Ty1 extrachromosomal circular copies in *Saccharomyces cerevisiae*

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The eukaryotic transposable element Ty1 is present in about 20–30 integrated copies per yeast aploid genome, variably localized in different strains. Here, we report the presence in yeast of circular extrachromosomal molecules homologous to Ty1, 6 kilobases in size (the same as integrated copies) present in about 1 circular copy/250–300 cells. This finding shows another analogy between eukaryotic-transposable elements and the pro-viral integrative form of retroviruses.

Yeast *Transposable element* *Circular extrachromosomal copy*

1. INTRODUCTION

Middle repetitive DNA sequences scattered in the genome and able to transpose have been described in eukaryotes (review [1]) and molecular characterization of such transposable elements has been reported [2–4]. The comparison of the structural feature of transposable elements in yeast and *Drosophila* has revealed substantial analogies between the two systems and with vertebrate retroviruses [5,6]. In addition, circular episomal copies of the transposable element 'copia' have been found in *Drosophila melanogaster* tissue culture cells [7]. This finding further strengthens the similarities between transposable elements and retroviruses which circularize at a stage preceding their integration into the host genome [8].

Circular DNA in yeast represents a population of molecules heterogeneous in size and only partially characterized. Besides the well-known 2 μm and 3 μm circles [9,10], other circular DNA molecules, which account for up to 8% of total circular DNA, have been observed [11]. As for the transposable element Ty1, copies of this element might be also present as extrachromosomal circles [2]. This hypothesis was based on the structure of a particular Ty1 genomic clone which could be interpreted as originating either from occasional intrachromosomal clusters of Ty1 elements or from extrachromosomal forms.

Here, we report on the presence in *Saccharomyces cerevisiae* of extrachromosomal circular DNA homologous to the transposable element Ty1.

2. EXPERIMENTAL

Saccharomyces cerevisiae DM(a/a) strain (20 g) in log phase was used for the yeast DNA preparation enriched in circular molecules performed as in [12]. The same amount of cells was used for an alternative kind of preparation we developed for yeast, adapting two methods [13,14] originally designed for prokaryotic plasmids. Cells were washed in sterile water, resuspended in 10% glucose, 25 mM Tris–HCl (pH 7.9), 10 mM EDTA digested by zymolase 6000 (Kirin Bewery) treatment (1 mg zymolase/g cells). The solution was made 0.1 M NaOH, 0.5% SDS and kept 10 min on ice to obtain a selective alkaline denaturation of chromosomal DNA [13]. The solution was then neutralized 1 h on ice by addi-

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tion of one-half of the total volume of 3 M Na-acetate (pH 4.8). The clear supernatant after a mild centrifugation was ethanol-precipitated, resuspended in 10 mM Tris–HCl (pH 7.9), 50 mM EDTA, treated 15 min with RNase, DNase free (20 μg/ml), 30 min with proteinase K (200 μg/ml) at 37°C, twice extracted by phenol, made 3% NaCl [14] to eliminate single-stranded DNA, ethanol-precipitated and resuspended in 50 mM Tris–HCl (pH 7.9), 1 mM EDTA for the EtBr–CsCl gradient. After the EtBr–CsCl gradient (last step in both kinds of circular DNA preparations), aliquots of each fraction were extracted with isoamylic alcohol and precipitated with 4.5 vol. 77% ethanol, resuspended in 10 mM Tris–HCl (pH 7.9), 1 mM EDTA and analyzed by agarose-gel electrophoresis (vertical apparatus in phosphate buffer). Gels were transferred to nitrocellulose filter by Southern technique [12], hybridized with specific nick-translated probes. The internal *Eco*RI–*Xho*I restriction fragment of a subclone (P_{30}) from TyD15 (J. Cameron) was purified by electro-elution. This probe was controlled for the lack of homology with the vector sequence (not shown). Hybridizations were performed in 3 × SSC, Denhardt 5 × [16], SDS 0.1%, calf thymus denatured DNA 100 μg/ml at 65°C for 18 h. Filters were washed in: 3 × SSC, 0.1% SDS 60 min; 1 × SSC, 0.1% SDS twice for 30 min; 0.1 × SSC for 30 min. The washed filters were exposed to X-ray Fuji film using an intensifying screen at −70°C. Topoisomerase I was prepared from chicken erythrocyte nuclei as in [17] and used at 1 μg enzyme/μg DNA in a buffer containing Tris–HCL 50 mM (pH 7.8), NaCl 0.1 M, Na_2EDTA 1 mM, 1 mg bovine serum albumin/ml for 60 min at 20°C.

