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A PIF-dependent recombinogenic signal in the mitochondrial DNA of yeast

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From their recombination properties, tandem rho$^-$ mutants of the mitochondrial genome of *Saccharomyces cerevisiae* were divided into two categories. In crosses between PIF-independent rho$^-$ and rho$^+$ strains, the recombination frequency is low and similar in *PIF/pif* and *pif/pif* diploids. In crosses between PIF-dependent rho$^-$ and rho$^+$ strains, the recombination frequency is stimulated 10–50 times in *PIF/pif* diploids and is drastically decreased in *pif/pif* diploids. These results suggest that a recombinogenic signal is present in the mitochondrial (mt) DNA of PIF-dependent rho$^-$ clones. This signal is not recognized in *pif* mutants. Sequence analysis of a series of small (<300 bp) overlapping tandem *rho*$^-$ genomes located in the *ery* region of the 21S rRNA gene led us to identify an essential element of this signal within a 41-bp A+T sequence exhibiting over 26 bp a perfect dyad symmetry. However the recombinogenic signal is not sequence-specific since the sequence described above does not characterize PIF-dependent rho$^-$ clones located in the *oli*1 region. Our results rather suggest that the recombinogenic signal is related to the topology of rho$^-$ DNA. Denaturated sites in the double helix or cruciform structures elicited by local negative supercoiling might be preferred sites of the initiation of recombination.

Key words: mtDNA/recombination/yeast/*pif* mutants

Introduction

The multiple-copy mitochondrial genome of *Saccharomyces cerevisiae* recombines very actively. Two types of recombination are observed. Site-specific recombination which involves reciprocal exchanges within two short homologous sequences of mitochondrial (mt)DNA, initiates the induction of rho$^-$ mutants by excision of mtDNA segments (Gaillard *et al.*, 1980; De Zamaroczy *et al.*, 1983) and probably generates, by inter and intramolecular events, oligomeric series of circular molecules (Faye *et al.*, 1973; Lazowska and Slonimski, 1976) and DNA rearrangments typical of rho$^-$ mutants. On the other hand, genetic exchanges between two different mtDNA molecules are mediated by the generalized recombination system. The frequency of recombination events depends on the input fraction of mtDNA molecules from each partner, the number of DNA mating rounds and the probability of DNA exchanges (Dujon *et al.*, 1974). Except for a few polar regions (*ω*, *var1*) (Dujon *et al.*, 1976; Strausberg *et al.*, 1978) in which a parental allele is asymmetrically converted into the other parent allele, no bias is observed in the transmission of mitochondrial alleles (Dujon *et al.*, 1974).

The rho$^-$ genomes cover a large variety of mtDNA sequences; they are of variable lengths (from < 100 bp to several thousand base pairs) and exhibit either tandem (abcabc) or palindromic (abcc'b'a') repetitions of their conserved sequence (Locker *et* al., 1974, 1979; Bos *et al.*, 1980). Therefore the rho$^-$ mutants offer the unique opportunity to analyze, in crosses with rho$^+$ strains, the influence of these parameters on heteroduplex formation and genetic exchanges. Palindromic organization of the repeating unit always elicits high recombination frequency (Foury and Kolodynski, 1983), possibly because the two short inverted homologous sequences located at the junction between two repeats (Sor and Fukuhara, 1983a) elicit cruciform Holliday structures which are used as recombination intermediates (Mizuuchi *et al.*, 1982). In crosses between rho$^+$ and tandem rho$^-$ strains, the recombination frequency varies highly from one rho$^-$ mutant to another (Bolotin-Fukuhara and Fukuhara, 1976; Perlman, 1976; Michel *et al.*, 1979). It might be expected that recombination increases with the size of the repeating-unit of the rho$^-$ mutant, since the formation of heteroduplex between rho$^-$ and rho$^+$ is thus facilitated. However, we have found that in many crosses between rho$^+$ and tandem rho$^-$ strains with a mitochondrial genome of < 1000 bp, the transmission of a genetic marker from the rho$^-$ clone to the rho$^+$ diploid progeny exceeds 50% (Foury and Kolodynski, 1983). This high level of recombination is surprising if we consider, for instance, that in yeast chromosomes, the recombination frequency between two genetic markers separated by 3 kb is ~1% (Mortimer and Schild, 1982). These observations support the hypothesis proposed by Michel *et al.* (1979) that specific sites in the mtDNA increase recombination between rho$^+$ and rho$^-$ genomes.

