Non-random distribution of the TY1 elements within nuclear DNA of *Saccharomyces cerevisiae*

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Ty1 homologous sequences appear to be non-randomly distributed among different density classes of nuclear yeast DNA. Characteristic patterns of Ty1 containing *Eco*RI fragments can be generated from the various DNA fractions. The sequences are particularly enriched in the A + T rich part of the main nuclear DNA fraction, while the frequency in the rDNA containing heavy satellite DNA is low. The transposon however, seems to be present in this dense fraction, at least for some strains.

Yeast *Transposable element* *DNA rearrangement* *DNA fractionation* *rDNA* *DNA–DNA hybridization*

1. INTRODUCTION

The Ty1 element [1] is repeated about 30-times in the genome of *Saccharomyces cerevisiae* and represents one family of transposable DNA sequences in yeast. It consists of a unique sequence of about 5 kilobasepairs (kb) flanked by a direct repeat of 338 basepairs, the delta element which also exists independently [1,2]. Some of the Ty1 copies appear as tandem duplications [1], and several subgroups with partial homology to Ty1 have been classified [3–5].

It is generally assumed that the Ty1 sequences are dispersed, and may transpose, throughout the genome. The element has been found inserted adjacent to a number of defined genes [1,3–8], and its many different locations are revealed by the characteristic distribution among restriction fragments generated from genomic DNA of various strains [1,4,9]. The transposon itself is transcribed [10] and it may cause positive, or negative, effects on the expression of neighbour genes, as reviewed in [11]. Like transposable elements from other organisms [11, 12], it may also promote a variety of DNA rearrangements as in [13].

From our earlier work on yeast, we know that the amount of rDNA varies from one strain to the other, and an increase in rDNA level was observed during subculturing of a haploid strain initially low in rDNA content [14]. It is an open question whether transposons could take part in such phenomena, although other mechanisms such as sister chromatid exchanges [15, 16] or involvement of extrachromosomal copies of the rDNA unit [17, 18] may be proposed. Here we have investigated how Ty1 homologous sequences are distributed within nuclear yeast DNA fractionated according to A + T content. Especially as we wanted to know whether any Ty1 sequences at all were present in the heavy satellite, the γ-DNA fraction, about 80% of which is rDNA [19]. The results show that there is a marked bias in the distribution of Ty1. In Ag^+/Cs_2SO_4 density gradients, most of the Ty1-homologous sequences band on the A + T rich side of the main nuclear DNA fraction, while transposition into the rDNA containing satellite is apparently suppressed. Sequences homologous to Ty1 are, however, also found in this G + C-enriched DNA fraction and show characteristic strain variations.

2. MATERIALS AND METHODS

2.1. *Strains*

Haploid strains of *S. cerevisiae* used for DNA

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isolation were among those described in [14] together with strain 309 obtained from G. Simchen. An *Eschericia coli* strain carrying the *Eco*RI fragment S13 of Ty1 inserted into pBR322 [1] was kindly provided by G.R. Fink. The S13 fragment is generated from a tandem-duplicated Ty1 and represents the prototype of Ty1.

2.2. *DNA preparation and hybridization*

Nuclear yeast DNA was isolated from cells grown in the presence of ethidium bromide and purified extensively before fractionation on Ag^+/Cs_2SO_4 density gradients as in [14, 19]. The average size of the DNA was about 50 kb, as estimated from agarose gels, and 150 μg were applied on each gradient. For development of hybridization spectra [1] across the gradient, fractions from different parts were pooled, dialyzed extensively after complexing silver ions with KCN [19] and concentrated. About 1/10 of each sample was digested with at least 5 units *Eco*RI/μg DNA for 4 h at 37°C. Restriction fragments were separated by electrophoresis on 4 mm thick 1% agarose gels. The gels were stained with ethidium bromide and photographed before blotting onto membrane filters according to Southern 20. A ^{32}P-labelled hydribization probe was derived by nick-translation of the S13 plasmid as in [1]. Hybridizations were carried out at 65°C for 24 h using a 10-fold concentration of the original Denhardt solution [21] in 0.9 M NaCl, 0.09 M sodium citrate (pH 7) after a preincubation of at least 5 h. Autoradiograms were exposed at room temperature using Kodak X-Omat RP films.

