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Chimeric mitochondrial minichromosomes of the human body louse, *Pediculus humanus*: Evidence for homologous and non-homologous recombination

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ABSTRACT

The mitochondrial (mt) genome of the human body louse, *Pediculus humanus*, consists of 18 minichromosomes. Each minichromosome is 3 to 4 kb long and has 1 to 3 genes. There is unequivocal evidence for recombination between different mt minichromosomes in *P. humanus*. It is not known, however, how these minichromosomes recombine. Here, we report the discovery of eight chimeric mt minichromosomes in *P. humanus*. We classify these chimeric mt minichromosomes into two groups: Group I and Group II. Group I chimeric minichromosomes contain parts of two different protein-coding genes that are from different minichromosomes. The two parts of protein-coding genes in each Group I chimeric minichromosome are joined at a microhomologous nucleotide sequence; microhomologous nucleotide sequences are hallmarks of non-homologous recombination. Group II chimeric minichromosomes contain all of the genes and the non-coding regions of two different minichromosomes. The conserved sequence blocks in the non-coding regions of Group II chimeric minichromosomes resemble the "recombination repeats" in the non-coding regions of the mt genomes of higher plants. These repeats are essential to homologous recombination in higher plants. Our analyses of the nucleotide sequences of chimeric mt minichromosomes indicate both homologous and non-homologous recombination between minichromosomes in the mitochondria of the human body louse.

1. Introduction

Mitochondrial DNA (mtDNA) has been the mainstay of molecular studies of the ecology, evolution, and population genetics of animals for over 30 years (Avise et al., 1987; Cann et al., 1987; Moritz et al., 1987; Boore and Brown, 1998; White et al., 2008). The popularity of mtDNA is attributable to three supposed unique features. Studies of animal mtDNA from the 1960s to 1980s showed that, in comparison to nuclear DNA, mtDNA had (i) a high mutation rate (Brown et al., 1979); (ii) a simple mode of inheritance, maternal inheritance (Ursprung and Schabtac, 1965; Dawid and Blackler, 1972; Hayashi et al., 1978; Francisco et al., 1979); and (iii) little or no recombination (Clayton et al., 1974; Zuckerman et al., 1984; Hayashi et al., 1985). These features of animal mtDNA have been re-assessed in the past two decades as more and more data became available. There is little controversy about the first feature, that the mutation rate is usually higher in mtDNA than in nuclear DNA. The second feature seems to be true for most animals, that maternal inheritance is the usual mode of mtDNA inheritance, although it is clear that paternal inheritance of mtDNA occurs in some animals (reviewed in Barr et al., 2005; White et al., 2008). There is much controversy, however, about the third feature, the supposed lack of recombination of mtDNA in animals. Take for example human mtDNA, which is the most studied among animals. Human mitochondria have the biochemical machinery for recombination (Thyagarajan et al., 1996; Lakshmipathy and Campbell, 1999a,b), and there is compelling evidence for recombinant mtDNAs in humans (Kraytsberg et al., 2004; Zsurka et al., 2005, 2007). It is still controversial, however, how often mtDNA recombines in humans. Indeed, two papers on recombination of mtDNA in humans came to different conclusions. Sato et al. (2005) showed that recombination of mtDNA was rare in human somatic hybrid cells. Zsurka et al. (2005,

Abbreviations: mt, mitochondrial; mtDNA, mitochondrial DNA; kb, kilobase; rRNA, ribosomal RNA; tRNA, transfer RNA; PCR, polymerase chain reaction; DNA, deoxyribonucleic acid; CR, coding region; NCR, non-coding region; CSB, conserved sequence block; DSB, double-strand break; SSB, single-strand break; ROS, reactive oxygen species; *atp6* and *atp8*, ATP synthase subunits 6 and 8; *cox1-3*, cytochrome *c* oxidase subunits 1, 2, and 3; *cob*, cytochrome *b*; *nad1-6* and *4L*, NADH dehydrogenase subunits 1 to 6, and 4L; *pnad1*, part of the gene for NADH dehydrogenase subunit 1; *patp6*, part of the gene for ATP synthase subunit 6; *pcox2*, part of the gene for cytochrome *c* oxidase subunit 2; *pcox3*, part of the gene for cytochrome *c* oxidase subunit 3; *rrnL*, large ribosomal RNA subunit; *rrnS*, small ribosomal RNA subunit; *trnA*, tRNA gene for alanine; *trnC*, tRNA gene for cysteine; *trnD*, tRNA gene for aspartic acid; *trnE*, tRNA gene for glutamic acid; *trnF*, tRNA gene for phenylalanine; *trnG*, tRNA gene for glycine; *trnH*, tRNA gene for histidine; *trnI*, tRNA gene for isoleucine; *trnK*, tRNA gene for lysine; $trnL_1$, tRNA gene for leucine (anticodon NAG); $trnL_2$, tRNA gene for leucine (anticodon YAA); *trnM*, tRNA gene for methionine; *trnN*, tRNA gene for asparagine; *trnP*, tRNA gene for proline; *trnQ*, tRNA gene for glutamine; *trnR*, tRNA gene for arginine; $trnS_1$, tRNA gene for serine (anticodon NCU); $trnS_2$, tRNA gene for serine (anticodon NGA); *trnT*, tRNA gene for threonine; *trnV*, tRNA gene for valine; *trnW*, tRNA gene for tryptophan; *trnY*, tRNA gene for tyrosine.

