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Effect of changes in the conventional carbon sources on the stability of minichromosome in the budding yeast, *Saccharomyces cerevisiae*

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Mitotic stability of a plasmid is defined as the fraction or percentage of plasmid-carrying cells in a transformant culture. Several mutants of the budding yeast *Saccharomyces cerevisiae* are available, called minichromosome maintenance (*mcm*), which cannot identify the genes required for stable propagation of minichromosomes and are hence defective in the stable maintenance of minichromosomes. The *mcm* mutants cause a decrease or loss of minichromosome stability, associated with defective replication or centromere-dependent chromosome segregation. This communication focuses on whether the plasmid stability in both wild type (WT) and *mcm* mutant transformants is related to any change in the carbon source of the growth medium, using the conventional dextrose and the altered glycerol-alcohol combination as the two sources. The results revealed that WT transformants showed better plasmid stability than the mutants, but the alteration in the carbon source practically had no or very negligible effect on minichromosome stability, whether in WT or mutant transformants. Thus, the plasmid stability in yeast is irrespective of the carbon supply in the medium.

Key words: Yeast, plasmid stability, minichromosome maintenance, dextrose, glycerol-alcohol

INTRODUCTION

Eukaryotic genomes are organized into multiple chromosomes. Each chromosome is replicated by the initiation of DNA synthesis at numerous initiation sites. The identification of autonomously replicating sequences (*ARS*) suggests that these initiation sites are specific sequences, and these initiation events are regulated. The budding yeast Saccharomyces cerevisiae possess approximately 400 replication origins distributed among 16 chromosomes, each of which is independently controlled. *ARS* are putative replication origins, most of which are present in single copies in the yeast genome. So far, only two families of repetitive *ARS* have been identified. ARS enable the plasmids to replicate autonomously in yeast. Chromosomal segregation in yeast occurs with high fidelity (Sinha *et al*, 1986). There are certain yeast mutants that are defective in the stable maintenance of circular minichromosome (*mcm*) and cannot identify genes required for the stable propagation of minichromosome. The *mcm* phenotype is due to single gene mutation (Maine *et al.*, 1984). Such mutants have been divided into two classes: 1. *ARS*-specific class: It leads to the loss of minichromosome depending upon a particular group of *ARS* cloned on them. They usually fail to initiate DNA replication at their origin of replication or *ARS* (Tye, 1994; Chong *et al.*, 1996; Merchant *et al.*, 1997). *MCM1 - MCM12* are *ARS*-specific mutants; and 2. *ARS*-non-specific or independent class: They are defective in the maintenance or stability of all minichromosome irrespective of *ARS* sequence present. They may be carrying mutations in genes, which control fidelity of minichromosome transmission. These defects could be in replication, in centromere-dependent segregation into daughter cells or other aspects of chromosome maintenance, *mcm 13 - mcm 23* belong to this group. All these genes are required for proper functioning of centromere (Roy *et al*, 1997).

Most autonomously replicating plasmids (YR_P) in yeast are mitotically unstable and are lost from the

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cells at a relatively high frequency showing mother cell bias due to nondisjunction. In the yeast Saccharomyces cerevisiae, the identification of specific *DNA* sequences, such as *ARS*, centromeric DNA (*CEN*) and telomeric DNA (*TEL*), has led to the construction of artificial linear and circular chromosomes which have been extremely useful for understanding the mechanisms by which chromosome stability is maintained. Minichromosomes are mitotically stable plasmid, each of which contains different *ARS*, and stabilized by the introduction of a functional *CEN*, *TEL*, and yeast genetic markers like *LEU2*, *URA3*, *HIS4* and *TRP1*. The plasmid vectors so generated are termed as YC_P, showing enhanced stability during mitosis and meiosis. The *cis*- and *trans*-acting mutations affecting *ARS*, *CEN* and *TEL* functions lead to chromosome instability (Newlon, 1988, 1993; Page and Snyder, 1993; Tye, 1994; Zakian, 1995). Mitotic and meiotic chromosome loss rates in yeast are once in every 10^5 and once in every 10^4 divisions respectively. Another important determinant of chromosome stability is its length (Roy *et al.*, 1997).

