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Stabilization of dicentric chromosomes in *Saccharomyces cerevisiae* by telomere addition to broken ends or by centromere deletion

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We introduced CEN6 DNA via integrative transformation into the right arm of chromosome II in a haploid *Saccharomyces cerevisiae* strain thus creating a dicentric chromosome. The majority of the transformed cells did not grow into colonies as concluded from control transformations with mutated CEN6 DNA. Five percent of the initial transformants with the wild-type centromere gave rise to well growing cells. We analysed the probable fate of the dicentric chromosome in two transformants by electrophoretic separation of chromosome sized DNA and by hybridizations with chromosome II DNA probes. We found two different mechanisms which generated cells lacking dicentric chromosomes. The first mechanism is breakage of the chromatid between the two centromeres and healing of the new ends to functional telomeres thus creating progeny cells with the chromosome II information split into two genetically stable new chromosomes one carrying CEN2 and the other CEN6. The second mechanism is loss of the resident CEN2 by a 30-50 kb deletion event which resulted in a genetically stable but shortened chromosome II. Both mechanisms operated in the two transformants studied.

Key words: centromere/dicentric chromosomes/telomere/pulsed field electrophoresis/yeast

Introduction

Genetically stable chromosomes carry one functional centromere. Chromosomes with two functional centromeres, so called dicentric chromosomes are usually unstable. The independent attachment of the two centromeres to spindle fibres from opposite poles can cause breakage of the chromosome between the centromeres at mitosis. In the daughter cells the resulting monocentric chromosome fragments are still unstable, most likely because they lack a telomere at their broken ends. Broken ends are highly recombinogenic as already observed by McClintock (1939,1941) in her classical cytogenetic studies with broken maize chromosomes. Broken ends, initiated a breakage-fusion-bridge cycle (BFB cycle). They could fuse to form a dicentric chromosome which, when pulled to opposite poles, formed a chromatin bridge with subsequent breakage. McClintock also found that stabilization could occur in several ways. Occasional loss of the dicentric chromosome by non-disjunction generated stable aneuploid cells. In other cells broken chromosomes could become healed to genetically stable monocentric chromosomes.

Molecular studies with respect to the stabilization processes of dicentric chromosomes have so far only been performed with the yeast *Saccharomyces cerevisiae*. This simple eucaryote has several advantages for such studies. It is genetically well characterized, tolerates extensive aneuploidy, can easily be transformed with recombinant DNA and it is the only organism for which cloned centromere DNA (CEN DNA) is available, thus allowing constructions of dicentric DNA molecules with predetermined sequence arrangements.

Three different experimental approaches have been used to construct dicentric chromosomes. In the first approach identical or non-identical CEN DNAs were cloned into plasmids capable of autonomous replication in *S.cerevisiae* (Mann and Davis, 1983; Oertel and Mayer, 1984; Koshland *et al.*, 1987). Such dicentric circular minichromosomes depress the rate of cell division and show increased mitotic non-disjunction. Well segregating derivatives isolated after prolonged growth carry deletions for either one of the centromeres.

A completely different approach which mimics a possible natural situation employed meiotic recombination between a circular and a linear chromosome III (Haber *et al.*, 1984; Haber and Thorburn, 1984). Unequal numbers of cross-over events generated a dicentric chromosome which can be described as a tandem duplication of almost the entire chromosome III. Colonies from viable haploid spores carrying such duplicated chromosome III were highly variegated. Genetic and Southern blot analysis demonstrated that stable cell lines arose from mitotic breakage of dicenteric chromosome followed by different healing events. Since the dicentric chromosome was a big duplication, the highly reactive broken ends could recombine with the homologous regions at the other end of the dicentric chromosome. This happened in the majority of healing events and genetically stable monocentric linear or circular chromosomes were generated.

In the third approach a cloned centromere DNA was integrated at a predetermined chromosomal location, thus avoiding the genetic complexity caused by the tandem duplications in the previously described approach. Surosky and Tye (1985) studied transformed haploid cells with CEN5 DNA inserted at HIS4, a locus on the left arm of chromosome III. Selection for the maintenance of CEN5 in chromosome III caused non-resolvable problems for most of the transformants. Three of the few viable transformants were analysed and shown to have lost CEN3, the original centromere, by a deletion event which involved copies of the mobile element Ty located at both sides of CEN3. A shortened chromosome III with CEN5 and a ring chromosome with CEN3 were generated. We have previously tested integrations of CEN6 wild-type and mutant DNA at LYS2, a locus on the right arm of chromosome II (Panzeri *et al.*, 1985). Only 5% of the initial transformants grew into colonies when we selected for the maintenance of the wild-