We have recently isolated three recessive allelic nuclear mutants, assigned to the *pif* locus, that affect the frequency of recombination between rho$^+$ and rho$^-$ genomes. Our conclusions were based on the comparison of the recombination frequency of mitochondrial genetic markers among the rho$^+$ diploid progeny issued from heterozygous PIF rho$^+$ × *pif* rho$^-$ and homozygous *pif* rho$^+$ × *pif* rho$^-$ crosses (Foury and Kolodynski, 1983). Mutations in the *PIF* gene prevent recombination between rho$^+$ strains and ~50 tandem rho$^-$ mutants located at various loci of the mtDNA. Such rho$^-$ clones have been called PIF-dependent. However, the *pif* mutations have no effect on the recombination frequency in crosses with palindromic rho$^-$ mutants. These palindromic mutants, which have been called PIF-independent, are characterized by their high level of recombination in both *PIF* rho$^+$ × *pif* rho$^-$ and *pif* rho$^+$ × *pif* rho$^-$ crosses (>80%). Curiously one tandem rho$^-$ mutant, located in the 21S rRNA gene, is also PIF-independent. However, in contrast with the PIF-independent palindromic rho$^-$ mutants, this PIF-independent tandem rho$^-$ mutant gives a moderate level of recombination in crosses with tandem rho$^-$ strains (25% for the E^R_{514} marker), suggesting that it recombines in a different way from palindromic rho$^-$ mutants. In addition, the isolation of this PIF-independent tandem rho$^-$ mutant suggests the existence of a signal which distinguishes it from PIF-dependent rho$^-$ mutants. To unravel this signal, we decided to obtain from this unique tandem PIF-independent, tandem rho$^-$ strain a set of new PIF-dependent and PIF-independent rho$^-$ clones. Eighteen new rho$^-$