The aim of this investigation is to find out whether a change in the conventional carbon source used for yeast growth (dextrose) in any way affects the minichromosome stability, i.e., the maintenance of minichromosome. We selected for our studies two wild type (WT) yeast transformants, namely 301-2B/YC_P lac III and 301-2B/pL2, and two *mcm* mutant transformants, namely 301-2BΔM16/YC_P lac III (carrying mutation in *mcm 16*) and 301-2BΔM39/pL2 (mutation in *mcm* 21).

MATERIALS AND METHODS

Yeast strains and their genotypes

301-2B — MATa leu 2-3, 112 ura 3-52, his 4A34, trpl;
301-2BAM76 — AM7a leu 2-3, 112 his 4A34, ura 3-52, trpl mcm!6-M:: URA3; and 301-2BAM39 — MATa leu 2-3, 112 his 4A34, ura 3-52, trpl mcm21-M::URA3.

Plasmids used

YC_P lacI 11 (CEN4 ARSI LEU2) and pL2 (ARSI LEU2 CEN5), constructed by digesting YC_P1 with Hind III, and self- ligating to remove the URA3 gene.

The WT yeast strain 301-2B was transformed separately with YC_P lac III and pL2 plasmids, whereas the mutant strains 301-2BAM/6 and 301-2BAM39 were transformed with YC_P lacI 11 and pL2 plasmids respectively.

Media used

The complete media used were YEPD, consisting of yeast extract - 0.3% (w/v), peptone -1% (w/v) and dextrose - 1% (w/v), together with uracil - 0.005% (w/v) and agar-agar -1.5% (w/v) for solid media only; or YEP + glycerol + alcohol, having the same composition as above except that glycerol - 3% (v/v) and alcohol - 2% (v/v) were added instead of dextrose.

The two selective media, CM-leu, i.e., all amino acids except leucine, supplemented by dextrose (conventional carbon source) or glycerol + alcohol (alternate carbon source) were used. The compositions of the two media are listed as follows: (i) CM-leu with dextrose: dextrose - 2% (w/v); CM consisting of salt mixture - 0.67% (w/v), amino acid mix - 0.1% (w/v), histidine, uracil, tryptophan and adenine - 0.005% each, except leu; agar-agar - 1.5% (w/v) only for solid media; vitamins - 0.1% (w/v) and trace elements -0.1% (w/v) added after autoclaving; and (ii) CM-leu with glycerol and alcohol: CM same as before, but instead of dextrose, glycerol - 3% (v/v) and alcohol - 2% (v/v) were used, together with agar-agar - 1.5% (w/v) for solid media only.

The stability assays were carried out essentially as described in Maine et al. (1984). The single colonies of yeast transformants 301-2B/YC_P lacIII, 301-2B/pL2, 301-2BAM7d/YC_p lacIII and 301-2BAM39/pL2, all growing on separate YEPD plates, were streaked in minimal medium lacking leucine (CM-leu), i.e., (CM-leu) + dextrose and (CM-leu) + glycerol-alcohol, and left for incubation at 30°C for about three days. A single isolated colony was picked up from each plate and inoculated in 5 ml of liquid culture media, either YEPD or YEP + glycerol + alcohol. The cell growth was allowed for two days at 30°C. Finally, streaking a small aliquot of the two cultures on the respective plates, single colonies were derived. The single colonies were then replica plated onto the respective selective media. After three days, the total number of cells (whether viable or not growing) as well as the number of growing cells was counted and the per cent stability was expressed as the ratio: (Number of colonies growing on CM-leu / Number of colonies growing on YEPD) x 100.