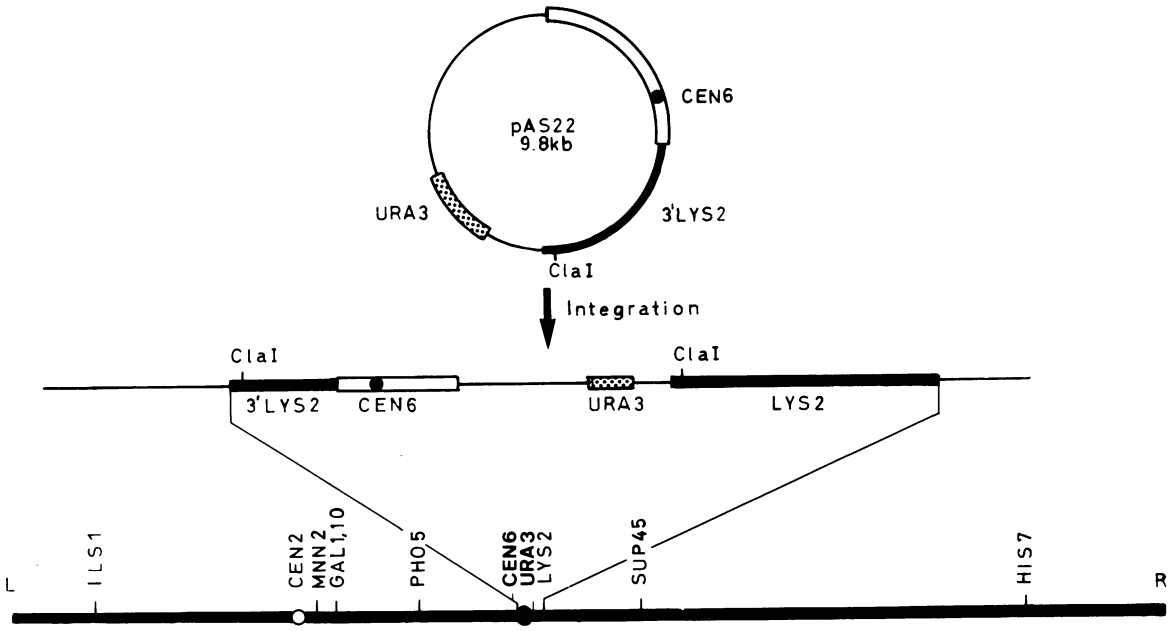

Fig. 1. Integration of CEN6–URA3 at LYS2. The top part shows sequence arrangements of the transforming plasmid and the LYS2 locus after integration of the *Cla*I cleaved plasmid. Sequences of *S.cerevisiae* origin are indicated by their genetic symbols. Thin lines represent pBR322 sequences with URA3 (1.1 kb *Hin*dIII fragment) at the *Ava*II site (Struhl *et al.*, 1979), CEN6 (2.65 kb *Eco*RI–*Bam*HI fragment) between the *Eco*RI and *Bam*HI sites (Panzeri and Philippsen, 1982) and 3′ half of LYS2 (2.3 kb *Bgl*II–*Bam*HI fragment) at the *Bam*HI site (Eibel and Philippsen, 1983). The location of the functional CEN6 sequences is indicated by the dot. The lower part shows a short version of the genetic map of chromosome II (Mortimer and Schild, 1981) including the orientation of CEN6, URA3 and LYS2. This orientation is based on the known transcription of LYS2 towards CEN2 (P.Hieter, personal communication). The integration of pAS22 does not lead to an inactivation of LYS2.

type CEN6 DNA at LYS2. Southern blot analyses did not allow firm conclusions with respect to breakage and healing events. A major drawback was the lack of a method for direct observation of chromosomal DNAs.

In this paper we investigate the fate of dicentric chromosome II in *S.cerevisiae*. As a major analytical tool we employ pulse field gel electrophoresis (Schwartz and Cantor, 1984) with the modification introduced by Carle and Olson (1984). Breakage and healing events can be directly analysed. Size determinations of chromosomal fragments together with hybridizations to chromosome II DNA probes allow the location of break-points. Genetic diversities in cell populations can be seen in one separation and stabilities of different rearrangements can be easily followed in outgrowth experiments.

Results

Transformations generating dicentric chromosome II

Figure 1 shows the plasmid pAS22 which was used for directed integration at the LYS2 locus of chromosome II. The plasmid carries a functional CEN6 DNA, URA3 as selectable marker, and sequences for the LYS2 gene. It cannot autonomously replicate in *S.cerevisiae*. After cleavage at the *Cla*I site and transformation of the haploid *S.cerevisiae* strain VB2-20A (α ura3 ade2 leu2 trp1) the plasmid will integrate at the LYS2 locus in a way shown in the middle of Figure 1 thus creating a dicentric chromosome II. Since the new centromere CEN6 is tightly linked to URA3, selection of transformants in medium lacking uracil will select for the presence of CEN6. When both centromeres of chromosome II attach to microtubules from opposite poles (*trans* attachment) the mitotic segregation can only proceed if microtubules break or lose their contact to one of the centromeres or if the chromosomal DNA between CEN2 and CEN6 breaks. The first solution would leave the dicentric chromosome intact the second not.

Transformation of VB2-20A with equal amounts of linearized pAS22 and pAS22-CEN6 Δ (a control plasmid with an inactivating deletion in CEN6) yielded 21 and 500 Ura^+ transformants, respectively, and in a second experiment 78 and 2700 Ura^+ transformants. This shows that ~3–4% of the original transformants with a dicentric chromosome managed to grow into colonies.

Cell populations evolving from transformants with a dicentric chromosome II

Cells from Ura^+ transformants T9 and T10 were streaked on selective plates. After 1 day of growth cells from the middle of the streak were inoculated into selective medium. The generation time at 30°C was 3 h. The same time was determined for the parent cells grown in selective medium supplemented with uracil.

When the chromosomal DNA of T9 and T10 cells was separated in alternating electrical fields and compared to the parent cell pattern several differences were observed. Figure 2B shows the ethidium bromide stained gel and Figure 2A the chromosome assignment of the chromosomal DNAs (solid lines) together with the approximate sizes of new chromosomal DNAs (broken lines). T9 cells no longer carry chromosome II but instead shortened chromosomes of 850, 550 (comigrating with chromosomes V and VIII, see below), 490 and 400 kb. T10 cells still contain chromosome II sized DNA (900 kb) in addition to shortened chromosomes of 850, 630, 410 and 300 kb. Some but not all of these fragmented chromosomes carry the newly introduced centromere as shown by hybridizations with radioactively labelled CEN6 DNA (Figure 2C).