3. RESULTS AND DISCUSSION

We probed preparations enriched in yeast circular molecules with a labelled Ty1 restriction fragment (see scheme in fig.1). The Southern picture of an EtBr–CsCl isopycnic gradient of such a preparation [12] is shown in fig.1A. Sequences homologous to the probe show up in the chromosomal DNA region of the gradient (fractions d–h) and in the higher density region (fractions k–m). Bands A and B (fractions k–m) have been interpreted, respectively, as the supercoiled and relaxed form of a circular molecule homologous to Ty1. The upper band (⟶) is due to a residual chromosomal DNA contamination.

To confirm the finding with a different extraction procedure, we adapted to yeast, two methods for bacterial plasmids [13,14]. The preparation enriched in circular DNA was spun to equilibrium in an EtBr–CsCl gradient and the pool of the circular fractions analyzed by gel electrophoresis for the presence of the circular DNA forms already described in yeast: the 2 μm plasmid of unknown function [9] and the 3 μm DNA which is the episomal form of the ribosomal DNA repeated units [10]. Fig.2 (track 1) shows the ethidium picture of an aliquot of the pool. To identify the supercoiled circles, we treated a duplicated sample of circular DNA with topoisomerase I, which relaxes the supercoiling, thereby reducing the mobility of circular DNA on agarose gel electrophoresis (track 2). To check for the presence of known circular DNAs in our preparation we serially hybridized a Southern transfer with a cloned 2 μm DNA (from C. Hollenberg) and with a cloned *X. laevis* ribosomal DNA fragment (from I. Bozzoni) which is known to cross-hybridize with yeast ribosomal DNA [18]. This allowed us to identify the various bands observed by fluorescence. In fig.2 (track 3), bands a,c,d,e,h,j hybridize with 2 μm DNA; bands a,e,j are the supercoiled form of monomeric, dimeric and trimeric 2 μm plasmids (track 2) described in [19]. Bands b,f,g (track 4) were identified as 3 μm DNA by further hybridization with a ribosomal probe.

Fig.3 shows the Southern picture of the Ty1 on two aliquots of a yeast circular DNA preparation without and with (track 2,3) nicking–closing enzyme treatment. Band A visible in track 2 is absent after the nicking–closing enzyme treatment, as expected for a circular supercoiled molecule. In fig.3 (tracks 2,3) an upper band C, partially covered by the chromosomal DNA, not sensitive to relaxing enzyme treatment, can be observed. We suggest that this band could be a multimeric form of Ty1 in a relaxed conformation. To summarize we define the circular nature of the discrete molecules homologous to Ty1 by the following criteria:

(1) The behaviour in an EtBr–CsCl gradient (fig.1);
(2) The comigration in the gradient with known yeast circular molecules (fig.2);