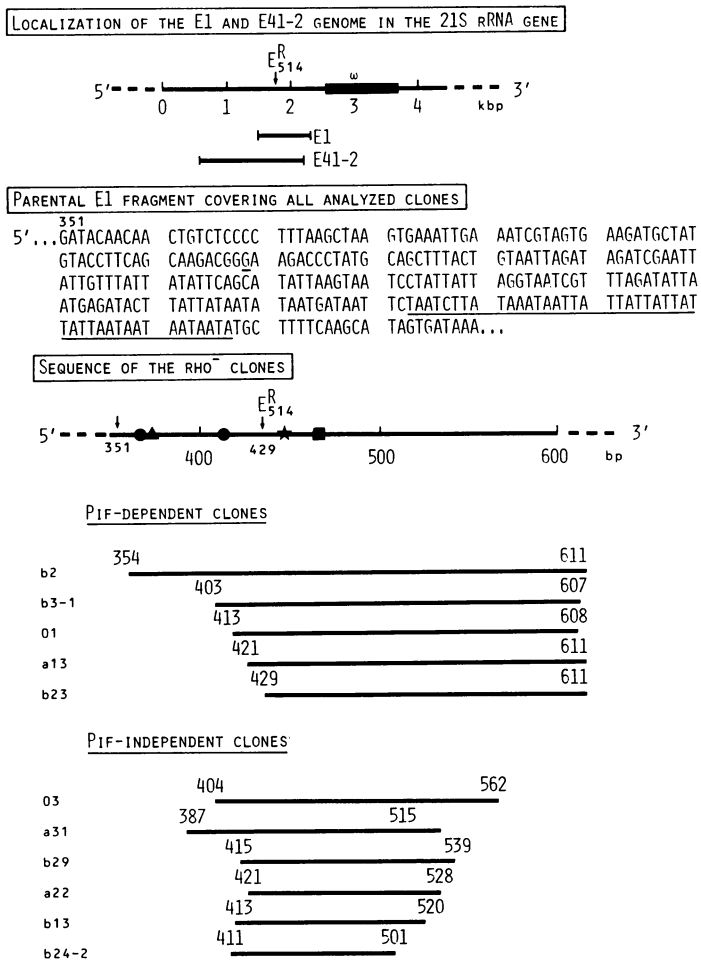

Fig. 1. Sequence of the repeating-units of the rho$^-$ clones derived from 394/E1. In the upper part, we show the localization in the exon of the 21S rRNA gene of the repeating units of the rho$^-$ clones E1 (between positions 1523 and 2220) and E41-2 (between positions 558 and 2125). The nucleotides are numbered from the first 5′ nucleotide of the 21S rRNA gene. The black box represents the intron ω present in ω^+ strains. In the second part of the figure, the sequence (non coding-strand) of the portion of the repeating unit of the rho$^-$ E1 which covers all rho$^-$ clones analyzed is given, and below, the localization and the deletion end points of each rho$^-$ clone. Thick lines represent the retained sequences in the rho$^-$ clones. The nucleotides are numbered from the first 5′ nucleotide of the repeating unit of E1. The genetic allele E^R_{514} (underlined at position 429) is characterized by the substitution in the E^S allele of an A by a G. All rho$^-$ genomes, except that of b2 were cleaved into a single fragment by *Sau*3A. In 394/E1/b2, DNA cleavage was achieved at the restriction site *Dde*I. After 5′ end labelling with [γ-^{32}P]ATP, either single strands were separated by acrylamide gel electrophoresis or the DNA fragments were subject to secondary restriction. Restriction sites in the E1 DNA fragment: ● *Alu*I; ▲ *Dde*I, ★ *Rsa*I; ■ *Sau*3A.

clones, with very short repeating-units, were isolated. In addition PIF-dependent and PIF-independent tandem rho$^-$ mutants were also obtained in the *oli*1 region of the mtDNA. Sequencing data show that the mtDNA of the five PIF-dependent isolates in the *ery* region contain a signal which stimulates recombination in *PIF* rho$^+$ × *pif* rho$^-$ crosses. An essential element of this signal is contained within a 41-bp A+T sequence exhibiting a perfect dyad symmetry over 26 bp. However the comparison of the sequences of the PIF-dependent rho$^-$ clones in *oli*1 and *ery* regions show that the recombinogenic signal is not sequence-specific but is rather related to DNA topology.

Table I. Recombination in crosses with PIF-dependent and PIF-independent rho$^-$ clones

pif rho$^-$ clones	rho$^-$ class	% E^R recombinants in crosses with	
		PIF rho$^+$	*pif* rho$^+$
394/E1/01	PIF-dependent	20.2	<0.2
394/E1/a13	PIF-dependent	10.3	<0.2
394/E1/b2	PIF-dependent	21.0	0.9
394/E1/b3-1	PIF-dependent	19.2	<0.2
394/E1/b23	PIF-dependent	0.015	0.0003
394/E1/03	PIF-independent	0.7	1.5
394/E1/a22	PIF-independent	0.2	0.3
394/E1/a31	PIF-independent	0.4	0.3
394/E1/b13	PIF-independent	0.3	0.2
394/E1/b24-2	PIF-independent	0.2	0.3
394/E1/b29	PIF-independent	0.1	0.1

The *pif* rho$^-$ clones were crossed on complete glucose medium with the rho$^+$ strains NW38-4C (*PIF*) and JF19-6D (*pif*). Prototrophic selection of rho$^+$ diploids was achieved by replication onto glucose minimal medium. The diploids were spread for single colonies on minimal medium and replicated on glycerol medium containing erythromycin (4 mg/ml) as reported by Foury and Kolodynski (1983). In crosses with PIF-dependent rho$^-$ clones (except 394/E1/b23), 400–600 rho$^+$ diploids were scored, while in crosses with PIF-independent rho$^-$ mutants, 5000–10 000 rho$^+$ diploids were scored. In the case of 394/E1/b23, some 10 000 rho$^+$ diploids per Petri dish were spread on minimal medium and replicated onto erythromycin medium in order to estimate the number of erythromycin-positive colonies. In parallel, the number of rho$^+$ diploids spread on minimal medium was accurately determined by serial dilutions of the initial sample followed by spreading ~200 colonies per Petri plate. Out of 425 000 *PIF*/*pif* rho$^+$ diploids, 62 E^R recombinants were scored, while out of 345 000 *pif*/*pif* rho$^+$ diploids, only one E^R recombinant was found.

Results

Transmission of the E^R_{514} marker by clones issued from a PIF-independent tandem rho$^-$ mutant (394/E1)

The mitochondrial genome of the tandem rho$^-$ mutant 394/E1 is characterized by a repeating-unit of 728 bp localized in the exon region of the 21S rRNA gene, some 500 bp upstream of the intron ω (Sor and Fukuhara, 1983b) and contains the genetic marker E^R_{514} (Sor and Fukuhara, 1982) (Figure 1). The recombination of the E^R allele in E^S rho$^+$ × E^R rho$^-$ crosses was estimated by the frequency of erythromycin-resistant clones among the rho$^+$ diploid progeny. About 25% E^R recombinants were observed with the rho$^-$ mutant E1, in *pif* rho$^+$ × *pif* rho$^-$ and in *PIF* rho$^+$ × *pif* rho$^-$ crosses (Foury and Kolodynski, 1983). The tandem rho$^-$ mutant 394/E1 is thus PIF-independent.