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2007), however, showed that recombination of mtDNA was common in human skeletal muscle.

Evidence for recombination of mtDNA has also been reported in animals other than humans, including reptiles, fish, molluscs, nematodes, and arthropods (reviewed in Barr et al., 2005; White et al., 2008). Indeed, it has been suggested that recombination of mtDNA is much more widespread than has been observed empirically (Ladoukakis and Zouros, 2001; Piganeau et al., 2004). Convincing evidence for recombination of mtDNA in non-human animals, however, remains scarce, possibly due to the difficulty in detecting recombination (Barr et al., 2005; White et al., 2008). The mtDNA of animals typically consists of a single circular chromosome that is ~16 kb long and has 37 genes; the thousands to millions of copies of this chromosome in individual animals are either identical or near identical. Recombination cannot be detected if recombinant mtDNA is indistinguishable from non-recombinant mtDNA (White et al., 2008).

Unlike most animals, the mt genome of the human body louse, *Pediculus humanus*, consists of 18 circular minichromosomes; each minichromosome is 3 to 4 kb long and has 1 to 3 genes. There is unequivocal evidence for recombination between different minichromosomes in *P. humanus* (Shao et al., 2009). It is not known, however, how minichromosomes recombine. Here, we report the discovery of eight chimeric minichromosomes in *P. humanus*. Each of these chimeric minichromosomes contains sequences that are from two different minichromosomes. Our analyses of the sequences of these chimeric minichromosomes indicate both homologous and non-homologous recombination between the minichromosomes in the mitochondria of the human body louse.

2. Materials and methods

2.1. Analyses of nucleotide sequence-reads from the Human Body Louse Genome Project

The Human Body Louse Genome Project generated sequence-reads from the nuclear genome and the mt genome of *P. humanus*, and the genome of the endosymbiotic bacteria of this louse (Kirkness et al., 2010; sequences available at ww.vectorbase.org). We identified the sequence-reads that contained mt genes by Mega BLAST searches (Zhang et al., 2000). These sequence-reads were assembled into contigs with Sequencher (Gene Code, Ann Arbor, MI); the parameters for assembly were minimum match percentage 90% and minimum overlap 200 bp. tRNA genes were identified with tRNA-Scan (Lowe and Eddy, 1997) and ARWEN (Laslett and Canbäck, 2008); protein-coding genes and rRNA genes were identified with BLAST searches of GenBank (Altschul et al., 1990).

2.2. DNA extraction, PCR test, and nucleotide sequencing of chimeric mt minichromosomes

Total DNA was extracted from individual first-instar nymphs of *P. humanus* of the Orlando (Culpepper) strain, which was the same strain used in the Human Body Louse Genome Project. There were two PCR tests for each type of chimeric mt minichromosome. Positive and negative controls were run for all PCR tests. Fourteen amplicons, ~500 to ~4000 bp in size, were obtained from PCR tests and were cloned and sequenced (Fig. 1); the sequences were assembled into contigs and the genes were identified as described above. The protocols for DNA extraction, primer design, PCR set-up, agarose-gel electrophoresis, cloning of PCR amplicons, and nucleotide sequencing were described in Shao et al. (2009). The nucleotide sequences of the chimeric mt minichromosomes of *P. humanus* are in GenBank (FJ821004–FJ821014).

3. Results

3.1. Chimeric mt minichromosomes revealed by sequence-read analysis

Shao et al. (2009) reported that (i) the mt genome of *P. humanus* consisted of 18 circular minichromosomes; each minichromosome was 3 to 4 kb long and had 1 to 3 genes; and (ii) 36,615 of the 1,480,551 sequence-reads generated by the Human Body Louse Genome Project contained mt genes from the 18 mt minichromosomes. Here, we report that (i) 516 sequence-reads contain parts of two different mt protein-coding genes (Types 1 and 2 in Table 1; Supplementary material); the full-length counterparts of these partial protein-coding genes are in two different types of minichromosomes; and (ii) two sequence-reads and their mate-pairs contain sequences from genes that are found in two different mt minichromosomes (Types 3 and 4 in Table 1) (Note: a sequence-read and its mate-pair are from two different ends of the same DNA insert in a sequencing vector). These 518 sequence-reads are evidence for four different types of chimeric mt minichromosomes in *P. humanus*, in addition to the 18 mt minichromosomes reported in Shao et al. (2009). We classify these chimeric mt minichromosomes into two groups according to their gene-content: Group I chimeric minichromosomes have parts of two different protein-coding genes that are joined together (see below), whereas Group II chimeric minichromosomes have two or two clusters of genes that are separated by a large non-coding region (Table 1).