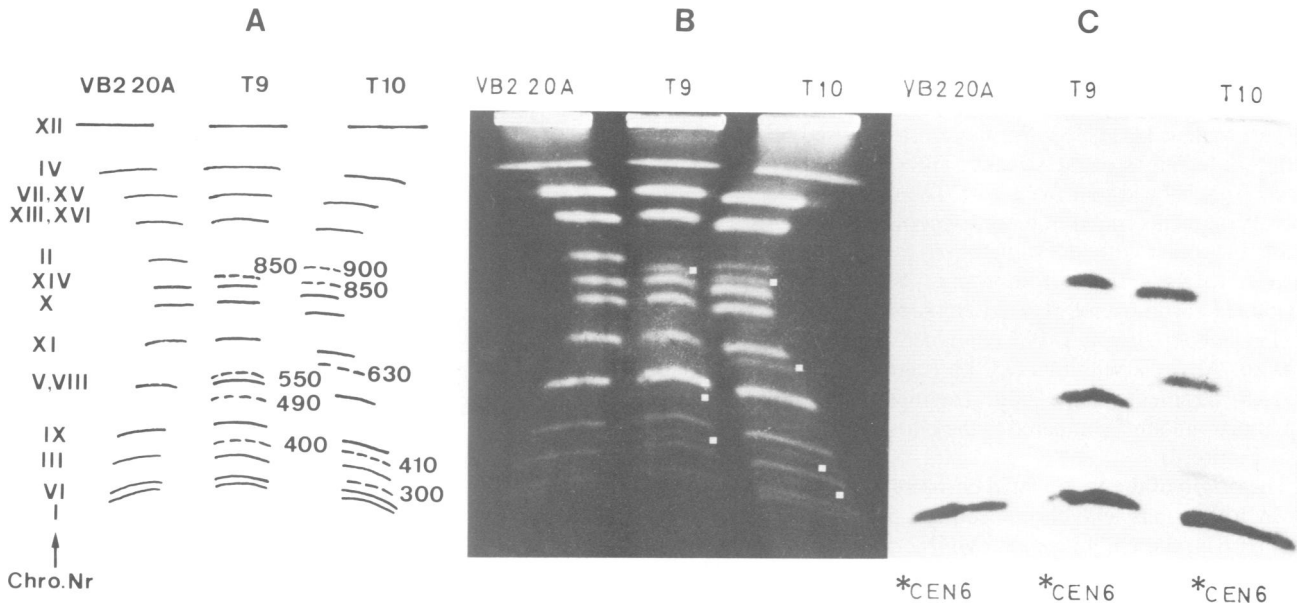

Fig. 2. Chromosomal banding pattern of VB2-20A and of transformant populations T9 and T10. (**A**) Schematic representation of the ethidium bromide banding pattern seen in (B). Lines represent the original karyotype of VB2-20A. Broken lines indicate the new chromosomal molecules found in the transformants T9 and T10. The chromosome assignment on the left is according to Carle and Olson (1985). The numbers at the fragment bands indicate their approximate sizes in kb as estimated from comparison with a ladder of λ DNA oligomeres. (**B**) Ethidium bromide stained chromosomal DNA pattern of VB2-20A and of the transformants T9 and T10. The white dots assign all chromosomal molecules that were generated from dicentric chromosome II. (**C**) Southern blot and hybridization with a radioactively labelled CEN6 probe (*CEN6) of the gel in (B). The lowest band hybridizing in all three lanes is chromosome VI. In T9 the two fragments CF850 and CF550 carry CEN6 and in T10 CF850, CF630 and only faintly visible CF410.

Table I. Results of hybridization with different chromosome II probes

Subpopulation[a]	Fragment[b]	Intensities[c]	DNA probes used for hybridization[d]							
			ILS1	MNN2	GAL1,10	PHO5	CEN6	LYS2	SUP45	Y'TEL
T9/1	CF850	0.9	+	−	+	+	+	+	+	−
	CF550	0.1	−	−	+	+	+	+	+	+
	CF400	0.1	+	0	−	0	−	0	0	+
T9/2	CF850	0.6	+	−	+	+	+	+	+	−
	CF550	0.3	−	−	+	+	+	+	+	+
	CF490	0.1	+	0	+	0	−	0	0	0
	CF400	0.3	+	0	−	−	−	−	0	+
T9/3	Chromoso-									
	me II	0.5	+	+	+	+	−	+	+	−
	CF850	0.5	+	−	+	+	+	+	+	−
T10/1	CF850	0.3	+	−	+	+	+	+	+	−
	CF630	0.7	−	−	+	+	+	+	+	+
	CF300	0.7	+	−	−	−	−	−	−	+
T10/2	Chromoso-									
	me II	0.4	+	+	+	+	−	+	+	−
	CF850	0.4	+	−	+	+	+	+	+	−
	CF630	0.4	−	−	+	+	+	+	+	+
	CF410	0.3	−	0	−	−	+	+	0	0
	CF300	0.4	+	−	−	−	−	−	−	+
T10/3	CF850	1.0	+	−	+	+	+	+	+	−
	CF600	1.0	+	+	+	−	−	−	−	−
T10/4	Chromoso-									
	me II	1.0	+	+	+	+	−	+	+	−
	CF850	1.0	+	−	+	+	+	+	+	−

[a]Subpopulations as described in Results.
[b]Numbers indicate size in kb.
[c]Relative intensities estimated from comparison in ethidium bromide stained gels and from autoradiograms of DNA−DNA hybridizations.
[d]Symbols: (+) hybridization signal, (−) no hybridization signal, (0) not determined.

By comparing the fluorescence intensities of the bands in Figure 2B it is immediately apparent that all new chromosomal DNAs are present in submolar amounts. This means that the cell populations assayed are heterogeneous with respect to their karyotype. We therefore analysed cells from different locations of the streaks. Three new cultures were made from T9 and four from T10. The sizes of the chromosomal fragments found in these subpopulations are listed in Table I together with an estimation of their molar ratio with respect to the other chromosomes. Subpopulation T9/3 contained chromosome II sized DNA previously not found in T9. Subpopulation T10/3 contained a new fragment of 600 kb. All cells with this 600 kb fragment also carry the 850 kb fragment, since both fragments are present in equimolar amounts compared to the other chromosomes (see also Figure 4).

