CHROMOSOME I AS A POSSIBLE SITE FOR SOME rRNA CISTRONS IN *SACCHAROMYCES CEREVISIAE*

T.B. ØYEN

Department of Biochemistry, University of Oslo, Blindern, Norway

Received 15 December 1972

1. Introduction

By hybridizing ribosomal RNA with DNA extracted from different disomic strains of *Saccharomyces cerevisiae*, Goldberg et al. [1] have shown that the 140 rRNA cistrons in yeast are non randomly distributed on the genome. None of their strains seemed to carry disomic chromosomes enriched for rRNA genes. However, one mutant that showed a significantly lower hybridization plateau than a wild type haploid, turned out to have at least seven chromosomes in double portion. Besides indicating the presence of only a few, if any, rRNA genes on these extra chromosomes, this illustrated some of the problems in identifying the aneuploids genetically. As also stated by Parry and Cox [2], it may be difficult to obtain stable yeast strains with disomy for one chromosome only.

Another approach to the mapping of rRNA cistrons in yeast would be to study rRNA–DNA hybridization for monosomics, that is diploid cells lacking one chromosome. A reduced hybridization level could then be attributed to the loss of one specific chromosome. In the present work, such studies have been carried out with two strains that were monosomic for chromosome I, one chromosome VI monosomic, and one strain possibly monosomic for chromosome V. The results indicate that chromosome I may carry about 70% of the rRNA genes.

2. Materials and methods

2.1. *Strains*

The parent and the monosomic strains of *S. cerevisiae* were obtained from dr. Ø. Strømnaes:

X951: a diploid with several heteroallelic markers.
X951-13: ad_1, monosomic for chromosome I.
X951-17: thr_3, supposed to be monosomic for chromosome V.
X951-20: ad_1, another chromosome I monosomic.
X951-22: his_2, monosomic for chromosome VI.

2.2. *Radioactive compounds*

[^{3}H]Adenine (27 Ci/mMole) was supplied by the Radiochemical Centre, Amersham,and ^{32}P inorganic phosphate (carrier free) by Institutt for Atomenergi, Kjeller.

2.3. *[^{3}H]DNA isolation*

Cells were grown in YEP medium (1% yeast extract, 2% peptone, 4% glucose) added 1 mCi[^{3}H]adenine/ℓ. At harvesting, samples were plated and tested for requirements and the ability to sporulate on acetate medium. The cells were broken in an Eaton press and the DNA isolated as described by Goldberg et al. [1]. The DNA preparations were purified by centrifugation on preparative CsCl gradients two times. The amount of mitochondrial DNA in each preparation was estimated by analytical CsCl centrifugation [1]. Alternatively, the cells were labeled in YEP medium containing 10 μg ethidium bromide/ml. DNA extracted from these cells showed no mitochondrial band in analytical CsCl gradients [3].

2.4. *[^{32}P]rRNA*

[^{32}P]Ribosomal RNA was prepared from X951 essentially as described by Schweizer et al. [4]. Ribosomes were isolated and washed in 0.05 M Tris buffer pH 7.3 containing 0.5 M NH_4Cl and 0.1 M $MgCl_2$. The RNA was deproteinized by phenol and purified on

sucrose gradients. Less than 0.2% of the radioactive material was stable in alkali.

2.5. *Hybridization*

Low temperature hybridization was carried out in 30% formamide, 2 X SSC and 0.4% SDS for 20 hr at 37° with shaking [5] using the membrane filter technique of Gillespie and Spiegelman [6]. Selectron-filters, BA 85, 27 mm (Schleicher and Schüll, Dassel) were loaded with 10 μg of denatured [^{3}H]DNA each, and cut into quarters. The filters were preincubated in the formamide system for 1 hr and then incubated with different concentrations of 26 S + 17 S [^{32}P]-rRNA in a volume large enough that the filters could move freely. Further treatment of the filters was that of Goldberg et al. [1]. Each filter was finally dissolved in 1 ml of ethyl acetate after removal of salts by 1 ml of distilled water and drying. 10 ml of toluene–PPO–POPOP were added, and the samples counted in a Packard Tri-Carb scintillation counter. The values were corrected for radioactivity bound to blank filters. Maximum hybridization values were determined from double reciprocal plots of % hybridization against rRNA concentration by extrapolating to infinite RNA concentration [7]. To obtain % hybridization of nuclear DNA, the values were corrected for the amount of mitochondrial DNA in the DNA preparations.

3. Results and comments

The experiments reported here were undertaken to see whether rRNA hybridization with DNA from monosomic mutants could give further information about the distribution of rRNA cistrons on the 16–18 chromosomes in *S. cerevisiae*.