The Human Body Louse Genome Project used a whole-genome random shotgun sequencing strategy (Kirkness et al., 2010). So the number of sequence-reads of a given chimeric minichromosome or a minichromosome generated by the project should indicate approximately its abundance in *P. humanus*. There are 518 sequence-reads for the four types of chimeric minichromosomes described above and 36,615 sequence-reads for the 18 mt minichromosomes (see above). Therefore, the relative abundance of the four chimeric minichromosomes to the 18 mt minichromosomes (Shao et al., 2009) is 518 to 36,615, or 1 to 71. Similarly, of the four chimeric minichromosomes, the Type 2 chimeric minichromosome (496 sequence-reads; Table 1) is much more abundant than the Type 1 (20 sequence-reads), Type 3 (1 sequence-read), and Type 4 (1 sequence-read) chimeric chromosomes.

3.2. Chimeric mt minichromosomes revealed by PCR tests and nucleotide sequencing

Our PCR tests and nucleotide sequencing confirmed the presence of Type 1, Type 2, and Type 3 chimeric minichromosomes, which were first revealed by our sequence-read analyses (above). Moreover, our PCR tests and nucleotide sequencing revealed four additional chimeric minichromosomes of Group II: Types 5 to 8 (Table 2; Fig. 1). The fact that there are no sequence-reads for Types 5 to 8 in the sequence-reads from the Human Body Louse Genome Project indicates that these four types of chimeric minichromosomes are less abundant than Types 1, 2, and 3. All of the seven types of chimeric minichromosomes revealed by our PCR tests and nucleotide sequencing are apparently circular because the two PCR amplicons from each type of chimeric minichromosome overlap at both ends, by 42 to 414 bp (Fig. 1).

3.3. Size and gene-content of chimeric mt minichromosomes

We sequenced entirely the two Group I chimeric minichromosomes and four of the five Group II chimeric minichromosomes revealed by PCR tests (Table 2). The two Group I chimeric minichromosomes, Types 1 and 2, are about 2300 and 2700 bp and the four Group II chimeric minichromosomes, Types 3, 5, 6, and 7, are about 6300, 5100, 6500, and 6100 bp, respectively (Fig. 1; Table 2). Each Group I chimeric minichromosome has one coding region (CR) and one non-coding region (NCR), whereas each Group II chimeric minichromosome has two CRs and two NCRs (Fig. 1). The sizes of

