Ribosomal DNA Is a Site of Chromosome Breakage in Aneuploid Strains of Neurospora

David K. Butler[1]

Department of Physiological Chemistry, University of Wisconsin, Madison, Wisconsin 53706

Manuscript received January 10, 1992
Accepted for publication March 21, 1992

ABSTRACT

In wild-type strains of *Neurospora crassa*, the rDNA is located at a single site in the genome called the nucleolus organizer region (NOR), which forms a terminal segment on linkage group (LG) V. In the quasiterminal translocation strain T(I;V)AR190, most of the right arm of LG I is moved to the distal tip of the NOR, and one or a few rDNA repeat units are moved to the truncated right arm of LG I. I report here that, in partial diploid strains derived from T(I;V)AR190, large terminal deletions result from chromosome breakage in the NOR. In most of these partial diploids, chromosome breakage is apparently frequent and the breakpoints occur in many parts of the NOR. The rDNA ends resulting from chromosome breakage are "healed" by the addition of new telomeres. Significantly, the presence of ectopic rDNA creates a new site of chromosome breakage in the genome of partial diploids. These results raise the possibility that, under certain conditions, rDNA is a region of fragility in eukaryotic chromosomes.

SPECIFIC regions of eukaryotic chromosomes are apparently prone to breakage. For example, the micronuclear chromosomes of the ciliate *Tetrahymena thermophila* contain hundreds of sites, defined by a 15-base pair (bp) sequence, that are subject to breakage during macronuclear development (YAO, ZHENG and YAO 1987; YAO, YAO and MONKS 1990). Of great significance to human health are the heritable "fragile sites" of human chromosomes. Breakage at some of these sites may be the initiating event in a range of nonrandom chromosomal abnormalities–including reciprocal translocations, inversions, interstitial deletions and terminal deletions–repeatedly observed in tumor cells of lymphohematopoietic origin (YUNIS 1983; HECHT and SUTHERLAND 1984; LEBEAU and ROWLEY 1984). Very little is known about the nature of fragile sites or about the *in vivo* conditions under which fragile sites may break.

Under certain genetic conditions, some chromosomes of *Neurospora crassa* can undergo large terminal deletions. Normally, the genome of Neurospora is haploid. However, it is possible to construct nontandem duplications involving chromosome arms, and these are called partial diploids. Partial diploids are found among the progeny of crosses of quasiterminal translocation strains to normal sequence strains (see PERKINS and BARRY 1977; see Figure 1 for an example). Some kinds of partial diploids are unstable during vegetative growth, that is, they rapidly become heterokaryons consisting of haploid nuclei and unaltered partial diploid nuclei. Cytological evidence is consistent with a model in which one of the duplicated chromosomal segments is deleted from the partial diploid genome to form a haploid genome (NEWMEYER and GALEAZZI 1977; PERKINS and BARRY 1977). In most kinds of unstable partial diploids, the translocated (or ectopic) segment is preferentially deleted so as to restore a normal, or wild-type, sequence (NEWMEYER and GALEAZZI 1977; see Figure 1 for an example). Only rarely, if ever, is the normal segment deleted. In the unstable partial diploid formed by one atypical translocation, however, the translocated segment and the normal segment are deleted equally frequently (TURNER 1977).

The mechanism by which such large chromosomal regions are deleted is unknown. NEWMEYER and GALEAZZI (1977) have suggested that the chromosome tip (either subtelomeric or telomeric DNA) and the original translocation breakpoint have common sequences. For terminal duplications, *intra*chromosomal homologous recombination between direct repeats at the chromosome tip and at the translocation breakpoint would cause precise deletion of the translocated segment. There probably are no sequences repeated at every one of the subtelomeric sites in the Neurospora genome (SCHECHTMAN 1987). Neither is the Neurospora telomeric repeat, TTAGGG, present in long stretches at nontelomeric sites (SCHECHTMAN 1990) as would be required by the NEWMEYER and GALEAZZI model. Thus it is possible that a more complicated mechanism of terminal deletion operates in some types of unstable partial diploids, perhaps one

[1] Present address: Division of Basic Sciences, M385, Fred Hutchinson Cancer Research Center, 1124 Columbia Street, Seattle, Washington 98104.

involving chromosome breakage at preferred sites and the eventual healing of the broken end.