The application of hybridization data in mapping studies is based on the assumption that there is no preferential loss of any nuclear DNA fraction during purification of the DNA samples to be compared. To make this assumption valid, all the DNA samples were treated in the same manner. Care was taken to prevent loss of DNA fractions on both the light and the heavy side of the main peak in the CsCl gradients, and repeated precipitations were avoided.

The hybridization curves for total DNA extracted from strain X951 and the segregants 13, 17, and 22, are shown in fig. 1. A significantly lower hybridization plateau was obtained for X951-13 and for X951-17 than for the parent diploid, X951. Segregant 22 hybridized to the same extent as the diploid.

The reduced % hybridization for the two strains was not due to an abnormal high content of mitochondrial DNA in these preparations. In table 1 are shown the corrected hybridization values after extrapolating to infinite RNA concentration. It is seen that the monosomics 13 and 17 might contain considerably less rRNA cistrons per cell than the diploid and the chromosomes VI monosomic.

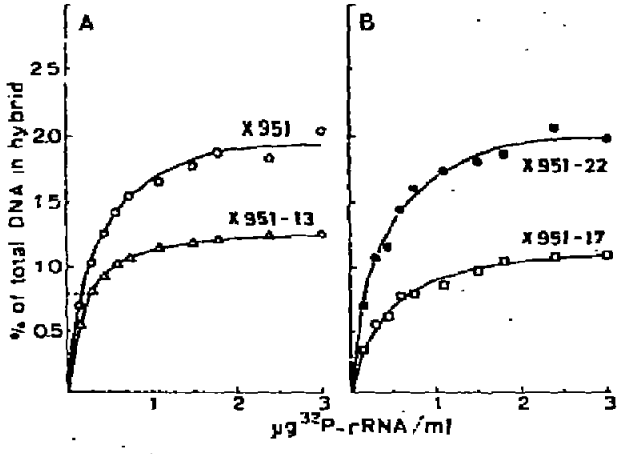

Fig. 1. Hybridization of rRNA with total DNA isolated from the diploid X951 and the monosomic segregants X951-13 (chrom. I), X951-17 (chrom. V) and X951-22 (chrom. VI).

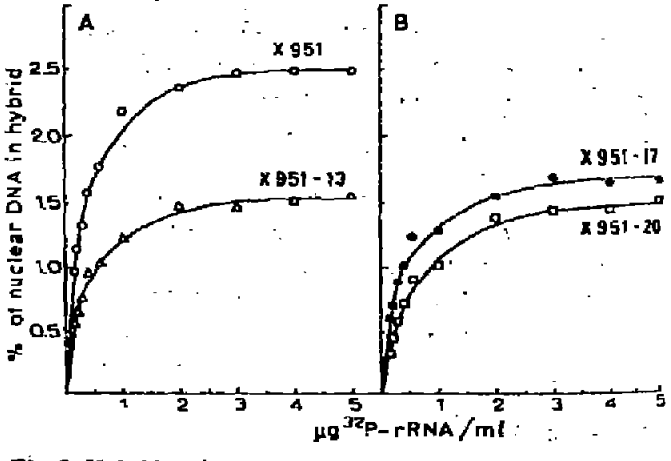

Fig. 2. Hybridization of rRNA with nuclear DNA isolated from ethidium bromide treated cultures of the diploid X951, the chromosome I monosomics X951-13 and X951-20, and the chromosome V monosomic X951-17.

Table 1

The maximum % of nuclear DNA in hybrid with rRNA estimated by extrapolating to infinite RNA concentration in double reciprocal plots of the curves in figs. 1 and 2.

Strain	Total DNA extracted (% mitochondrial DNA)	Total DNA extracted (% of nuclear DNA in hybrid)	Nuclear DNA extracted from ethidium bromide treated cells: (% DNA in hybrid)	Average % hybridized	% of wild type
X951	8	2.4	2.6	2.5	100
X951-13	13	1.6	1.6	1.6	64
X951-17	12	1.4	1.8	1.6	64
X951-20	–	–	1.7	1.7	68
X951-22	10	2.6	–	2.6	104

The values obtained with the total DNA preparations were corrected for the presence of mitochondrial DNA.

Chromosome I monosomics seem to be of the more stable among such aneuploids and are relatively easy to obtain [9]. Another strain with monosomy for chromosome I (X951-20) was therefore included in this study to see whether the rRNA hybridization level had changed. Essentially the same hybridization value as for strain 13 was obtained. This indicates that both strains might have lost the same rRNA gene containing element, most likely chromosome I.