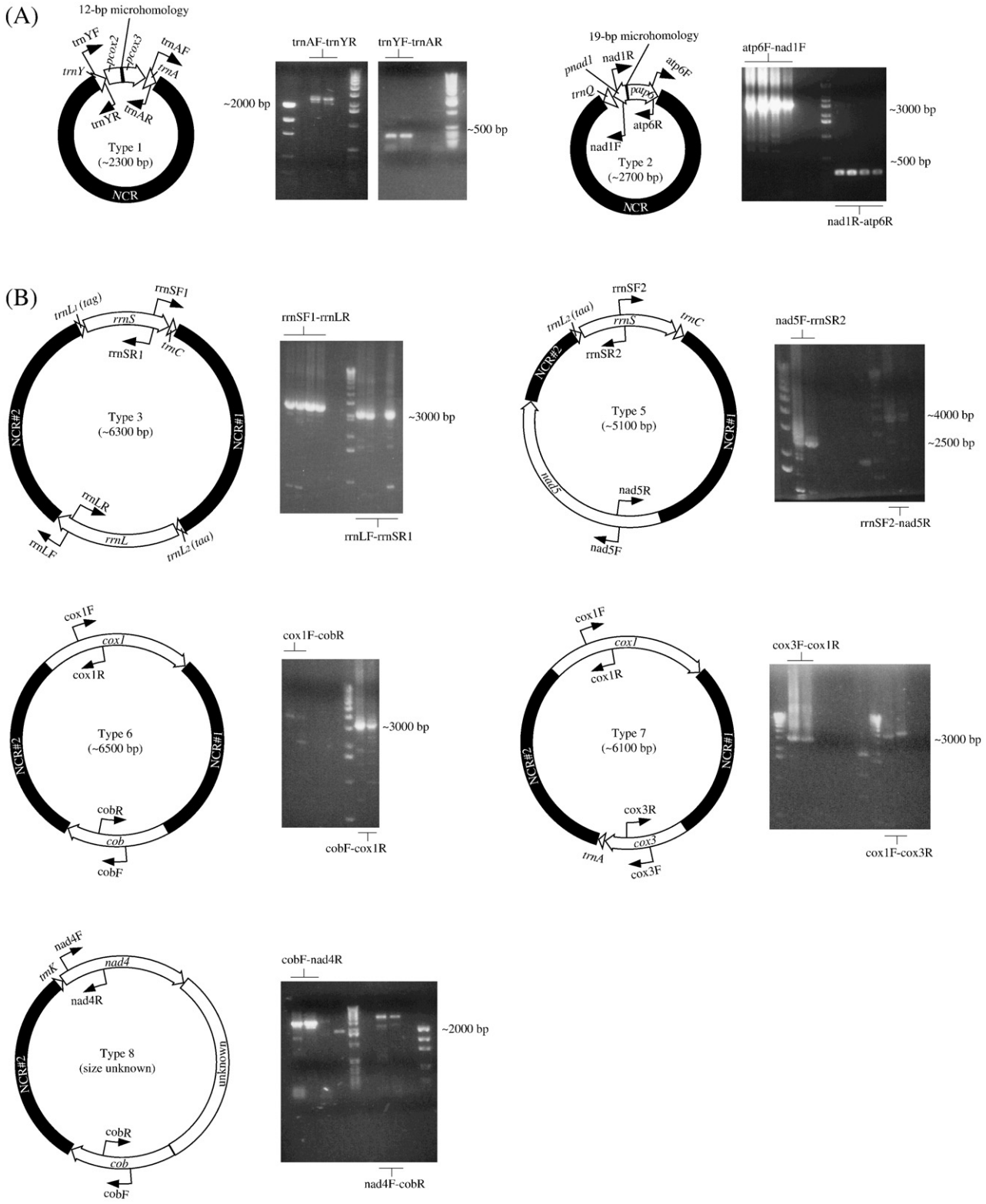

Fig. 1. Group I (A) and Group II (B) chimeric mt minichromosomes of *Pediculus humanus* revealed by PCR tests and nucleotide sequencing. Each chimeric minichromosome was amplified by PCR with two pairs of primers. Bent arrows indicate the location and the orientation of PCR primers. The non-coding regions (NCR) are in black. Protein-coding genes are abbreviated as *atp* (for ATP synthase), *cox* (for cytochrome *c* oxidase), *cob* (for cytochrome *b*), and *nad* (for NADH dehydrogenase), followed by the number of the subunit. *rrnL* and *rrnS* are for large and small rRNA subunits. tRNA genes are shown with the single-letter abbreviations of their corresponding amino acids. Note that *trnQ* is downstream of *nad1* and has an abnormal secondary structure (D-arm replacement loop). *trnQ* was misannotated to be upstream of *trnN* and *trnE* in Shao et al. (2009); this misannotation is corrected here.

chimeric minichromosomes vary, even among different minichromosomes of the same type, due to variation in the size of the NCR (Table 2; see below). Each Group I chimeric minichromosome has parts of two different protein-coding genes that are joined together (see below). In the Type 1 chimeric minichromosome, the parts of the two protein-coding genes, *pcox2* and *pcox3* (note: *p* for partial), have only 14% and 12% of the full-length of *cox2* and *cox3*. The two tRNA genes, *trnA* and *trnY*, in the Type 1 chimeric minichromosomes,

Table 1
Chimeric mitochondrial minichromosomes of *Pediculus humanus* revealed by analysis of sequence-reads from the Human Body Louse Genome Project.

Group	Type	Gene-content[a]	Identifier number of sequence-read[b]	Identifier number of mate-pair[b]	Template number
I	1	*trnY*, *pcox2*,[c] *pcox3*, *trnA*	(20 sequence-reads; see Supplementary material)	N.A.	N.A.
	2	*pnad1*, *trnQ*, *patp6*	(496 sequence-reads; see Supplementary material)	N.A.	N.A.
II	3	*rrnL*, *NCR*, *rrnS*, *trnC*	1101336098660	1101336969410	1061028611138
	4	*nad2*, *NCR*, *nad1*	1101306792505	1101306610395	1061028323577

Note: [a]See Fig. 1 for a list of the abbreviations of the names of genes; [b]sequence-read and mate-pair: the two sequence files from the two ends of the same DNA insert (i.e., template) in a sequencing vector; [c]"p" for partial.

Table 2
Chimeric mitochondrial minichromosomes of *Pediculus humanus* revealed by PCR tests and nucleotide sequencing.

Group	Type	PCR test 1			PCR test 2			Gene-content and gene-arrangement[a]	Size of NCR #1 (bp)	Size of NCR #2 (bp)
		Forward primer	Reverse primer	Size of amplicon (bp)	Forward primer	Reverse primer	Size of amplicon (bp)			
I	1	trnAF	trnYR	~2000	trnYF	trnAR	~300	*trnY-pcox2-pcox3-trnA-NCR*	1922, 2361	N.A.
	2	atp6F	nad1F	~3000	nad1R	atp6R	~300	*trnQ-pnad1- patp6-NCR*[b]	1908	N.A.
II	3	rrnSF1	rrnLR	~3800	rrnLF	rrnSR1	~3000	*$trnL_1(tag)$-rrnS-trnC-NCR#1-$trnL_2(taa)$-rrnL-NCR#2*	2115	2079, 2080
	5	rrnSF2	nad5R	2700-4000	nad5F	rrnSR2	~2500	*$trnL_2(taa)$-rrnS-trnC-NCR#1-nad5-NCR#2*	1835	634
	6	cox1F	cobR	~4000	cobF	cox1R	~3000	*cox1-NCR#1-cob-NCR#2*	1947	1992, 1777
	7	cox1F	cox3R	~4000	cox3F	cox1R	~3000	*cox1-NCR#1-cox3-trnA-NCR#2*	1696	1755, 1993, 1994
	8	nad4F	cobR	~4000	cobF	nad4R	~3000	*?-cob-NCR-trnK-nad4-?*[c]	?	1249, 2023