3. RESULTS AND DISCUSSION

Yeast DNA can be fractionated according to the A+T content in preparative Ag^+/Cs_2SO_4 density gradients [19]. A heavy satellite, the γ-DNA, represents 10–15% of the nuclear DNA and contains all the rDNA. Fig. 1 shows how Ty1 homologous sequences are distributed across such gradients upon fractionation of nuclear DNA from two haploid strains. In both cases, the major part of the homologous sequences was found more or less regularly distributed across the main DNA fraction with a marked enrichment on the A+T rich side of the peak. The same picture was also found for diploid strains, and the presence of mitochondrial DNA, which bands in the upper part of the gradient [14,19], did not change the pattern. In all cases, the γ-DNA showed very low content of Ty1 sequences. Hence, the rDNA must be protected against transposition, or maintenance, of the transposon.

This characteristic distribution might reflect a preference for insertion of Ty1 at A+T-rich sequences. Authors in [9] found about 70% A+T in the sequences surrounding two cloned Ty1 elements, and for bacterial transposons [12] and in *Drosophila* [22], target sequences seem to be enriched in A+T. The distribution of Ty1 within the main nuclear fraction resembles that of tRNA genes [23], and in fact, Ty1 has been found adjacent to several tRNA genes. A high degree of linkage could then explain the similar banding patterns. On the other hand, the base composition of the element itself might contribute significantly to the banding position, provided that a certain clustering of elements existed. The flanking delta elements contain about 70% A+T [24], while the composition of the internal part of TY1 has not been published so far.

To analyze the distribution further, we developed hybridization spectra [1] across the gradients. Fractions from different parts of the gradients were pooled as indicated by the bars in fig. 1, and the appearance of Ty1-homologous sequences among *Eco*RI fragments was investigated. The spectra shown in fig. 2 illustrate that within the main DNA peak there is a clear subfractionation of Ty1-containing sequences. The band pattern represents sequences of both full and partial homology to the Ty1 probe in the size range from about 1 kb up to 13–20 kb. The dominating band in all fractions is the one of about 5.4 kb corresponding to the size of the probe, S13. This fragment is therefore probably derived from tandem-duplicated copies of Ty1. Such duplications seem to be present in different density classes but are particularly enriched in the A+T rich fractions. Characteristic strain variations could also be traced to certain fractions, such as the band of about 5 kb seen in fraction IV of strain 309 (fig. 2b). This is not found for strain S288C (fig. 2a). We also observed that the 2 μm DNA, seen on ethidium-stained gels, consistently banded in fraction IV; i.e., within the heavy part of the main nuclear DNA peak.