RESULTS AND DISCUSSION

Minichromosome maintenance (*mcm*) mutants were isolated as chromosomal mutations that led to the instability of small, circular *ARS* and *CEN*-carrying minichromosome (Maine *et al.*, 1984; Gibson *et al.*, 1990). The *MCM 16* and *MCM 21* were both minichromosome-maintenance genes, required for the stable propagation of minichromosomes in yeast. Mutations in these genes caused a defect in the segregation of minichromosomes due to the malfunctioning of the microtubule or kinetochore, lowered the stability of minichromosome and loss of minichromosomes from cells (e.g., the *mcm16* mutation caused a high rate of loss of chromosome III), being associated with an increase in the copy number of the minichromosome in the cells carrying it and also causing the replicated copies to accumulate in the mother cells (Maine *et al*, 1984; Sinha *et al*, 1986; Koshland *et al*, 1987; Roy *et al*, 1997; Sanyal *et al*, 1998; Poddar *et al*, 1999). That the two genes *MCM 16* and *MCM 21* affected chromosome segregation was supported earlier by several observations like increased non-disjunction of the minichromosome in the mutants, structural intactness and increased stabilization of dicentric plasmids in the mutants, less obstructed and increased transcription of a gene in mutants due to more relaxed kinetochore assembly and higher sensitivity of the mutant cells to anti-mitotic microtubule-depolymerizing drugs such as benomyl, thiabendazole and nocodazole. A pre-existing defect in the function of the microtubule or in its interaction with the kinetochore could render the cell more sensitive than the WT to these drugs. The *mcm 16* and *mcm 21* mutation decreased the tolerance of the corresponding strains to defects in microtubule assembly.

On the basis of all these observations, we were interested to find out whether this defect in the kinetochore-microtubule mediated process of chromosome segregation and hence maintenance of minichromosome both in WT and particularly in mutants was dependent on carbon source. We used dextrose and glycerol-alcohol combination as the two carbon sources. The results of the experiment are given in Tables 1 and 2.

It was found that the plasmids showed substantially higher mitotic stability in the two WT strains than in both *mcm 16* and *mcm 21* mutants, since the rate of loss of the minichromosome (defined as the incidence of loss of the mini-chromosome per cell per generation) was much lower in WT cells. The *mcm 21* mutation caused a more severe effect (3-4 times lesser plasmid stability) than *mcm 16* mutation in both the media. The mitotic stability of the WT transformants was 5-6 times higher than *mcm 16* mutants and 16-17 times higher than *mcm 21* mutants in YEPD medium (Table 1), while in YEP-glycerol-alcohol medium (Table 2), the WT transformants showed 4 times higher stability than *mcm* 16 mutants and 16-17 times higher than *mcm 21* mutants. It also appears from the data that the plasmid stability of both the WT transformants is

Table 1: Plasmid stability of transformants grown in YEPD medium

Transformants	No. of observations	No. of colonies in CM-leu plates / No. of colonies on YEPD	Per cent stability	Mean stability
301-2B/YC_P	1	56/82	68.29%	66.04%
lac 111	2	84/125	67.20%	
	3	57/91	62.63%	
301-2B/pL2	1	62/100	62.00%	61.43%
	2	58/96	60.41%	
	3	57/92	61.90%	
301-2B ΔM16/	1	8/86	9.30%	11.51%
YCp lac111	2	12/92	13.04%	
	3	11/90	12.20%	
301-2B ΔM39/	1	4/94	4.20%	3.81%
pL2	2	1/85	1.17%	
	3	6/99	6.06%	

Table 2: Plasmid stability of transformants grown in YEP + glycerol-alcohol medium

Transformants	No. of observations	No. of colonies in CM-leu plates / No. of colonies on YEPD	Per cent stability	Mean stability
301-2B/YC_P	1	74/99	74.74%	69.49%
lac III	2	40/77	51.94%	
	3	63/77	81.80%	
301-2B/pL2	1	65/91	71.42%	72.90%
	2	57/73	78.08%	
	3	61/88	69.31%	
301-2B ΔM16/	1	10/100	10.0%	15.93%
YC_plac III	2	18/90	20.0%	
	3	15/84	17.8%	
301-2B ΔM39/	1	9/95	5.20%	4.16%
pL2	2	3/94	3.00%	
	3	4/92	4.30%	

almost unaffected in YEP media containing glycerol-alcohol and YEPD medium. Similarly, both the mutant transformants showed only a very little increase in stability (only 1.1-1.4 times) in medium containing glycerol-alcohol combination than that with dextrose. This provides the conclusion that minichromosome maintenance or plasmid stability is practically unaffected by or independent of the change in carbon source (whether dextrose or glycerol-alcohol) used in the growth medium. Similar observations have been reported earlier by Maine *et al.* (1984), Maiti and Sinha (1992) and Roy *et al.* (1997).

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