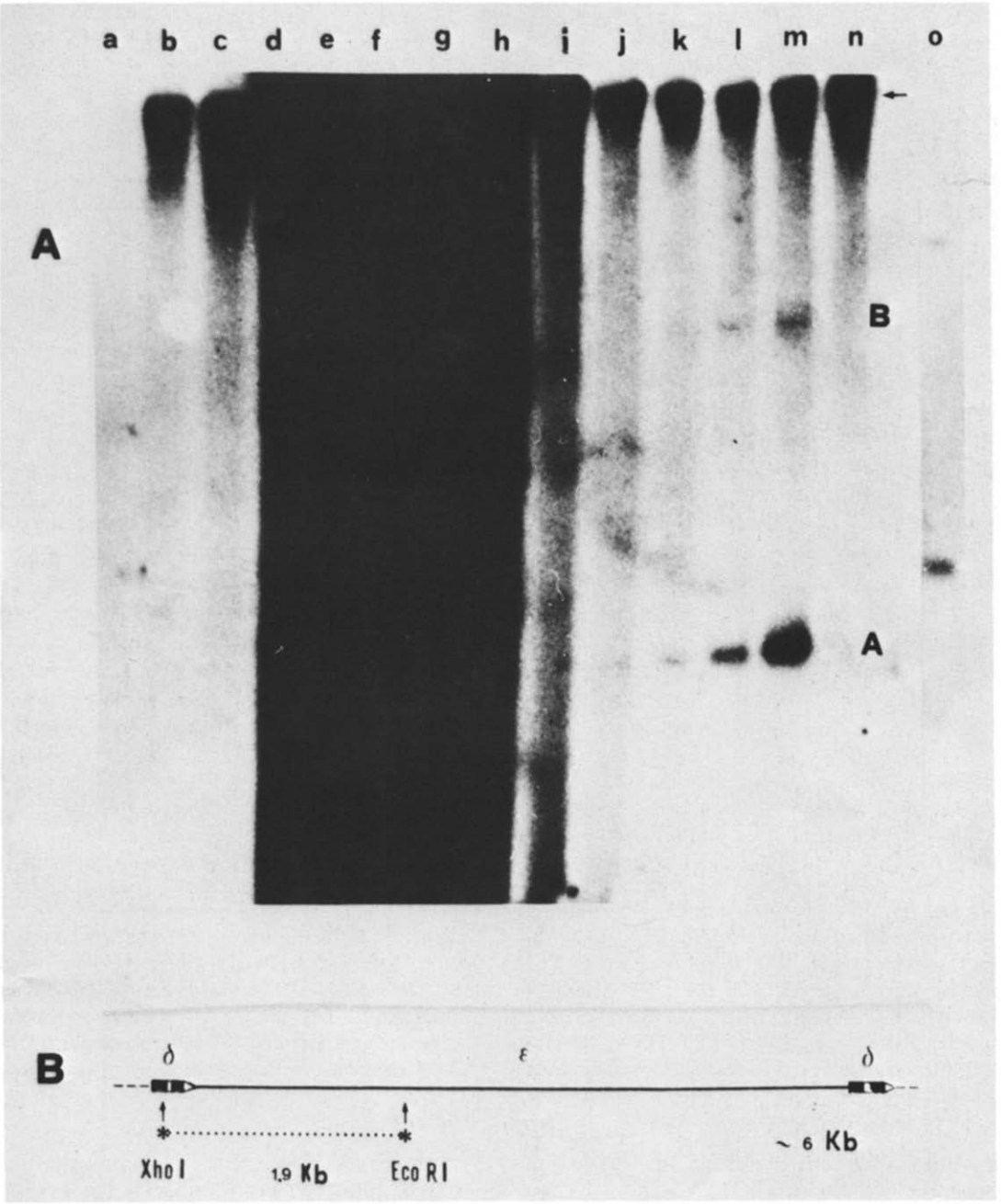

Fig.1. Autoradiogram of single fractions from an EtBr–CsCl gradient probed with a Ty1 internal fragment. (A) The nitrocellulose filter obtained by Southern blotting of gel electrophoresis (35 × 18 cm, 0.7% agarose, 20 h, 100 V) hybridized with a Ty1 *Eco*RI–*Xho*I restriction fragment (spec. act. 1.5×10^7 cpm/μg DNA. (a–n) fractions from the top to the bottom of the EtBr–CsCl gradient (1/3 of the total DNA recovered by this preparation); (o) 0.05 ng plasmid P_{30} (7.5 kb) containing the Ty1 sequence used as probe. (B) General structure of Ty1: (*——*) *Eco*RI–*Xho*I internal fragment used as probe.

Fig.2. Characterization of the yeast circular DNA population. Pools of the circular DNA fractions from an EtBr–CsCl gradient were analyzed by gel electrophoresis (20 × 18 cm, 1% agarose, 20 h, 30 V): (1) ethidium bromide staining of an aliquot of pooled fractions; (2) the same amount treated with topoisomerase I. The nitrocellulose filter obtained from track 1 was serially hybridized with: (3) nick-translated 2 μm DNA (BTyP1 clone, spec. act. 2.2 × 10^7 cpm/μg DNA, 20 min exposure); (4) nick-translated *X. laevis* ribosomal DNA (Xcr3 clone, spec. act. 1 × 10^7 cpm/μg DNA, 12 h exposure).