In normal sequence strains of Neurospora (*e.g.*, wild-type strains), the nucleolus organizer region (NOR) is a terminal segment on linkage group (LG) V (PERKINS and BARRY 1977). The rDNA at the NOR is arranged as a series of tandem repeat units; each repeat unit is 9.3 kbp (RUSSELL *et al.* 1984). In the reciprocal translocation strain T(I;V)AR190, most of the right arm of LG I is moved to the distal tip of the NOR, and one or a few rDNA repeat units are moved to the truncated right arm of LG I (*i.e.*, the translocation breakpoint is within the NOR). Thus, in T(I;V)AR190, the NOR lies deep within a compound chromosome (see Figure 1 for a diagram of the relevant chromosomes). Among the progeny of a cross between T(I;V)AR190 and normal sequence are partial diploids for the right arm of LG I (see Figure 1). Partial diploids derived from T(I;V)AR190 are very unstable (see PERKINS and BARRY 1977). During vegetative growth, the partial diploids rapidly become heterokaryons, consisting of unaltered partial diploid nuclei *and* haploid normal sequence nuclei (see Figure 1). The haploid normal sequence nuclei apparently arise by deletion of the translocated segment. Despite continuing selection for an allele on the translocated segment (*i.e.*, selection for partial diploidy), more than 90% of the nuclei are typically haploid normal sequence (it is important to keep in mind that, even under selection, a "partial diploid" culture is a mixed population of haploid nuclei and partial diploid nuclei in which the partial diploid nuclei are roughly analogous to stem cells). I report here that, in partial diploid strains derived from T(I;V)AR190, loss of the translocated segment results from chromosome breakage in the NOR. In most partial diploids, chromosome breakage is frequent, so that few partial diploid nuclei retain the translocated segment. The regions of the NOR in which breakage may occur are widely distributed. The breakage sites are also heterogeneous with respect to their position in the rDNA repeat unit. The rDNA ends resulting from chromosome breakage are "healed" by the addition of canonical telomeric repeats. Significantly, an ectopically located block of rDNA is also a site of chromosome breakage under conditions of partial diploidy.

MATERIALS AND METHODS

Strains, genetic methods, plasmids and enzymes: The origin and structure of the translocation strain T(I;V)AR190 are described in (PERKINS and BARRY 1977). T(I;V)AR190 was obtained from the Fungal Genetic Stock Center (FGSC #1952). (I have changed the notation from that used elsewhere to reflect the fact that the translocation is recipocal; see below.) The origin and structure of the translocation strain T(I;V)OY321 are described in (PERKINS, RAJU and BARRY 1984). The *mep*^{r} derivatives of T(I;V)OY321 were constructed by ROBERT L. METZENBERG. The origin and structure of the QNS strains are described in (PERKINS *et al.*, 1986). I constructed the *leu-3* derivative of QNS. DB203 is a *nic-2* derivative of NS-1 (BUTLER and METZENBERG 1989). All crosses were made on synthetic crossing medium (WESTERGAARD and MITCHELL 1947) at 25°. The rDNA plasmids and molecular weight standards used in this study were described in BUTLER and METZENBERG (1989). The oligonucleotides TEL1, TEL2, rD5 and rD17 were synthesized at the University of Wisconsin Biotechnology Center. All of the enzymes used in this study were purchased from the Promega Corporation.

Oligonucleotide labeling and hybridizations: Oligonucleotide labeling with [γ-^{32}P]ATP and T4 polynucleotide kinase was carried out by standard procedures. The Southern blots were prepared as before BUTLER and METZENBERG (1989), except that the gels were exposed to UV light for 2 min, instead of 0.25 M HCl, prior to transfer. The membranes were incubated in hybridization solution (5 × SSC, 5 × Denhardt's soluction, 0.1% sodium dodecyl sulfate (SDS), 0.1% Na-pyrophosphate) without oligonucleotide for 2 to 4 hr at 65°. The radiolabeled oligonucleotide was added to the solution and hybridization was allowed to proceed for 3 hr at 65°. Following hybridization, the membranes were washed twice at room temperature for 10 min and once at 65° for 10 min in 4 × SSC, 0.1% SDS.

DNA preparations, restriction digestions and Bal-31 digestions: Preparation of Neurospora low molecular weight genomic DNA, intact Neurospora chromosomes and intact *Saccharomyces cerevisiae* chromosomes were as described in BUTLER and METZENBERG (1989). Restriction digestions of intact chromosomes were carried out as described BUTLER and METZENBERG (1989).