The origin of the fragmented chromosomes was analysed by hybridizations with chromosome II probes from the left arm (ILS1), the CEN2 region (MNN2), the DNA between the two centromeres (GAL1,10 and PHO5), the site of CEN6 integration (LYS2) and the region right of the new centromere (SUP45). The probes were subcloned into M13mp8 to avoid cross-hybridization with the pBR322 sequences in the rearranged chromosome. The hybridization data, which are summarized in Table I allow the following conclusions to be drawn. The chromosome II sized DNA is chromosome II. The 850 kb chromosome originated from chromosome II by an ~50 kb deletion of the CEN2 region. The other chromosomal fragments originated from chromosome II by breakages between CEN2 and CEN6. Because of the size differences such breakages must have occurred at different locations.

Genetically stable descendants from cells with dicentric chromosome II

The heterogeneity found in most of the cell populations could be due to a mixture of genetically stable cells or could reflect continuous breakage and fusion processes. The hybridization analysis presented in Table I favours the first interpretation but the second interpretation cannot be completely excluded. Cultures from single cell isolates of the subpopulations T9/1 and T10/1 were therefore analysed. A typical result is shown in Figure 3A. Cells carrying either the 850 kb chromosome II derivative or pairs of chromosomal fragments with complementing parts of chromosome II can be cloned. From 12 single cell isolates of T9/1 nine carried the short chromosome II CF850 and three carried the pair CF550/CF400. For T10/1 similar distributions were found. These altered chromosomes are genetically stable, i.e. the sizes did not change during growth periods in complete and selective medium of up to 100 divisions (D.Jäger, unpublished data, see also below). Loss of 50 bp per cell division would have been detected after 100 generations. Figure 3B compiles those stable fragments or pairs of fragments which were observed in our analysis of single cell isolates.

Telomere healing at chromosome breaks

Genetic stability of fragmented chromosomes demands functional origins of replication, a centromere and, at both ends, telomeres. Since the disruption of the DNA between the two centromeres generates fragments with origins of replication, a centromere but only one telomere, one has to postulate that the broken ends acquired functional telomere

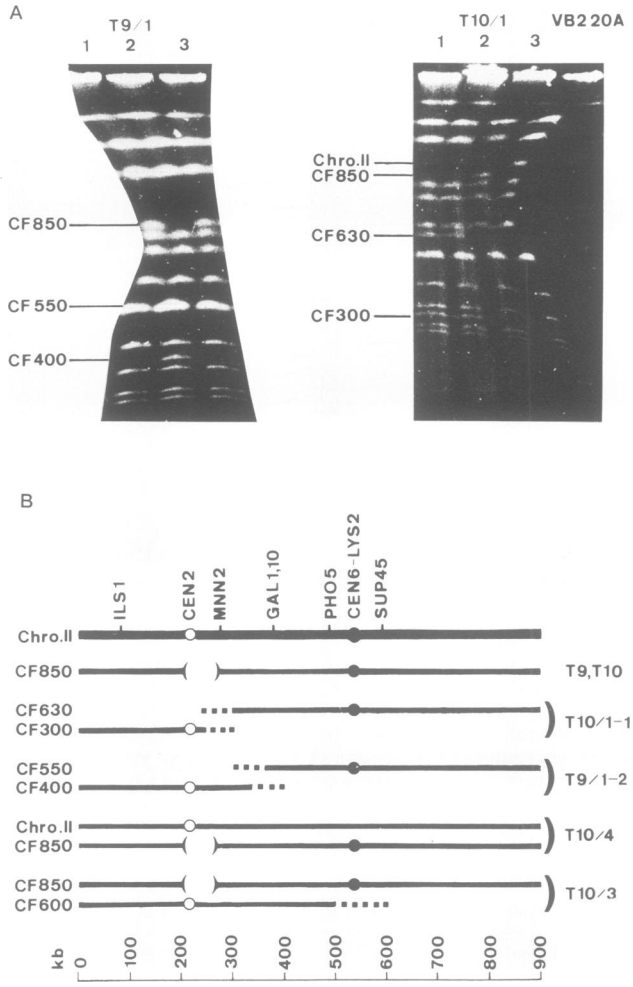

Fig. 3. Karyotypes of stable cell lines. (**A**) Chromosomal DNA separations of representative single cell isolates from populations T9/1 and T10/1. (**B**) Genetically stable chromosome II derivatives found in single cells. The location of the genetic loci are adjusted to physical measurements from this paper. Open circles represent CEN2, closed circles CEN6, brackets the CEN2 deletion and dots represent the apparently healed ends of broken chromosomes.

sequences. Telomeres in *S.cerevisiae* have heterogeneous sequence compositions (Chan and Tye, 1983; Button and Astell, 1986; Walmsley, 1987). The very terminal sequences of all chromosomes consist of specific CA repeats which are preceded in most chromosomes by one to four copies of a 6.7 kb so called Y′ sequence. The other chromosomes carry so called X sequences of variable length next to the CA repeats. X sequences are also found adjacent to Y′ sequences and both contain origins of replication. Since the chromosome II of VB2−20A is one of the few chromosomes in *S.cerevisiae* laboratory strains which does not carry telomere Y′ sequences (D.Jäger, unpublished data), telomere addition to broken ends of chromosome II can be fairly easily tested. Hybridizations with a telomere Y′ probe showed no signal with the 850 kb chromosome II derivative, in agreement with its origin by a deletion event. But the chromosome fragment pairs 550/400 kb and 630/300 kb hybridize with the Y′ probe (Table I). One of the hybridization experiments is presented in Figure 4, which shows an analysis of the parent strain and of three genetically stable cell lines from transformant T10. It is also apparent from lane T10/3 in