When the hybridizations were repeated with DNA extracted from cells treated with ethidium bromide, the results shown in fig. 2 were obtained. At the growth conditions used, ethidium bromide blocks the synthesis of, and causes the degradation of preformed, mitochondrial DNA, but has no effect on the nuclear genes [3]. At harvesting, the cells were all petites, but showed no altered growth requirements as compared to the untreated cells. The extrapolated hybridization values for these nuclear DNA preparations are also summarized in table 1. It is seen that the values were essentially the same as those obtained for the preparations of total DNA after corrections for the contents of mitochondrial DNA. Strains 13, 17, and 20, thus, seem to carry significantly less rRNA cistrons than the parent diploid.

From a nomogram similar to that of Goldberg et al. [1] it can be concluded that the strains 13, 17, and 20 have lost from 1 to 9 chromosomes carrying from about 70 to 100% of the haploid number of rRNA cistrons. Strain 22, on the other hand, might have lost 1 or 2 chromosomes that carried no rRNA genes.

In his study of the *p*-fluorophenylalanine induced chromosome aberrations in strain X951, Strømnaes [10] found that none of the segregants used here gave tetrads with more than 2 viable spores. Segregant 22 showed also a large portion of 1:3 and 0:4 viability and was interpreted as a chromosome VI monosomic with some additional lethal. This is also consistent with the hybridization data, which then indicate that chromosome VI carries no rRNA genes. Both segregant 13 and 20 satisfied the criteria of being monosomic for chromosome I. Hence, no more than one chromosome could be lost, and the reduction in the number of rRNA genes per cell, about 35%, might therefore be a result of the loss of one chromosome I homolog.

Segregant 17 was more difficult to interpret genetically. It could possibly be a chromosome V monosomic, or it could be homozygous for one chromosome V homolog and a lethal. The hybridization level found for this mutant was about the same as for strain 13 and strain 20. If, therefore, 70% of the genes are located on chromosome I, the low level obtained with X951-17 could not be due to the loss of one chromosome V only. One possible explanation would be that X951-17 has a deletion on rRNA genes. It is known from other organisms that unequal crossing-over may occur within the nucleolar organizer [11]. As there is now evidence for a nucleolus also in yeast [12], an organizer region could possibly be linked to chromosome I. In the genetic map presented by Hawthorne and Mortimer [13], chromosome I represents less than 1% of the genome. Extension of this chromosome with about 2/3 of the rRNA cistrons would then not give a chromosome of abnormal size.

From their studies of the γ-band of high-molecular weight DNA prepared from yeast nuclei, Cramer et al. [14] calculated that no more than 10 to 30 rRNA genes could be clustered together. The finding that 70%, or about 100 cistrons, may be located on chromosome I, would then mean that several such clusters appear on that chromosome. If each chromosome is represented by only one DNA molecule [15],

the clusters of rRNA genes may be interspaced by other DNA regions.

References

[1] S. Goldberg, T. Øyen, J.M. Idriss and H.O. Halvorson, Molec. Gen. Genet. 116 (1972) 139.
[2] E.M. Parry and B.S. Cox, Genet. Res. 16 (1970) 333.
[3] E.S. Goldring, L.I. Grossman, D. Krupnick, D.R. Cryer and J. Marmur, J. Mol. Biol. 52 (1970) 323.
[4] E. Schweizer, C. MacKechnie and H.O. Halvorson, J. Mol. Biol. 40 (1969) 261.
[5] J.N. Hansen, G. Spiegelman and H.O. Halvorson, Science 168 (1969) 1291.
[6] D. Gillespie and S. Spiegelman, J. Mol. Biol. 12 (1965) 829.
[7] J.O. Bishop, F.W. Robertson, J.A. Burns and M. Melli, Biochem. J. 115 (1969) 353.
[8] E. Schweizer and H.O. Halvorson, Exp. Cell Res. 56 (1969) 239.
[9] J. Bruenn and R.K. Mortimer, J. Bact. 102 (1970) 548.
[10] Ø. Strømnaes, Hereditas 59 (1968) 197.
[11] M.L. Birnstiel, M. Chipchase and J. Speirs, Progress in Nucleic Acid Res. and Mol. Biol. 11 (1971) 351.
[12] W.W. Sillevis Smitt, C.A. Vermeulen, J.M. Vlak, T.H. Rozijn and I. Molenaar, Exp. Cell Res. 70 (1972) 140.
[13] D.C. Hawthorne and R.K. Mortimer, Genetics 60 (1968) 735.
[14] J.M. Cramer, M.M. Bhargava and H.O. Halvorson, J. Mol. Biol. 71 (1972) 11.
[15] J. Blamire, D.R. Cryer, D.B. Finkelstein and J. Marmur, J. Mol. Biol. 67 (1972) 11.