Note: [a]See Fig. 1 for a list of the abbreviations of the names of genes; [b]underlined genes are transcribed from right to left; genes not underlined are transcribed from left to right; [c]question marks indicate unknown gene-content and size.

however, have the same sequence and length as their counterparts in the mt minichromosomes. In the Type 2 chimeric minichromosome, the two partial protein-coding genes, *pnad1* and *patp6*, have 20% and 48% of the full-length of *nad1* and *atp6*. *trnQ* in the Type 2 chimeric minichromosome, however, has the same sequence and length as its counterpart in the mt minichromosome. Each of the four Group-II chimeric minichromosomes that we sequenced entirely had the genes of two different types of mt minichromosomes combined; these genes had the same arrangement and the same length as their counterparts in the minichromosomes.

3.4. Non-coding regions (NCR) of chimeric mt minichromosomes

We sequenced entirely 17 NCRs from Group I and Group II chimeric minichromosomes. These 17 NCRs were 634 to 2361 bp long; the length of the NCR varied substantially among different types of chimeric minichromosomes and even among different chimeric minichromosomes of the same type (Table 2). Shao et al. (2009) reported that the NCRs of the mt minichromosomes of *P. humanus* shared three highly conserved sequence blocks (CSB): (i) a 523-bp CSB adjacent to the 3′ end of the coding region; (ii) a 302-bp CSB in the middle of NCR; and (iii) a 101-bp CSB adjacent to the 5′ end of the coding region (Fig. 2A). Although the NCRs of the chimeric mt minichromosomes we sequenced entirely varied substantially in size, all of the 17 NCRs had the 101-bp CSB. Sixteen of the 17 NCRs had the 302-bp CSB. Fifteen of the 17 NCRs had the 523-bp CSB, whereas the other two NCRs had ~330 bp of the 523-bp CSB (Fig. 2B).

4. Discussion

4.1. Microhomologous sequences at the gene junctions in Group I chimeric mt minichromosomes are hallmarks of non-homologous recombination

Each Group I chimeric mt minichromosome has parts of two different protein-coding genes: (i) *pcox2* and *pcox3* in the Type 1 chimeric minichromosome and (ii) *pnad1* and *patp6* in the Type 2. In both types of chimeric minichromosome, the two parts of the protein-coding genes are joined at a short homologous sequence: 12 bp long (5′-TGTTTGTGTGGG-3′) between *pcox2* and *pcox3* in the Type 1 and 19 bp long (5′-ATGCGAGGGTTTCTAGCTC-3′) between *pnad1* and

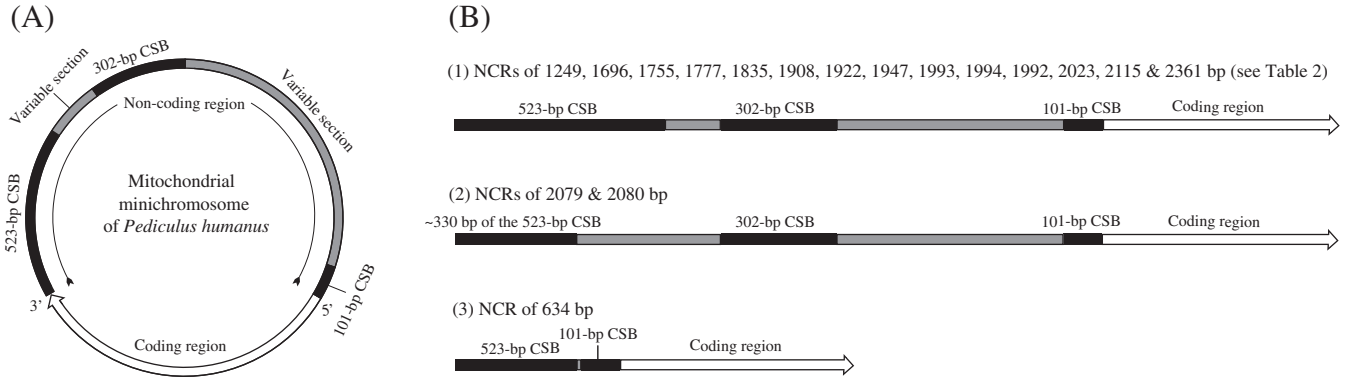

Fig. 2. Conserved sequence blocks (CSB) in the non-coding regions (NCR) of the 18 mt minichromosomes (A) of *Pediculus humanus* (Shao et al., 2009) and the Group I and Group II chimeric mt minichromosomes (B) of *P. humanus* reported in the present article. For the convenience of illustration, the circular chimeric mt minichromosomes were linearized at the 3′-end of the coding regions. CSBs are in black; variable sections in the non-coding region are in grey.