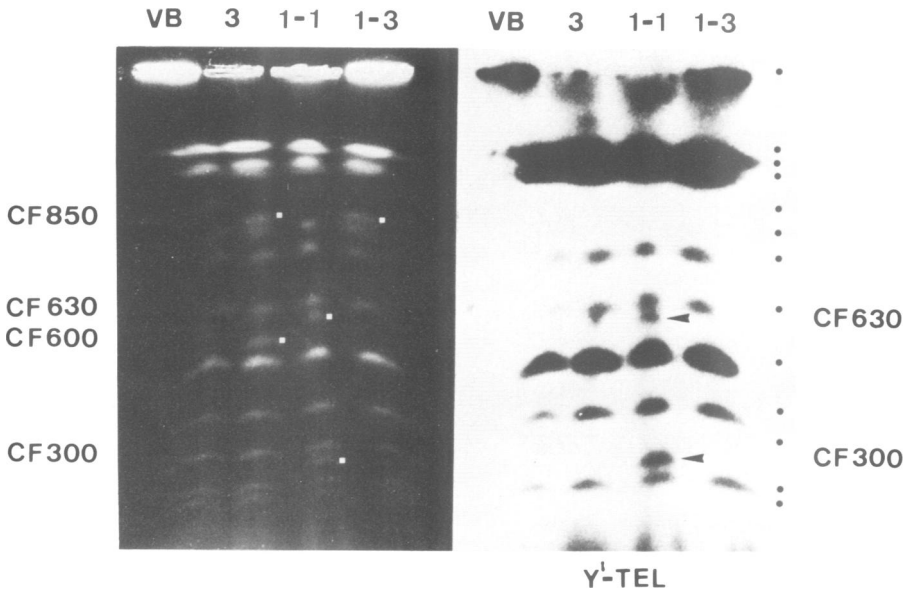

Fig. 4. Hybridization of a Y′ telomere probe to separated chromosomal DNA. The ethidium bromide stained gel (left) shows from the left to the right the karyotype of the parent strain, of the subpopulation T10/3 and of the single colony isolates T10/1-1 and T10/1-3 (indicated as 3, 1-1 and 1-3, respectively). The gel shown here was run for 15 h at 300 V and 50 s pulsing at 8°C. This leads to a better separation of the DNA molecules in the size range between 100 and 700 kb. The chromosome II rearranged molecules are marked by white squares. Chromosomes with Y′ telomeres are detected in the autoradiogram (right) after hybridization with the Y′ probe. The arrows point to the hybridization of the 630/300 kb fragment pair. The dots mark the position of the chromosomal DNAs from the parental strain. The black squares refer to the position of the rearranged chromosome II molecules. The hybridization clearly shows that not all chromosomal DNA bands show a signal.

Figure 4 that the genetically stable 600 kb chromosome fragment has no Y′ sequences.

Growth characteristics of cells with divided chromosome II

Subpopulations T9/1 and T10/1 consist of cells containing the chromosome II information either in a shortened 850 kb chromosome II or in two chromosomal fragments. It is an open question whether cells with divided chromosome II suffer any growth disadvantages or not. In order to answer this question subpopulations T9/1 and T10/1 were continuously grown and were analysed by DNA hybridization after 10, 50 and 100 generations for the presence or absence of fragmented chromosomes. This should allow the detection of slight growth differences among the cell types. Figure 5 shows the result after 10 and 50 generations grown in selective and complete medium. The result after 100 generations is indistinguishable from the result after 50 generations and is therefore not shown in the figure. At 0 generations the relative amount of cell types in T9/1 population was 90% for the 850 kb and 10% for the 550/400 kb fragment pair carrying cells and in the T10/1 population 30% for the 850 kb and 70% for the 630/300 kb fragment pair carrying cells (data not shown). Under growth conditions which select for the presence of the CEN6 linked URA3 gene, none of the cell types has a growth advantage. Even after 50 generations the ratio between the 850 kb and the CEN6 carrying fragment of 550 and 630 kb, respectively has not significantly changed, as evident from the hybridizations in Figure 5A. In complete medium cells with the 850 kb chromosome II derivative have growth advantages over those cells with divided chromosome II. Already after 10 generations only a trace of the 630 kb CEN6 carrying fragment can be detected by hybridization in the T10/1 population. No signal is seen for the 550 kb CEN6 carrying fragment in the T9/1 population. After 50 generations only cells with the 850 kb chromosome can be detected (Figure 5B).

Discussion

Dicentric chromosomes are sometimes formed spontaneously by recombination processes but quite frequently after X-irradiation or similarly DNA damaging agents. Dicentric chromosomes cause in most cases unwanted genetic instability. In mammals a mechanism of unknown nature is operating which keeps one of the centromeres in apparently dicentric chromosomes functionally silent (Earnshaw and Migeon, 1985). Such a mechanism has not been found in other organisms and chromosomal rearrangements are the only way to generate stable monocentric derivatives. In this paper we could demonstrate with direct methods that stabilization of dicentric chromosomes in *S. cerevisiae* occurs by deletion of one centromere or by breakage plus healing at the newly generated ends.