The preparation and restriction digestion of norDNA (nucleolus organizer region DNA) were as follows. Intact chromosomal DNA was digested with *Bcl*I and fractionated by contour-clamped homogeneous electric field (CHEF) gel electrophoresis through a 1% low melting temperature agarose gel (SeaPlaque from FMC BioProducts). The gel was stained with ethidium bromide for 10 min and destained in distilled water for about 45 min in the dark. The DNA was examined under UV light and the appropriate bands were excised with a razor blade. The gel slices were further destained in 4 ml of TE (10 mM Tris, 1 mM EDTA, pH 8.0) at 4° for 24–36 hr. Prior to restriction digestion, the gel slices were incubated in the appropriate 1 × restriction buffer for 1 hr at 0°. The buffer was removed and 150 μl of buffer containing 60 units of restriction enzyme was added. Digestion was allowed to proceed for approximately 6 hr, after which the digested norDNA was purified with GENECLEAN (Bio 101) and loaded onto a one-dimensional gel for electrophoresis.

For Bal-31 digestions, each sample (approximately 15 μg of total genomic DNA) was incubated with 11 units of Bal-31 in 100 μl total volume at 30°. At various times, a 33-μl aliquot was removed to 67 μl water and immediately extracted once with phenol-chloroform and once with chloroform. Following this, the DNA was ethanol-precipitated and digested with the restriction enzyme *Bam*HI.

CHEF gel electrophoresis: The apparatus used was that described by CHU, VOLLRATH and DAVIS (1986). Pulsed-field CHEF gel electrophoresis was performed for 24 hr in 0.5 × TBE at 10–11°, with 1% agarose gels cast in 0.5 × TBE (CHU, VOLLRATH and DAVIS 1986) unless otherwise stated in the figure legend. The pulse times and voltages are given in the appropriate figure legends. These were varied from experiment to experiment to optimize resolution of pertinent fragments (VOLLRATH and DAVIS 1987).

Polymerase chain reaction and sequencing: The rDNA

termini were amplified from approximately 10 ng of total genomic DNA by the polymerase chain reaction (PCR) using either of two rDNA primers (rD5 or rD17) and a telomere primer (TEL2). The sequence of rD5 is 5′-AATGACGCTCGAACAGGC-3′. The sequence of rD17 is 5′-GCGGTACCCAATTGAATACTGATGCC-3′. This primer includes a *Kpn*I site (underlined) beginning 2 bases from the 5′ end. The sequence of TEL2 is 5′-TATGATCACCCTAACCCTAACCCTAACCC-3′. This primer includes a *Bcl*I site (underlined) beginning 2 bases from the 5′ end. Amplification was carried out in a Perkin Elmer Cetus DNA Thermal Cycler. The cycling parameters were as follows: one cycle of denaturation at 94° for 5 min, annealing at 60° for 1 min, extension at 72° for 3 min; 28 cycles of denaturation at 94° for 1 min, annealing at 60° for 1 min, extension at 72° for 3 min; one cycle of denaturation at 94° for 1 min, annealing at 60° for 1 min, extension at 72° for 10 min. For cloning, the PCR products were digested with *Bcl*I and either *Eco*RI (rD5 amplifies rDNA that includes an *Eco*RI site) or *Kpn*I and the fragments were ligated to either *Bam*HI-*Eco*RI or *Bam*HI-*Kpn*I cut pGEM3Zf(+) vectors (Promega Corporation). The plasmids were purified by CsCl-ethidium bromide gradient centrafugation and the inserts were sequenced from the double-stranded templates with Sequenase (United States Biochemical).

RESULTS

Analysis of NOR size in partial diploid and haploid translocation sequence progeny of T(I;V)AR190: Previously, it had been shown that the size of the NOR, that is, the number of rDNA repeat units, changes frequently during the sexual phase of the life cycle (BUTLER and METZENBERG 1989, 1990). The progeny of a typical cross inherit only one NOR, and the size of the NOR is generally stable during vegetative growth. During routine strain construction, I noticed that some individual progeny of a cross between T(I;V)AR190 and normal sequence were very unusual, in that they harbored many different sizes of the NOR. The unusual progeny had invariably inherited their NOR from the T(I;V)AR190 parent. As shown in Figure 1, there are two classes of progeny that inherit their NOR from T(I;V)AR190. One class, the partial diploid progeny, have received the LG V;I from T(I;V)AR190 and LG I from the normal sequence parent. The second class, the haploid translocation sequence progeny, have received both LG I;V and LG V;I from the T(I;V)AR190 parent.

I hypothesized that the NOR size heterogeneity within single progeny of T(I;V)AR190 might be related to the unusual behavior of partial diploids (see Introduction). To test this hypothesis, I crossed T(I;V)AR190 to normal sequence, recovered progeny that had inherited their NOR from T(I;V)AR190 (*i.e.*, partial diploids and translocation sequence haploids) and examined whether the NOR size heterogeneity was confined to partial diploid progeny.