patp6 in the Type 2 (Fig. 1A). Short homologous sequences like these have been called "microhomology" (Chu, 1997) and are typical of the gene junctions formed by non-homologous recombination (Chu, 1997; Ricchetti et al., 1999). So, we propose that a Group I chimeric mt minichromosome of *P. humanus* was created by non-homologous recombination of mt minichromosomes in two steps. First, there were two double-strand breaks (DSB) in each of two mt minichromosomes: one DSB in the coding region and another in the non-coding region. These DSBs split each circular minichromosome into two fragments. Second, non-homologous recombination joins a fragment from one

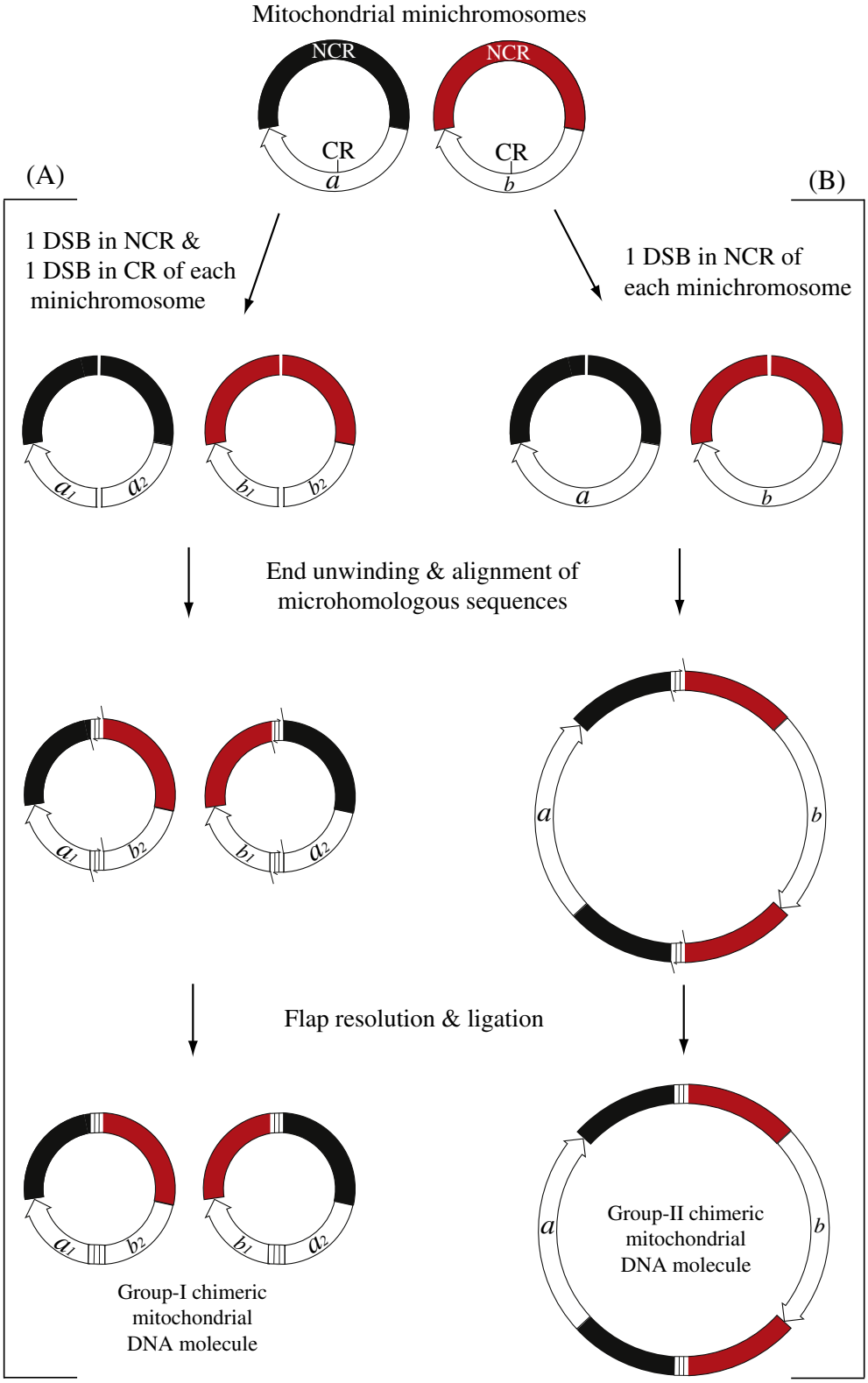

Fig. 3. Our model of non-homologous recombination between mt minichromosomes to generate a Group I (A) and a Group II (B) chimeric mt minichromosome in *Pediculus humanus*. This model is grounded in the models of nuclear DNA repair by non-homologous recombination in mammals and yeasts (reviewed in Chu, 1997). NCR is for non-coding region, CR for coding region, and DSB for double-strand break. *a* and *b* are for genes in the coding region.

minichromosome with a fragment from the other minichromosome to create a Group I chimeric minichromosome (Fig. 3B).