Chromosome breaks in haploids are generally considered to be lethal events (Weiffenbach and Haber, 1981). Most studies with dicentric chromosomes have therefore been performed in diploid or polyploid cells. In such genetic backgrounds the unbroken homologue plays a dominant role in healing events. We induced in this and in a previous paper (Panzeri *et al.*, 1985) genetic instability in a haploid *S. cerevisiae* by integrative transformation of a second centromere in the right arm of chromosome II. It is not possible to describe precisely what happened on the DNA level during the first divisions. However, the analysis of new arrangements of chromosome II information in genetically stable descendants of two transformants together with common knowledge on recombination and repair in

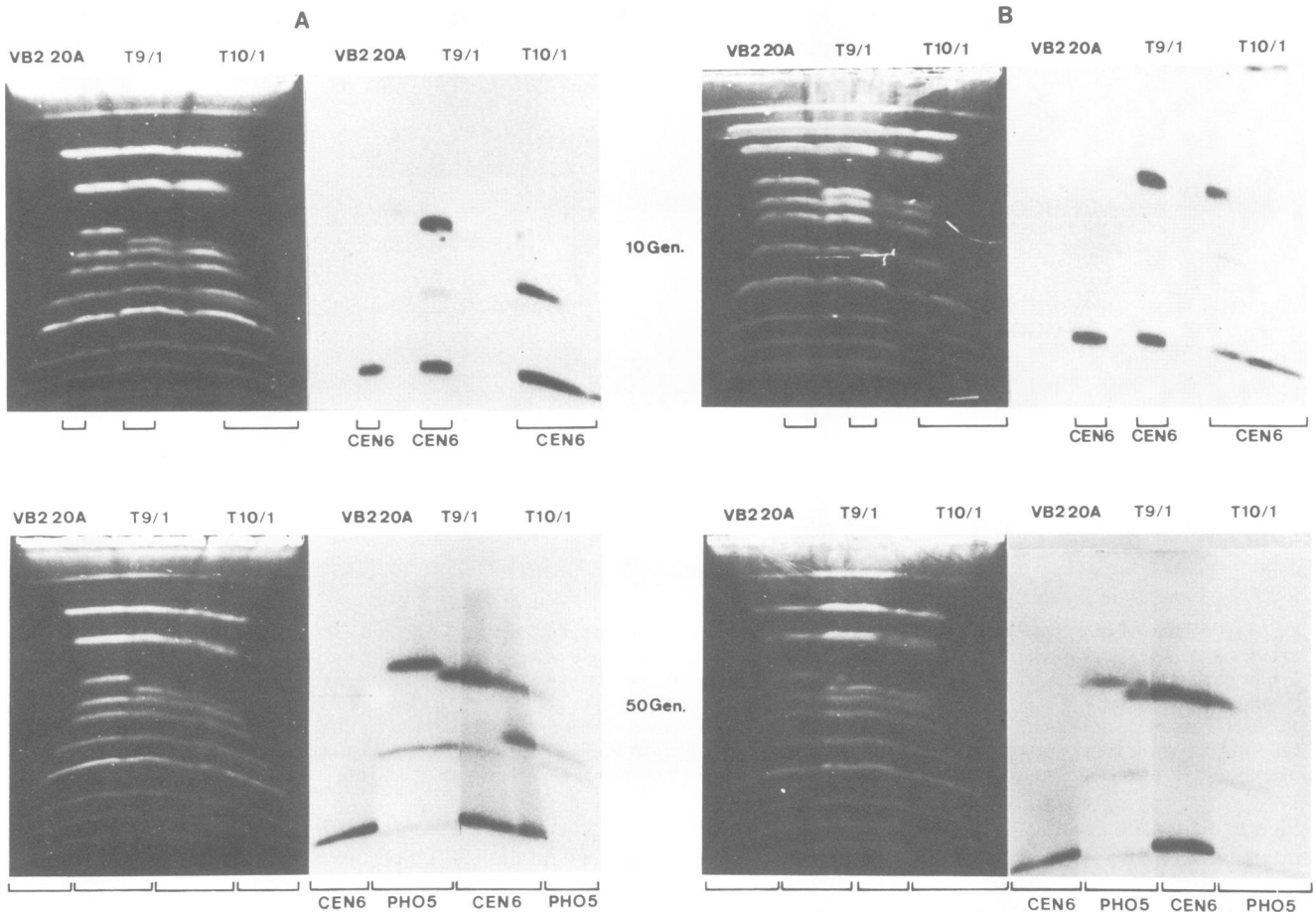

Fig. 5. Outgrowth of VB2-20A and subpopulations T9 and T10. Ethidium bromide stained gels from chromosomal DNA separations are shown together with the autoradiogram from hybridizations with CEN6 DNA (10 generations) or CEN6 DNA and PHO5 DNA (50 generations). Filters with blotted DNA were cut at the indicated positions to allow hybridizations with several probes. (**A**) Outgrowth for 10 and 50 generations in selective medium. The bands hybridizing with the CEN6 probe are from top to bottom: lanes VB2-20A: chromosome VI. Lanes T9/1: CF850, CF550 (co-migrating with chromosomes V and VIII) and chromosome VI. Lanes T10/1: CF850 (only faintly visible due to the cutting of the filter), CF630 and chromosome VI. (**B**) Outgrowth in rich medium (YPD) for 10 and 50 generations, respectively. The bands seen are: lanes VB2-20A: chromosome VI. Lanes T9/1: CF850 and chromosome VI. Lanes T10/1: (10 Gen.) CF850, CF630 (faint band visible only at 10 generations) and chromosome VI. The hybridizations with the chromosome II PHO5 probe at 50 generations were done as control for intensity correlations. The weak signals seen on the double band of chromosomes V, VIII and on chromosome VI are due to homology of the PHO5 promoter to other PHO genes.

S.cerevisiae allows us to reconstruct the most likely events. Due to the observed complexity (Table I), the occurrence of stabilizing rearrangements either during or directly after the first division of the initial transformants can be excluded.

Theoretically four possible outcomes have to be discussed for the first mitosis. These are outlined in Figure 6 and serve as a reference for the following part. Dependent on the timing of CEN6 integration, before or after (during) replication of the LYS2 locus, two dicentric chromosomes or one normal and one dicentric chromosome will be generated in the initial transformants (Figure 6A and B, respectively). A spindle *trans* attachment in case A causes problems. Mitosis can only proceed if the chromatids of both dicentric chromosomes break as indicated at the thin bars. The chromatids will most likely be disrupted at non-identical positions. Consequently one daughter cell has lost genetic information because it received the 'shorter' halves of the disrupted chromatids (cell A1) and the other daughter cell has duplicated information because it received the 'longer' halves (cell A2). A1 type cells will stop dividing if the deletion includes an essential gene. The two halves in A2 type cells will repair to a dicentric chromosome by recombination of the highly reactive ends with homologous sequences on the other chromosome fragment (Orr-Weaver *et al.*, 1981; Rudin and Haber, 1988). A spindle *cis* attachment in case A does not cause any problems and two cells (A3 and A4) each carrying a dicentric chromosome will form.