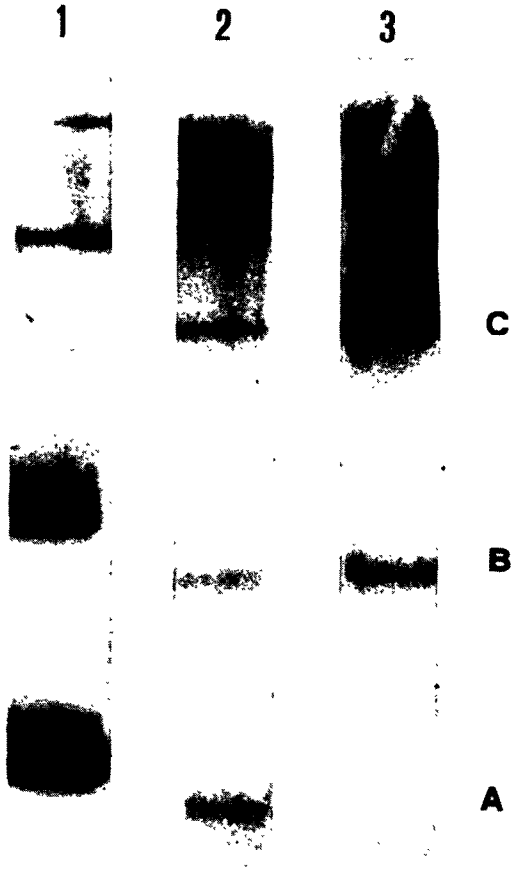

Fig.3. Nicking–closing enzyme treatment on Ty1 circular copies. Southern blot hybridization on aliquots of a circular DNA preparation (as in fig.2) run on agarose gel electrophoresis (20 × 18 cm, 0.6% agarose, 20 h, 30 V): (1) labelled 2 μm DNA (BTyP1, spec. act. 6 × 10^6 cpm/μg DNA, 5 h exposure); (2) *Eco*RI–*Xho*I fragment (spec. act. 7 × 10^6 cpm/μg DNA, 30 h exposure) on circular DNA preparation; (3) the same Ty1 fragment on the circular DNA preparation treated by topoisomerase I.

(3) The alkali resistance during the extraction procedures;
(4) The variation of conformation from the supercoiled to the relaxed one, due to the increase in linking number, after the nicking–closing treatment (fig.3, tracks 2,3).

The Ty1 circular copies are about 6 kilobases as judged by the migration of marker supercoiled molecules (fig.1, track 0) and the 2 μm internal marker (fig.3, track 1). This value is within the average size range of the chromosomal copies of Ty1 family [20]. The number of Ty1 extrachromosomal copies present in DM strain was evaluated by photometric scanning of the intensity of Ty1 hybridizing signals, compared with those of marker species of varying dilutions (see fig.1) and correct for the average recovery (10% of circular DNA calculated from the approximated known number of 2 μm copies) [21]. We estimate that there is about 1 Ty1 circular molecule/250–300 cells.

The question of whether circular copies of Ty1 are the expression of an excision event and/or are the nomadic copies which are involved in the process of transposition is still open. It should be stressed that this report points out the presence of circular episomal DNA homologous to an eukaryotic transposable element in a wild-type diploid yeast strain grown under physiological conditions. Similar findings in *Drosophila melanogaster* [7] refer to tissue culture cells where additional events take place with respect to the embryo genome [22]. Two other observations should be mentioned on the presence of *Alu*-type sequences, thought to be mammalian transposable elements, in a circular form in Green monkey cell culture [23] and in human leukocytes [24]. The identification of circular Ty1 copies in yeast, in addition to [7,23,24] suggests that the presence in eukaryotes of extrachromosomal circular DNA molecules related to transposable elements and retroviruses [8] may involve an evolutionary conserved function.

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