Out of 16 000 clones from the mutant 394/E1 mutagenized by ethidium bromide, we isolated two classes of tandem E^R_{514} rho$^-$ clones, different from 394/E1. Five rho$^-$ clones were PIF-dependent, since the transmission frequency of the E^R marker was 10–20% in *PIF* rho$^+$ × *pif* rho$^-$ crosses and lower than 0.2% in *pif* rho$^+$ × *pif* rho$^-$ crosses (except with the clone b2 where it reached 0.9%) (Table I). In the other 13 clones, the recombination frequency was equal in *PIF*/*pif* and *pif*/*pif* diploids, and therefore these clones were defined as PIF-independent. Curiously, however, the recombination frequencies were very low (1% or less) (Table I). Our results show therefore that in the *PIF* background, the transmission of the E^R_{514} marker to the rho$^+$ diploid progeny was higher with PIF-dependent clones than with PIF-independent clones. The PIF-dependent clone b23 was an exception to this rule, since it produced only 0.015% recombinants in a cross with a *PIF* rho$^+$ strain. Sequence data (see

Table II. Suppressiveness of rho⁻ mutants

pif rho⁻ clones	Number of diploids scored	% rho⁻ in *PIF* rho⁺ × *pif* rho⁻ cross
394/E1/01	316	85.4
394/E1/a13	634	81.2
394/E1/b2	408	89.7
394/E1/b3-1	342	85.9
394/E1/b23	298	71.8
394/E1/03	310	26.1
394/E1/a22	335	3.0
394/E1/a31	252	10.7
394/E1/b13	319	16.6
394/E1/b24-2	367	1.1
394/E1/b29	221	13.1

The rho⁻ clones were crossed on complete glucose medium with NW38-4C (rho⁺ *PIF*) and allowed to mate for 6 h at 30°C. The cell mixtures were replicated onto minimum glucose medium and incubated for 13 h. They were spread for single colonies on complete glucose medium. The colonies were replicated on complete glycerol and minimum glucose media to determine the number of rho⁻ and rho⁺ diploids.

below) show that, in this mutant, the E^{R}_{514} marker is located exactly at the deletion end point of the repeating unit. Therefore the extremely low recombination frequency can be readily explained by a boundary effect which hampers strand exchanges and heteroduplex resolution.

Suppressiveness of rho⁻ clones issued from 394/E1

Suppressiveness, first discovered by Ephrussi (Ephrussi *et al.*, 1955), is an estimate of the fraction of rho⁻ zygotic clones in a cross between rho⁺ and rho⁻ strains. Hypersuppressiveness (95 – 100% rho⁻ zygotic clones) has been correlated with short, highly amplified rho⁻ repeating units containing specific sequences (*ori* or *rep*) (De Zamaroczy *et al.*, 1979, 1981; Blanc and Dujon, 1980; Baldacci *et al.*, 1984) which are thought to be replication origins of the mtDNA. We observed that PIF-dependent rho⁻ clones exhibited high suppressiveness (from 70 to 90%) in crosses with wild-type *PIF* rho⁺ strains (Table II). However the parental strain 394/E1 from which they derived did not possess a replication origin and was not suppressive (Table II). The rho⁻ diploids issued from *PIF* rho⁺ × *pif* rho⁻ crosses had the same mitochondrial genome as the original haploid parent (data not shown), suggesting replicative advantage as in the case of hypersuppressive rho⁻ mutants (Blanc and Dujon, 1980; De Zamaroczy *et al.*, 1981). As previously reported (Foury and Kolodynski, 1983), suppressiveness was always higher in *pif* rho⁺ × *pif* rho⁻ crosses than in *PIF* rho⁺ × *pif* rho⁻ crosses, for PIF-independent as well as for PIF-dependent rho⁻ clones.