4.2. Conserved sequence blocks in the non-coding regions of Group II chimeric mt minichromosomes resemble the "recombination repeats" in the mt genomes of higher plants

Like Group I chimeric mt minichromosomes, Group II chimeric mt minichromosomes may also be created by non-homologous recombination. We propose that Group II chimeric minichromosomes could be created by non-homologous recombination between minichromosomes in two steps. First, there is a DSB in the NCR of each of two minichromosomes. These DSBs linearize each minichromosome. Second, non-homologous recombination joins the two linearized minichromosomes together and thus creates a Group II chimeric minichromosome (Fig. 3B).

Alternatively, Group II chimeric mt minichromosomes may be created by homologous recombination. Our model for the formation

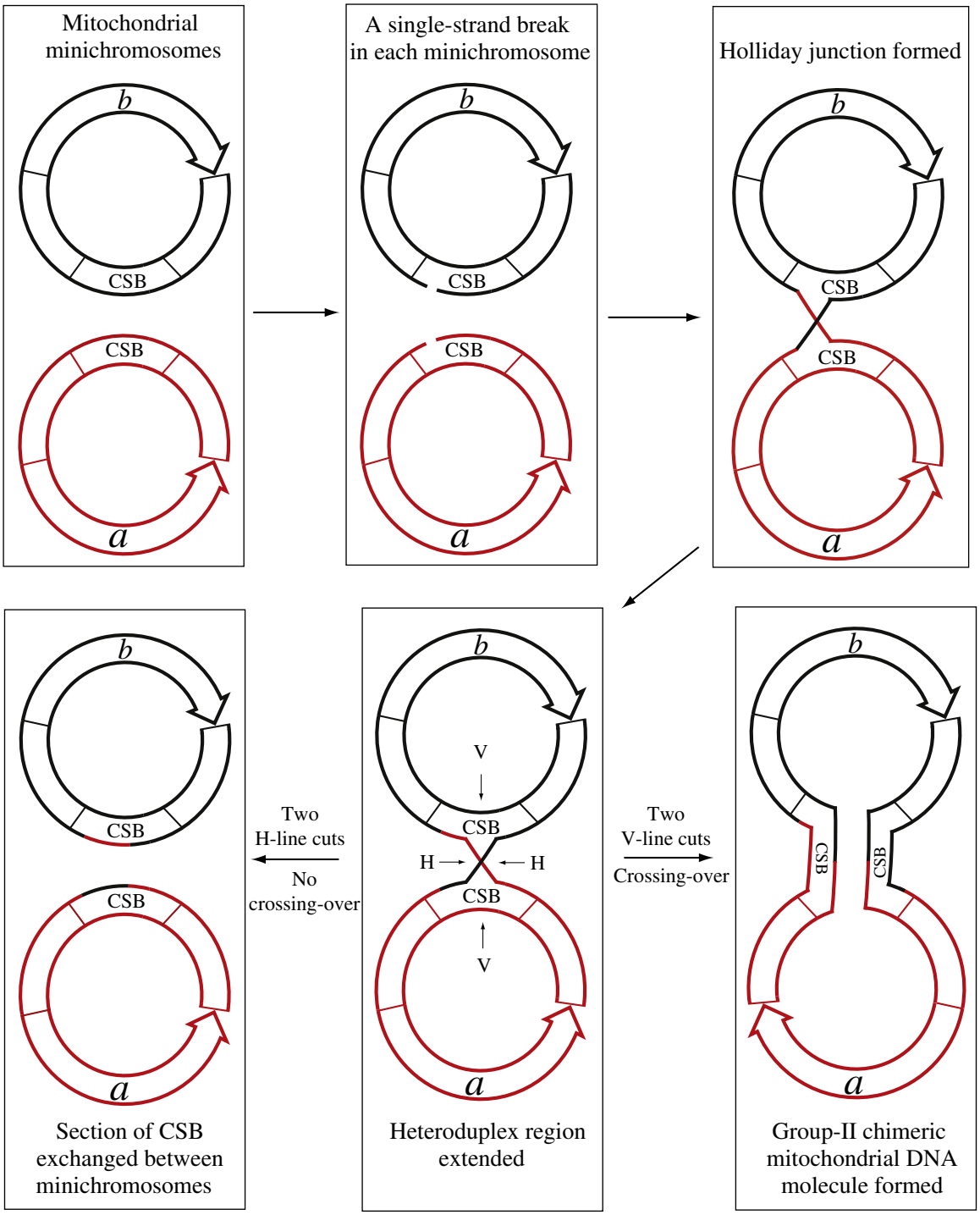

Fig. 4. Our model of homologous recombination between mt minichromosomes to generate a Group II chimeric mt minichromosome in *Pediculus humanus*. This model is grounded in the models of homologous recombination of nuclear DNA in eukaryotes (Holliday, 1964) and a model of recombination of mtDNA in higher plants (Hanson and Folkerts, 1992; Fauron et al., 1995). The two mt minichromosomes are in black and red. CSB stands for conserved sequence block in non-coding regions, *a* and *b* for genes in coding regions, V for vertical-line cut, and H for horizontal-line cut.

of Group II chimeric mt minichromosomes by homologous recombination has three steps (Fig. 4). First, there is a single-strand break (SSB) in conserved sequence blocks (CSBs) in each of two different mt minichromosomes. Second, the CSBs facilitate the formation of a four-chained structure, a Holliday junction (Holliday, 1964), between the two minichromosomes. Third, the Holliday junction is resolved by crossing-over; this creates a Group II chimeric mt minichromosome that has all of the genes and thus the size of two mt minichromosomes combined. The CSBs in the non-coding regions of Group II chimeric mt minichromosomes resemble the repeats in the non-coding regions of the mtDNA of higher plants; these repeats are required for homologous recombination and thus have been called "recombination repeats" (Hanson and Folkerts, 1992; Fauron et al., 1995). The mtDNA of higher plants typically consists of a master chromosome and several sub-chromosomes. The master chromosome usually has two recombination repeats, whereas each sub-chromosome has one recombination repeat. Recombination repeats are essential to homologous recombination both within a master chromosome and between two sub-chromosomes in these plants. Homologous recombination within a master chromosome generates two sub-chromosomes, whereas recombination between two sub-chromosomes generates a master chromosome (Fauron et al., 1995).