A spindle *trans* attachment in case B can be solved in mitosis by one break. One daughter cell (B2) receives the normal chromosome II and the right half of the disrupted chromosome together with the CEN6 linked URA3 gene. The other daughter cell (B1) only gets the left half of the disrupted chromosome. In B2 type cells repair processes will regenerate a dicentric chromosome II by recombination between the broken end and its homologous sequences on chromosome II. A spindle *cis* attachment in case B yields one cell (B3) with a dicentric chromosome and one cell (B4) with a normal chromosome II, which does not carry the essential URA3 gene. The final outcome of the four possible mitoses of Figure 6 are therefore either cells with dicentric chromosome II or cells which are non-viable due to the lack of genetic information.

The very likely scenario during the following cell divisions

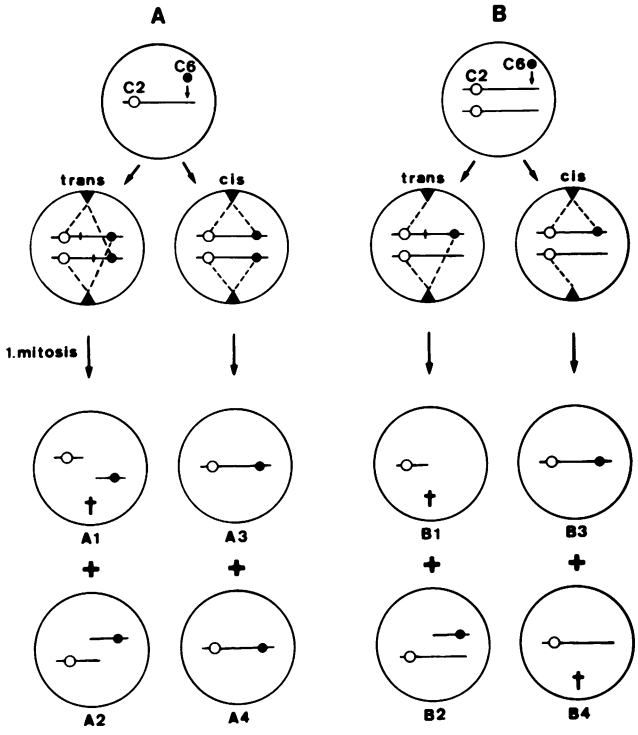

Fig. 6. Chromosome transmission pathways in the initial transformants. (**A**) Integration of CEN6 into chromosome II prior to DNA replication. (**B**) Integration of CEN6 into chromosome II after DNA replication. The positions of CEN2 (open circle) and CEN6-URA3 (closed circle) are not drawn to scale. *Cis* and *trans* refer to the two possible spindle fibre attachments during the first mitosis. Progeny cells lacking essential information are marked with a cross. For details see text.

is: unproblematic segregation with spindle *cis* attachments, and with equal likelihood breakages of the chromosomes due to spindle *trans* attachment followed by regeneration of the dicentric chromosome. It is very likely that a number of cell divisions with breakage and fusion events can proceed in *S.cerevisiae* before growth ceases or before resolving rearrangements occur. Survival may also depend on an initially high number of successive *cis* attachments in order to sufficiently increase cell numbers before one of the following *trans* attachments induces resolving rearrangements. About 3% of the initial transformants will encounter five successive *cis* attachments generating 32 cells with a dicentric chromosome II. This number should guarantee further successive *cis* attachments which increase the chances for stabilizing rearrangements.

Cells with the shortened 850 kb chromosome II can arise from breakage right of CEN2 and repair of the break by recombination with homologous sequences on the left side. Alternatively CEN2 may be lost by recombination excision which would also generate a circular DNA carrying CEN2. This is reminiscent of the centromere deletions observed by Surosky and Tye (1985) but in our gels we never found an indication of circular chromosome fragments. The presence of an intact chromosome II in addition to the 850 kb fragment in some of these cells can be explained in two ways dependent on whether both are present in equimolar amounts (T10/4) or half-molar amounts (T9/3). Non-disjunction of a dicentric chromosome followed later by CEN2 loss in one of the two chromosomes and excision of the CEN6–URA3 DNA in the other chromosome via the repeated LYS2 sequences (see Figure 1) could generate T10/4 cells. Fusion of a transformed cell with an untransformed cell followed later by a CEN2 deletion in the dicentric chromosome could generate T9/3 cells.

The other observed mechanism, telomere healing of broken ends, may involve short repeats present at the break and on one of the 22 chromosome ends with Y′ telomere sequences thus allowing recombination. It should be noted that such a reaction has to compete with the repair of the ends to dicentric chromosomes. As long as the 'long' halves in A2 type cells (Figure 6) show substantial overlaps repair with the homologous chromosome II sequences in the other half will occur. If the overlap is short or nonexisting, homology to ends of other chromosomes can lead to the healing. The missing MNN2 sequences in both fragments of the cell line T10/1 (Figure 4 and Table I) support this view.

Recently Matsumoto *et al.* (1987) reported telomere healing by *de novo* synthesis at ends caused by X-irradiation in *Schizosaccharomyces pombe*. We found so far no evidence for such a mechanism in healing broken ends in *S.cerevisiae* but cannot exclude that the 600 kb fragment in transformant T10/3 was stabilized by such an event. Independent of whether healing occurs by recombination or by *de novo* synthesis the obvious frequency of this process in stabilizing broken ends is surprisingly high. This reaction must be considered in the construction of very long DNA clones with the YAC system (Burke *et al.*, 1987). Fusion of functional centromeres to both ends of DNA fragments cannot be avoided. The breakage after transformation may be unwanted but the stabilization by telomere healing can be viewed as helpful in this very promising genome mapping approach.