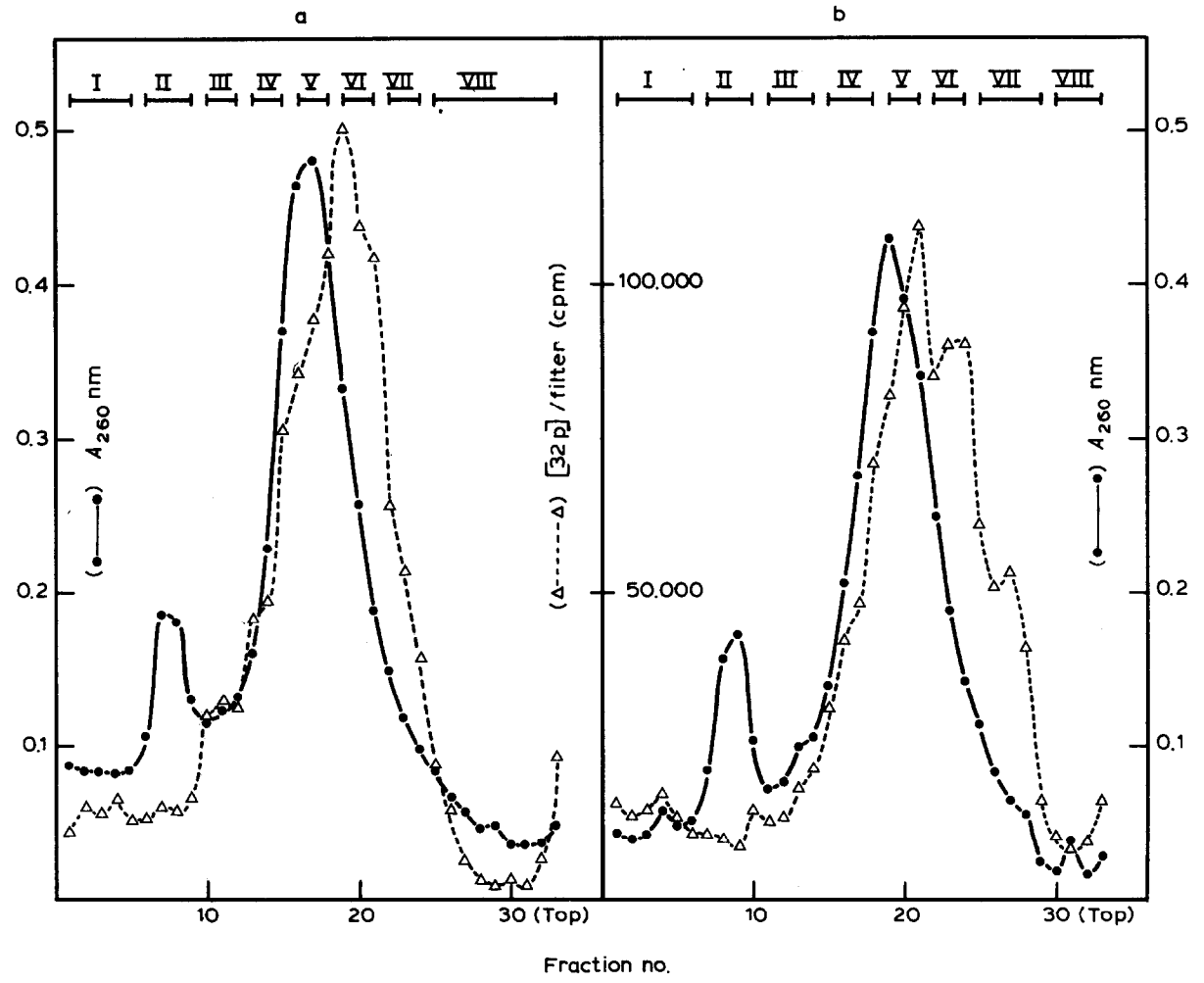

Fig.1. Banding of Ty1-homologous sequences upon fractionation of nuclear yeast DNA in preparative Ag^+/Cs_2SO_4 density gradients. After centrifugation, fractions were collected and the A_{260} (○---○) monitored. Aliquots were fixed to membrane filters and annealed to a ^{32}P-labelled S13 probe of Ty1. Radioactivity bound to the filters (Δ---Δ) was registered by scintillation counting. DNA from strain: (a) S288C; (b) 309.

One important question was whether Ty1 sequences were at all present in the γ-DNA fraction. About 80% of this component is made up of the repetitive rDNA unit [19], while the remaining part may represent DNA linked to rDNA, together with other G+C-enriched segments banding in this fraction. Changes in the number of rRNA genes could possibly, under certain conditions, be promoted by transposable elements linked to rDNA. The frequency of Ty1 in the γ-DNA fraction is very low (fig. 1). However, longer exposures after hybridization of the S13 probe to *Eco*RI fragments generated from the γ-DNA peak (fraction II, fig. 2) from different strains, revealed distinct band patterns in most cases. Five of the strains tested carried form I rDNA, the most common form, which gives 7 *Eco*RI fragments, from 2.8 kb (A) to 0.28 kb (G), in the order -BGCDAFE- [25,26]. Two strains harboured form II rDNA, which lacks fragment B (2.4 kb) and E (0.6 kb) of form I, but gives 6 *Eco*RI fragments with the largest, 3 kb, being a fusion of B and E [26]. For these two strains, we were not able to detect any significant band with homology to Ty1 in the γ-DNA. Form I

