair was performed by PCR directly on cells using 20-mer oligonucleotides whose 5′-end coordinates are 2725 and 6106 for FYBL2-5D *Δykl222c*::*URA3* I-*Sce*I, and 632491 and 633481 for FYBL2-5D *Δykr098c*::*URA3* I-*Sce*I (see Fig. 1). In both cases the primer distal to the centromere was biotinylated. Polymerase chain reaction products were separated on 1.5% agarose gels. Under these conditions insertions or deletions of at least 50 bp are detectable. These PCR-amplified DNA fragments were cut out from the gel and purified with QIAquick PCR Purification kit (Qiagen). The appropriate single-strand DNA was separated with streptavidine and then sequenced.

The *Δyme1* mutant of the strain FYBL2-5D *Δykr098c*::*URA3* I-*Sce*I was obtained with the one-step gene-disruption technique by replacing *YPR024w* (*YME1*) with the KANMX module[29]. The presence of the KANMX module instead of *YPR024w* was molecularly checked by PCR amplification of the specific fragment and by the restriction pattern of the amplified fragment with different restriction enzymes. As described for the *yme1* mutant[19], the double mutant *Δyme1*, *Δykr098c* constructed here did not form ρ^- or ρ^0 colonies and was unable to grow on glucose at 14 °C and on glycerol at 37 °C. The ρ^- and ρ^0 mutants of the FYBL2-5D *Δykr098c* strain were obtained as described[30]. By definition, ρ^- strains retain only a fraction of the wild-type mitochondrial genome, often in an amplified and rearranged configuration, and ρ^0 strains have no mitochondrial DNA. The ρ^- mutant was partially characterized by PCR amplification of short mitochondrial sequences and by Southern blot analysis using purified mitochondrial DNA digested with different restriction enzymes. These last two techniques were also used to reveal the absence of mitochondrial DNA in the ρ^0 mutants.

DNA sequence comparison

Sequences of PCR inserts were compared first with the Genbank and EMBL databases and, subsequently with the mitochondrial yeast genome[4] using BLAST2. The systematic sequence comparison between the mitochondrial and the nuclear genome of FY1679 was performed using BLAST2. The nuclear yeast genome sequence was retrieved from MIPS (http://speedy.mips.biochem.mpg.de/mips/yeast) on 23 December 1998. The sequence of the mitochondrial genome of strain FY1679 was kindly provided by F. Foury[4]. Mitochondrial DNA sequence was fragmented into 200-bp-long sequences to serve as query. Two different fragmentations shifted by 100 bp with respect to each other were used. Defined mitochondrial sequences matching the nuclear genome were then compared once more with the nuclear genome and classified by increasing BLAST2 probability scores. Sequences with probability $\leqslant 6.0 \times 10^{-3}$ are shown in Table 2 and discussed in the text.

In addition to this first group of sequences, 137 other short mitochondrial sequences (18–21 bp in length) have 100% identity with nuclear sequences. They were excluded from the previous list based exclusively on BLAST scores but have statistical significance ($<10^{-3}$) if the length of the fragment, the size of the nuclear genome and the nucleotide composition of both the mitochondrial and the nuclear genome are taken into account. These sequences originate from both coding and non-coding regions of the mitochondrial genome. Although statistically significant, the fact that these sequences have such a small size and that a third of them are present in coding regions of chromosomes (compared with only 9% for the longer sequences) suggests that some of these may correspond to random coincidence rather than actual transfer. These sequences have not been taken into consideration in our conservative analysis.

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Mechanical unfolding intermediates in titin modules

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The modular protein titin, which is responsible for the passive elasticity of muscle, is subjected to stretching forces. Previous work on the experimental elongation of single titin molecules has suggested that force causes consecutive unfolding of each domain in an all-or-none fashion[1–6]. To avoid problems associated with the heterogeneity of the modular, naturally occurring titin, we