Materials and methods

Strains and media

The *S.cerevisiae* strain VB2-20A (MATα ura3-52 trp1 leu2-3 2-112 ade2) was obtained from L.Panzeri. Integrative transformation experiments were performed with plasmids pAS22 and pAS22Δ (Panzeri *et al.*, 1985). Both plasmids were grown in *Escherichia coli* K12 strain 294 (thi endo1 hsdR hsdM$^+$ srlR-recA 36). *E.coli* K12 strain JM101 (lac pro supE thi F′traD36 proAB lacI^q Z M15) was used for all the subcloning constructs with phage M13mp8. Yeast cells were grown at 30°C in YPD (1% yeast extract, 2% peptone, 2% dextrose) or in SD minimal medium (0.67% yeast nitrogen base without amino acids, 2% dextrose, and the required supplements adenine, leucine and tryptophan, and if necessary uracil with a final concentration of 30 μg/ml). *E.coli* strain 294 was grown in LB medium (0.5% yeast extract, 1% tryptone, 1% NaCl) and *E.coli* strain JM101 in YT medium (1% yeast extract, 1.4% tryptone, 0.5% NaCl). When required ampicillin was added to a final concentration of 50 μg/ml. For plates, 1.3% agar (Gibco) was added.

Transformation

E.coli transformation was performed according to Mandel and Higa (1970). Yeast transformation was as described by Hinnen *et al.* (1978) and Beggs (1978) for the spheroplast procedure.

Nucleic acids and enzymes

Plasmid and yeast DNA were isolated according to established procedures described by Davis *et al.* (1980). M13mp8 was handled and used essentially as described by Sanger *et al.* (1977). Restriction endonucleases were purchased from Biolabs or Pharmacia. Proteinase K was obtained from Merck and Zymolyase 20′000 from Kirin Breweries or Miles Scientific.

Chromosomal DNA separation

Preparation of DNA was done as described by Carle and Olson (1985). The molten agar containing the chromosomal DNA was loaded into the preformed slots (mostly 0.2 × 2 × 0.3 cm) of 10.5 × 10.5 cm 1% agarose gels (0.4 cm thick). Electrophoresis was performed at 300 V for 22 h with 50 s switch interval with a buffer temperature of 8°C in the apparatus described by Carle and Olson (1984). The buffer concentration for the gel

runs was 0.4 × TB (1 × TB = 0.089 M Tris, 0.089 M boric acid) plus EDTA, pH 8, at a final concentration of 1 mM. After the run the gel was stained in an ethidium bromide bath (1 mg/l), destained for 1 h in deionized water and photographed under UV at 300 nm. Gel runs with different conditions are mentioned in the figure legends.

DNA transfer and hybridization

The DNA containing gels were treated for 30 min with 0.25 M HCl at room temperature to allow partial depurination to generate sufficient short fragments for efficient transfer. After alkali denaturation (20 min) and neutralization (20 min) in high salt buffer (1 M ammonium acetate, 0.02 M sodium hydroxide) the DNA was transferred to nitrocellulose filters and processed according to Southern (1975). DNA, labelled with ^{32}P by primer extension (Sanger *et al.*, 1977), was hybridized to the filters at 65°C in 6 × SSPE (1 M NaCl, 0.06 M NaH_2PO_4, 0.006 M EDTA, 0.06 M NaOH) for 12–16 h. The washing of the filters was performed at 55°C in 2 × SSPE. The washed filters were placed under X-ray films (Kodak XAR-5 or Fuji with Kyokko intensifying screens) for 1–10 days.

Hybridization probes

ILS1: 2.3 kb *Bam*HI–*Pst*I fragment in M13mp8 (donated from P.Nellböck) from the original 5.1 kb *Bam*HI fragment carrying the isoleucyl-tRNA synthetase gene (Meusdoerffer and Fink, 1983). MNN2: 1.35 kb *Bam*HI–*Sal*I fragment from cosmid clone 308 (donated from P.Nellböck) subcloned into pUC13. This subclone was cleaved with *Eco*RI plus *Pst*I and integrated into M13mp8. GAL1,10: 0.685 kb *Eco*RI–*Bam*HI promoter fragment from plasmid pBM293 (Johnston and Davis, 1984) subcloned into M13mp8. PHO5: 0.63 kb *Bam*HI–*Sal*I promoter fragment in M13mp8, obtained as clone 8M4 from H.Rudolph. LYS2: 0.65 kb *Eco*RI–*Bgl*II promoter fragment obtained from U.Fleig subcloned into M13mp8. CEN6: 2.3 kb *Eco*RI–*Bam*HI clone in M13mp8, constructed by J.H.Hegemann. SUP45: 1.3 kb *Bam*HI–*Bgl*II fragment isolated from plasmid p3030 (obtained from W.Pipersberg) subcloned into M13mp8. Y′TEL: 1.37 kb *Pvu*I–*Kpn*I probe isolated from plasmid pCE-T27 (donated by W.Oertel) subcloned into M13mp8. The Y′ telomere probe was originally isolated on the plasmid pSZ219-5 (Szostak and Blackburn, 1982) as *Pvu*I–*Sca*I clone.

Outgrowth of yeast transformant populations

Appropriate dilutions of exponentially growing cultures from SD minimal medium (0 generations) were incubated in prewarmed medium and grown for 10 generations at 30°C. The different generation times for growth in minimal and full medium were taken into account. After 10 generations, when the cultures were still in their logarithmic growth phase, further dilutions were made until 50 and 100 generations were reached.

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