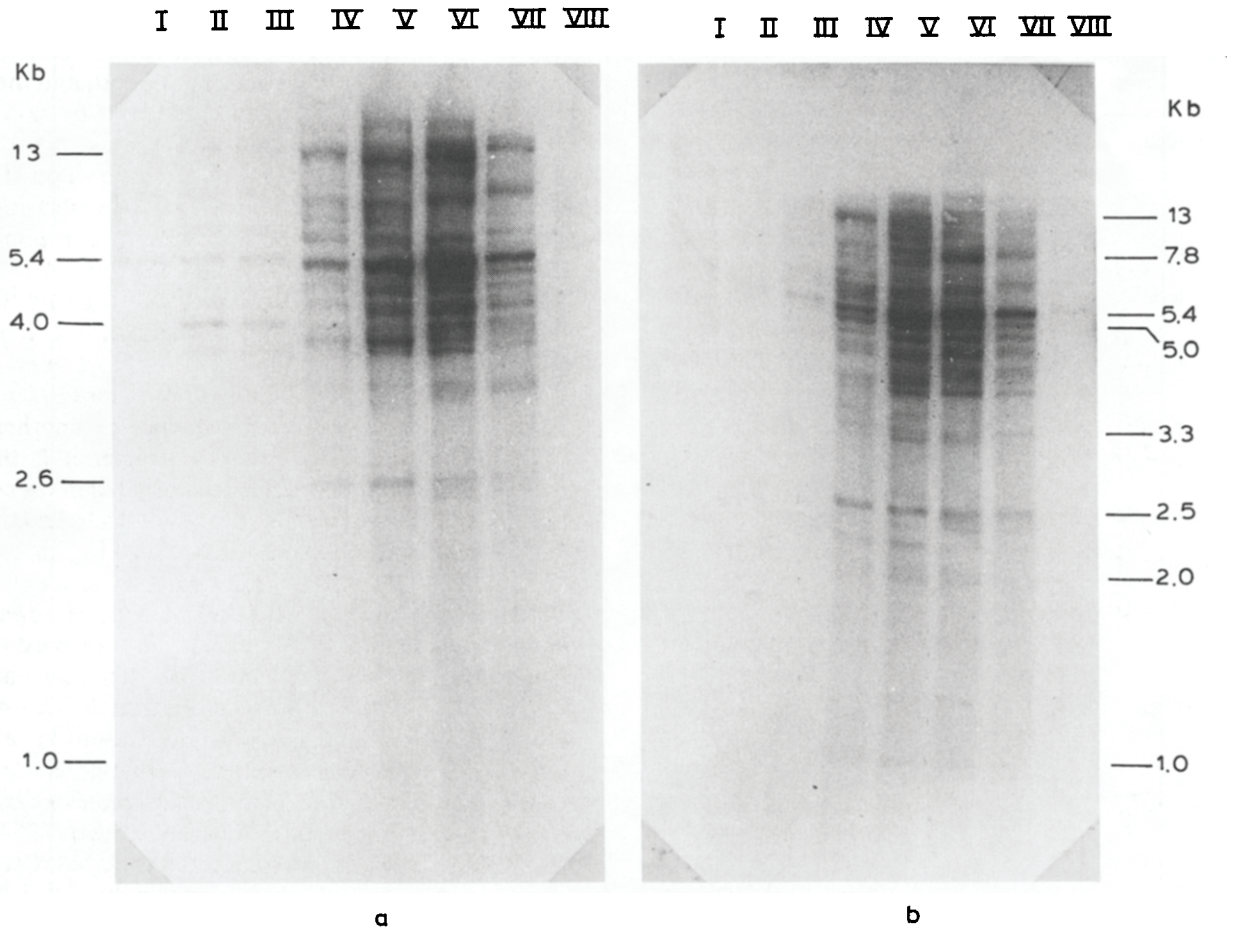

Fig.2. Distribution of Ty1-homologous sequences among *Eco*RI fragments generated from different fractions of yeast DNA. Gradient fractions were pooled as indicated by the bars in fig. 1. The hybridization spectra were developed as described in section 2. DNA from strain: (a) S288C; (b) 309.

rDNA strains, however, showed one or two prominent bands in this DNA fraction. No hybridization was seen in the size range of the *Eco*RI fragments of rDNA. A 5.4 kb band, apparently from tandem duplications, was present in all these strains, but in different amounts. Strain S288C carried another clear band at 4 kb (fig.2a-II). Here, the hybridization signal was stronger in fraction II than in III or I, indicating that the 4 kb fragment was generated from sequences banding in the γ-DNA fraction. Inverted duplications of Ty1 could give rise to such a band. This 4 kb band was absent from the γ-DNA of strain 309 and 12B, while for strain D5–7C it was the major one. The variety of patterns is clearly illustrated in fig. 3 which shows the hybridization spectra after treatment of γ-DNA from the 4 mentioned strains with *Pst*I and *Eco*RI. *Pst*I does not cleave rDNA, but has two sites in Ty1, generating an internal fragment of 0.8 kb. In the double digests, distinct bands at 2.8 and 2.1 kb and the internal fragments of 1.9 and 0.8 kb were seen for all strains. Additional bands were found at 3.9 and 1.6 kb for strain S288C and D5-7C, which would be consistent with inverted duplications in both orientations.