Sequences of the rho⁻ clones issued from 394/E1

All rho⁻ clones derived from 394/E1 possess a very short repeating unit of <300 bp (Figure 1), confined to the same area of the E1 genome. Electrophoresis in agarose gels showed that their DNA molecules were organized in highly reiterated repeating units similar in *PIF* and *pif* backgrounds (data not shown). Sequence analysis of these genomes was interesting for two reasons. First, the repeating-units of PIF-dependent and PIF-independent rho⁻ genomes overlapped and thus could be easily compared. Secondly, because of the small size of the repeating units, the length of heteroduplex between rho⁺ and rho⁻ is limited, and therefore the probability of genetic exchanges is low. In these conditions, the effect of a recombinogenic signal on the frequency of recombination events mediated by the generalized recombination should be stronger than in the case of repeating

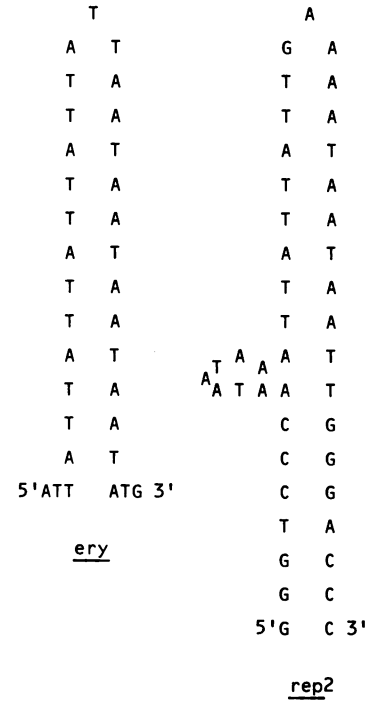

Fig. 2. Comparison of the structures of the 26-bp A+T and *rep*2 palindromes.

units of several thousand base pairs.

A few clones were sequenced. The results are given in Figure 1. The sequence shown in the upper part of the figure contains a portion of the repeating unit of 394/E1, from nucleotides 351 to 630, which covers all rho⁻ genomes derived from 394/E1. The location of the repeating units of the different rho⁻ clones and their deletion end points are indicated in the lower part of the figure. (The sequence of the rho⁻ genome E1 has been communicated to us by Dr. H. Fukuhara.)

Sequencing data show that all rho⁻ genomes were tandemly arrayed and co-linear with the E1 genome. No rearrangement was observed in the junction regions. In the repeating units of PIF-independent rho⁻ clones, no specific sequence, absent from PIF-dependent clones, was detected. In contrast all PIF-dependent clones had one striking feature, absent from all PIF-independent clones. They all terminated their 3′ end by the same A+T rich sequence, located in the E1 genome between positions 562 and 607 (Figure 2). This tail contains a pure 41-bp A+T sequence exhibiting, over 26 bp, a perfect dyad symmetry. Because PIF-dependent and PIF-independent genomes strongly overlapped (see for instance clones 01 and 03) except in this A+T region, we concluded that the 41-bp A+T sequence contains the signal, or an essential element of the signal, which distinguishes PIF-dependent and PIF-independent rho⁻ clones derived from 394/E1. In the absence of this signal there is a low background of recombination, independent of the *PIF* gene. The signal stimulates recombination, and this stimulated recombination is dependent on the *PIF* gene.

In addition, the 3′ end of the PIF-dependent genomes contains an essential element of the signal responsible for their high suppressiveness. This signal is certainly constituted of the 26-bp A+T palindrome which exhibits a high degree of homology with the palindromic sequences present in the replication origins of yeast or HeLa cells mtDNA (Figure 2) (Bernardi, 1982; Blanc and Dujon, 1980). These results strongly suggest that this dyad symmetry is the essential feature of the *rep (ori)* sequences, even