DNA double-strand breaks (DSB) and single-strand breaks (SSB) can be induced by exogenous factors such as radiation, UV light, chemicals, and endogenous factors such as reactive oxygen species (ROS). DSB and SSB in nuclear DNA are repaired by homologous recombination and non-homologous recombination (Thyagarajan et al., 1996; Lakshmipathy and Campbell, 1999a; Morel et al., 2008). DSB and SSB are common in mtDNA due to the abundance of ROS produced in mitochondria by the oxidative phosphorylation process (Croteau and Bohr, 1997). It has been thought for many years that mtDNA, in particular, animal mtDNA, does not have recombinational activities (Thyagarajan et al., 1996). An increasing body of evidence, however, indicates that mtDNA of animals recombines. Thyagarajan et al. (1996) and Lakshmipathy and Campbell (1999a) showed that protein extracts from human mitochondria could catalyze both homologous and non-homologous recombination. Further, Morel et al. (2008) showed that DSBs in the mtDNA of *Drosophila* cells could be repaired as efficiently as DSBs in the nuclear DNA, which indicated active DNA recombination activities in the mitochondria of *Drosophila*. The chimeric minichromosomes we discovered in this study add more evidence to DNA recombination in animal mitochondria. Unlike in humans and several other animals (see Lopez et al., 1996; Richly and Leister, 2004; Kim et al., 2006; Pamilo et al., 2007), our analyses of the nuclear DNA supercontigs (available at www.vectorbase.org/) generated by the Human Body Louse Genome Project found no evidence for nuclear-encoded mtDNA in the human body louse (Shao and Barker, unpublished). Thus, it is unlikely that the chimeric minichromosomes we observed in this study were generated by recombination of nuclear-encoded mtDNA; the only likely explanation is that these chimeric minichromosomes were generated by recombination between the minichromosomes in the mitochondria of the human body louse.

It has been suggested that human DNA ligase III plays a major role in mtDNA repair via homologous and non-homologous recombination; further, this ligase is present in both the mitochondria and the nuclei in humans (Lakshmipathy and Campbell, 1999b). The human body louse has a putative DNA ligase (PHUM186980-PA; sequence available at www.vectorbase.org/) that has 48% identity to the human DNA ligase III. Furthermore, the mitochondria-specific forms of several other enzymes that participate in nuclear DNA repair have been identified in mammals, including methyltransferase, glycosylase, and endonuclease (Lakshmipathy and Campbell, 1999a). The Human Body Louse Genome Project revealed 40 putative methyltransferases, 5 glycosylases, and 11 endonucleases that are encoded by the nuclear genome of the human body louse. It would be interesting to know whether or not any of these enzymes are present in the mitochondria of the human body louse and, further, whether or not they have roles in the generation of the chimeric mt minichromosomes we observed in this study.

Supplementary materials related to this article can be found online at doi:10.1016/j.gene.2010.11.002.

Acknowledgments

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