These experiments demonstrate that Ty1 homologous sequences are present in the γ-DNA satellite at least for some strains. The hybridization spectra from the γ-DNA show strain variations as typical as for the main DNA fraction. Hence, the

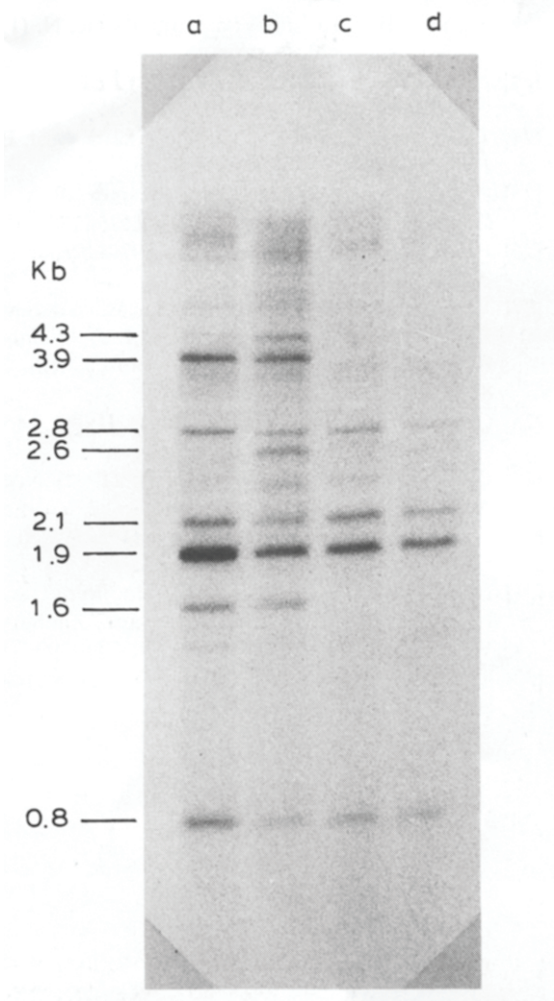

Fig.3. Hybridization spectra of Ty1 for *Pst*I plus *Eco*RI-digested γ-DNA. Gradient fractions containing the γ-DNA peak (fraction II, fig. 1) from 4 different strains were subjected to restriction with *Pst*I and *Eco*RI and the hybridization spectra developed. The lanes represent γ-DNA from strain: (a) S288C; (b) D5-7C; (c) 309; (d) 12B, all carrying form I rDNA.

γ-DNA seems to contain targets for transposition of Ty1.

So far, we do not know whether Ty1 sequences are linked to the rDNA region. The finding of strains carrying Ty1 sequences in the γ-DNA however, provides a marker for non-rDNA sequences in this fraction. By molecular cloning of the element with flanking regions, it should be possible to map how the element is organized and if it is related to rDNA. Within the rDNA unit, the *Eco*RI fragment B plays a special role. It carries the start of the 35 S rRNA gene at one end, and the 5 S RNA gene in the middle is flanked by non-transcribed sequences [27]. It is the most A+T-rich fragment [28,29], and would hence be a possible target for transposition of Ty1. It is also involved in the junction between rDNA and non-rDNA sequences on chromosome XII [30]. Apart from these junctions, the length of fragment B seems to be constant within one strain, but may vary from one strain to the other. Authors in [31] reported a 24 basepair insertion in one cloned fragment B as compared to the sequence of another clone [27], and in our case, the fragment B of strain 309 is about 50 basepairs shorter than those of the other strains (unpublished). Such length variations could represent scars following insertions and excisions of transposable elements; rRNA gene orphons, which might exist in some strains [32], could be the result of transposon-mediated translocations. But the low frequency of Ty1 in the γ-DNA could also mean that the rRNA is somehow protected against transpositions in the same way as general meiotic recombination is suppressed within rDNA [26]. Or, the mechanisms involved in the conservation of homogeneous rRNA units might efficiently eliminate any transposed sequence so that Ty1 elements are not maintained in rDNA.

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