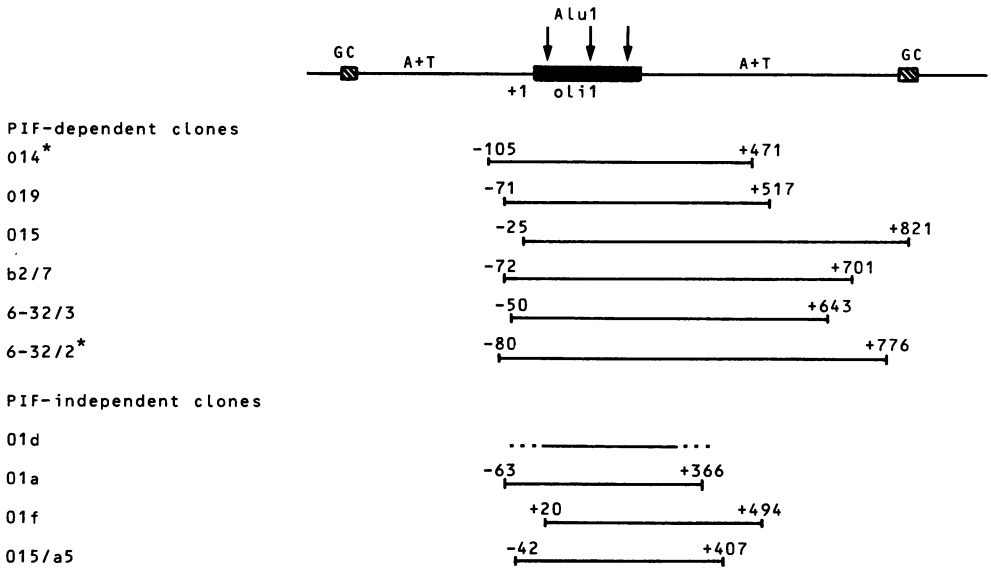

Fig. 3. Restriction maps of PIF-dependent and PIF-independent clones located in the *oli*1 region. In the upper part, we show the *oli*1 gene (black box) flanked by A+T sequences (thin lines) interrupted by GC clusters (hatched box). The nucleotides are numbered from the first 5′ nucleotide of the *oli*1 gene. The genetic allele O^R_{625} is located at position +159. In the second part of the figure, the retained sequence and deletion end points (determined by DNA sequencing) of each rho$^-$ clone is given. The deletion end points of the PIF-independent clone 01d which has not been sequenced are not indicated. It has a repeating unit of ~365 bp. In the clones 014 and 6-32/2, marked with an asterisk, rearrangements were observed. The sequences located between positions −100 and −43 for the clone 014, and between −80 and −59 for the clone 6-32/2 correspond to the inverted complementary sequence of the wild-type genome. In addition, at the junction of the repeating units of the latter clone, six additional bases absent from the wild-type genome, are observed. The rho$^-$ clones 014, 019, 015, 01d, 01a, 01f and 015/a5 were spontaneously derived from the rho$^-$ mutant JF2-2B/2-31/01. The rho$^-$ clone b2/7 was derived from the rho$^-$ mutant JF2-2B/1-42/b2 and the rho$^-$ clones 6-32/3 and 6-32/2 were derived from the rho$^-$ mutant JF11-2A/6-32.

though the presence of the complete *rep* sequence in a rho$^-$ genome still increases the degree of suppressiveness. It is possible that there is no direct relationship between the replicative and recombinogenic roles of the A+T palindromic sequence.

The 41-bp A+T sequence is located at the junction between two tandem repeating units in all PIF-dependent clones sequenced. Is this junction location fortuitous? It must be noted that the parental rho$^-$ genome E1 contains this A+T sequence inside its repeating unit at ~100 bp from its 3′ end and is PIF-independent. In contrast, the tandem rho$^-$ clone 394/E41-2 (1600 bp long), spontaneously derived from the palindromic clone E41 (Foury and Kolodynski, 1983) terminates at its 3′ end by the 41-bp A+T sequence, and is PIF-dependent. (The sequence of E41-2, not shown here, has been kindly performed by Drs Sor and Fukuhara.) Although these results would suggest that the A+T sequence must be located close to the junction, it cannot be excluded that the rho$^-$ mutant 394/E1 is PIF-independent because of some structural feature of its repeating-unit indirectly related to the absence of the terminal location of the A+T sequence.

A recombinogenic signal in the oli1 region

A major point was to determine whether the recombinogenic signal was sequence specific. We analyzed the sequences and recombination properties of tandem rho$^-$ clones spontaneously derived from unstable palindromic rho$^-$ mutants located in the *oli1* region (Foury and Kolodynski, 1983). The latter is characterized by long A+T stretches of several hundred bases flanking the 230-bp *oli*1 gene (Tzagoloff *et al.*, 1980). Even though some 50 PIF-dependent rho$^-$ clones were isolated, only four PIF-independent clones were obtained. The recombination properties of the two classes of clones were similar to those of the clones located in the *ery* region (data not shown). We concluded that a recombinogenic signal was also present in the *oli*1 region. The mtDNA of a few clones was sequenced. Their repeating units were 400−850 bp long and overlapped (Figure 3). Sequence analysis revealed, between positions +473 and +486 a 14-bp A+T palindrome homologous to that found in the *ery* region (Figure 4). The 14-bp palindrome however was absent in the PIF-dependent clone 014 and present in the PIF-independent clone 01f. We concluded that this palindrome was not necessary *per se* for the expression of PIF dependence. Many other short inverted sequences were observed in both PIF-dependent and PIF-independent clones. These results suggest that PIF dependence is not the result of a unique sequence, but is more likely related to the topology of the rho$^-$ DNA.

Discussion

The high level of recombination observed in crosses beween rho$^+$ and some rho$^-$ strains cannot be explained by the rules governing the frequency of genetic exchanges between rho$^+$ strains (Dujon, 1981). The work presented here has revealed a signal in the mtDNA of tandem rho$^-$ clones which enhances the frequency of transmission of the rho$^-$ alleles to the rho$^+$ diploid progeny in rho$^+$ × rho$^-$ crosses. The stimulation of recombination is dependent on the *PIF* gene.

The characterization of a variety of overlapping tandem rho$^-$ clones located in the *ery* region of the 21S rRNA gene and in the *oli*1 region has allowed us to classify them into two categories. In the PIF-independent class, the recombination level is low, especially in crosses with the rho$^-$ clones located in the *ery* region presumably because shortness of the rho$^-$ repeating-units (<300 bp) severely limits heteroduplex formation between rho$^+$ and rho$^-$ DNA. Recombination frequency does not depend on the *PIF* gene. In the PIF-dependent class, the level of recombination is, in *PIF/pif* diploids, 10−50 times higher than in the first class, and is drastically reduced in *pif/pif* diploids.

These results suggest the existence of a recombinogenic signal, in the mtDNA of PIF-dependent rho$^-$ clones, which is recogniz-

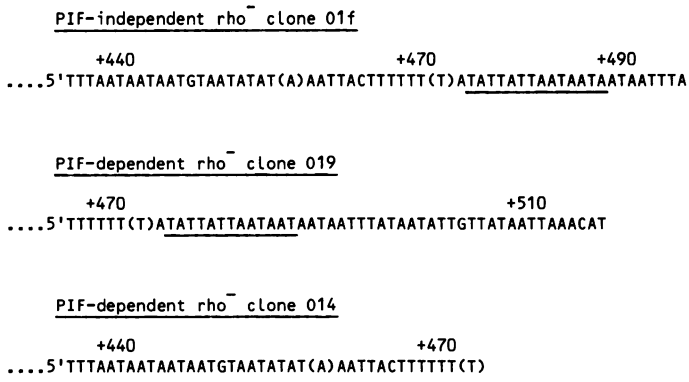

Fig. 4. Comparison of the 3′ end of the repeating units from PIF-independent and PIF-dependent rho⁻ clones located in the *oli*1 region. The nucleotides were numbered from the first nucleotide of the *oli*1 gene. The sequence of the non-coding strand is given. The A+T palindrome exhibiting homology with that located in the *ery* region is underlined. This palindrome, present in the PIF-dependent clone 019 and in the PIF-independent clone 01f, is absent in the PIF-dependent clone 014. ( ) Uncertain base.

ed by the *PIF* gene product or by a protein under the control of the *PIF* gene. All the PIF-dependent clones analyzed in the *ery* region terminate at their 3′ end by the same sequence composed of 41 A+T bases and exhibiting, over 26 bp, a perfect dyad symmetry. This sequence must contain an essential element of the recombinogenic signal. The latter, however, is not sequence-specific, since in the *oli*1 region, no unique sequence can distinguish PIF-dependent and PIF-independent rho⁻ clones. However dyad symmetry might be an essential element of the signal.

Our results suggest that the enhancement of recombination frequency observed in crosses with PIF-dependent rho⁻ clones is related to the topology of the rho⁻ mtDNA. However it is not the result of the organization of the mtDNA molecule in *pif* rho⁻ mutants which exibits, as in *PIF* rho⁻ strains, high reiteration of the repeating-unit eliciting the formation of multimeric structures (Foury and Kolodynski, 1983).

An attractive hypothesis is that the difference in recombination efficiency observed in crosses with PIF-dependent and PIF-independent clones is related to negative supercoiling of the rho⁻ DNA molecule. Requirement for negative supercoiling has been invoked in the recombination process catalyzed by the Rec1 protein in *Ustilago* (Kmiec *et al.*, 1983) and in λ integrative recombination (Kitts *et al.*, 1984). The torsional stress imposed by negative supercoiling favors local denaturation of the double helix and transition of inverted repeats to cruciform structures (Hsieh and Wang, 1975; Benham, 1979; Panayotatos and Wells, 1981). Local melting and cruciform extrusion, highly dependent on the base composition of the DNA molecule, are favored by a rich A+T environment.

We suggest that the PIF-dependent clones have the ability to form and to stabilize local strand separation, possibly as cruciform structures, since we found that an A+T palindromic sequence distinguishes the DNA of PIF-dependent and PIF-independent rho⁻ clones located in the *ery* region. In addition, many short inverted sequences are present in the *oli*1 region. In contrast PIF-independent clones could not form or stabilize these structures, because of the different size and base composition of their repeating units.

The local denaturations in the double helix of the mtDNA of PIF-dependent clones would be preferred sites of recombination initiation. What might be the role of the *PIF* gene product? (i) It might contribute to the destabilization of the double helix, by increasing local supercoil density. In this case, the *PIF* gene product might be related to the enzyme class of topoisomerases. (ii) It might contribute to the stabilization of the denatured regions or cruciform structures formed in the double helix of the PIF-dependent genomes. In this case, it might be a DNA-binding protein. (iii) Finally, the *PIF* gene product might recognize structures such as cruciforms and participate in the initiation of the recombination between rho⁺ and rho⁻ DNA molecules.

Whatever the final answer is, the present work points out the important role of DNA topology in recombination. It raises new questions about the role of supercoiling and cruciform structures in the initiation of mtDNA recombination which seems to occur at preferred sites, as is the case in many organisms.

Materials and methods

Strains

All *pif* mutants were isogenic to D273-10B/A1 (*α met2 PIF rho⁺ ω⁺*), the parental strain. Cytoductants with the nucleus of *pif* mutants and various rho⁻ genomes were constructed as previously reported (Foury and Kolodynski, 1983). The following rho⁻ clones were isolated from the PIF-independent tandem rho⁻ mutant 394/E1 (*α pif1-1 rho⁻* E^R_{514}): 394/E1/01, 394/E1/a13, 394/E1/b2, 394/E1/b3-1 and 394/E1/b23 (PIF-dependent); 394/E1/03, 394/E1/a22, 394/E1/a31, 394/E1/b13, 394/E1/b29 and 394/E1/b24-2 (PIF-independent). In addition, the following strains were used: NW38-4C (*a his PIF rho⁺*) (Foury and Goffeau, 1979), and JF19-6D (*a his pif1-1 rho⁺*), a meiotic segregant issued from the cross D273-10B/A1/can^R_1 × JF2-2B (Foury and Kolodynski, 1983).

PIF-dependent and PIF-independent tandem rho⁻ clones in the *oli*1 region were spontaneously derived from the palindromic rho⁻ mutants JF2-2B/2-31/01 (*a his pif1-1 rho⁻* O^R_{625}), JF2-2B/1-41/b2 (*a his pif1-1 rho⁻* O^R_{625}) and JF11-2A/6-32 (*a met2 pif1-1 rho⁻* O^R_{625}) described previously by Foury and Kolodynski (1983).

Isolation of rho⁻ mutants from 394/E1

The rho⁻ mutant 394/E1 in the exponential phase of growth was inoculated in liquid 2% glucose, 0.5% yeast extract (Difco), 50 mM sodium phosphate pH 6.4, in the presence of 25 μg/ml ethidium bromide. The cells (5×10^6 cells/ml) were shaken at 30°C in the dark for 16–24 h and spread from single colonies on complete glucose medium. The retention of the E^R_{514} marker was determined by crossing the rho⁻ clones with the rho⁺ strains NW38-4C (*PIF*) and JF19-6D (*pif*). Those rho⁻ clones which gave on erythromycin medium confluent growth in crosses with NW38-4C and no growth or only papillae in crosses with JF19-6D were selected because they had PIF-dependent phenotype. In addition those rho⁻ clones which gave on erythromycin medium papillae in crosses with both NW38-4C and JF19-6D were also selected because they had PIF-independent phenotype and had acquired most likely a repeating unit other than 394/E1. The isolation of tandem rho⁻ clones retaining O^R_{625} marker was performed by the same procedure, except erythromycin was replaced by oligomycin. The rho⁻ clones were crossed with rho⁺ strains which had appropriate mating-types and auxotrophies. Small-scale extraction of the mtDNA from the rho⁻ clones was carried out as reported by Jacquier and Dujon (1983).

Mitochondrial DNA isolation and DNA sequencing

Mitochondrial DNA was prepared as reported (Foury and Kolodynski, 1983) except that the CsCl gradient was omitted. After mtDNA cleavage with appropriate restriction enzymes, the fragments were separated by acrylamide gel electrophoresis, cut out from the gel and eluted as described by Maxam and Gilbert (1980). They were 5′ end-labelled with [γ-^{32}P]ATP in the presence of T4 polynucleotide kinase, chemically modified and sequenced by the method of Maxam and Gilbert (1980).

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