Two markers are important in the cross: *nic-2* and a *Hin*dIII restriction fragment length polymorphism

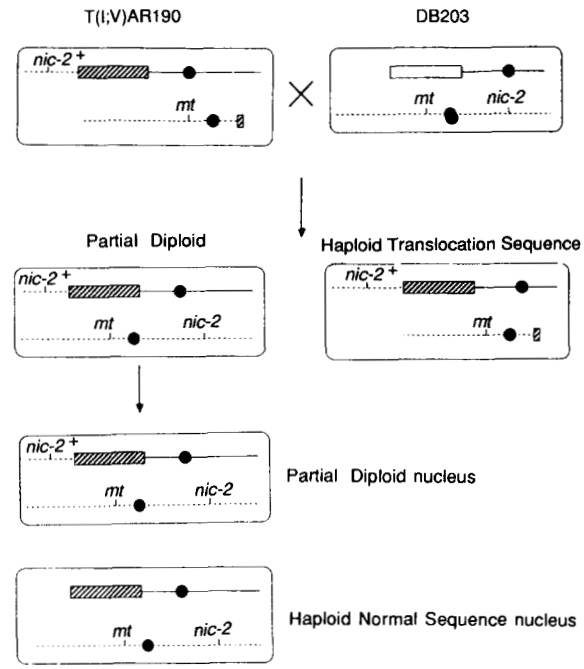

FIGURE 1.—Schematic representation depicting a cross between T(I;V)AR190 and DB203 and the two classes of type II rDNA progeny. The parental chromosomes are shown at the top of the figure. For T(I;V)AR190, LG V;I and LG I;V are shown. LG V;I has most of the right arm of LG I translocated to the distal tip of the NOR. LG I;V has the tip of the NOR, including the telomere, translocated to the truncated right arm of LG I. For DB203, normal sequence LG V and normal sequence LG I are shown. The "translocated segment" is the LG I sequence distal to the NOR in T(I;V)AR190. The "normal segment" is the homologous region of LG I in DB203. The two classes of progeny that inherit the NOR from T(I;V)AR190 are the partial diploid and the haploid translocation sequence. All "partial diploid strains" are actually heterokaryons consisting of two types of nuclei. Below the second arrow are the two types of nuclei in the heterokaryon arising from the partial diploid. The haploid normal sequence nuclear type is derived from LG V;I by loss of the translocated segment. The haploid nucleus is effectively a nicotinic acid auxotroph, because the *nic-2* gene on the intact LG I is uncovered by loss of the translocated segment. The chromosomes are not drawn to scale. Symbols: The solid lines represent LG V sequences; the dotted lines represent LG I sequences; the hatched rectangular box in T(I;V)AR190 and progeny represents a NOR composed of type II rDNA repeat units; the open rectangular box of DB203 represents a NOR composed of type I rDNA repeat units; the filled circles represent centromeres. The distances between markers are not drawn to scale. Markers: *nic-2*$^+$, nicotinic acid prototrophy; *nic-2*, nicotinic acid auxotrophy; *mt*, mating type.

(RFLP) in the rDNA. The normal sequence parent, called DB203, is a nicotinic acid auxotroph. This is due to a recessive allele, *nic-2*, located on the right arm of the normal sequence LG I. The T(I;V)AR190 parent is a prototroph. The translocated segment of LG I carries the wild-type (and dominant) allele of *nic-2*$^+$, approximately 15 cM distal to the NOR. DB203 and T(I;V)AR190 can also be distinguished on the basis of a *Hin*dIII RFLP in the rDNA. DB203 has only type I rDNA repeat units at the NOR and T(I;V)AR190 has only type II rDNA repeat units at

the NOR (data not shown). Type I rDNA repeat units have one *Hin*dIII site in the nontranscribed spacer region, while type II rDNA repeat units have two *Hin*dIII sites in the nontranscribed spacer region. Even though the number of rDNA repeat units in the NOR changes during a cross, the *type* of rDNA repeat units in the NOR does not (BUTLER and METZENBERG 1989). Thus, the *Hin*dIII RFLP can be used to identify those progeny that inherit their NOR from T(I;V)AR190.

The cross is set up so that most of the prototrophic progeny will have inherited their NOR from T(I;V)AR190 (see Figure 1). Only a relatively rare meiotic crossover, involving the right arm LG I between *nic-2*$^+$ and the NOR will lead to a prototrophic segregant that inherited its NOR from DB203. I selected about 20 prototrophic progeny from this cross and scored them for rDNA type. As expected, all of the recovered progeny were type II for their rDNA, indicating that they had inherited their NOR from T(I;V)AR190 (data not shown). To distinguish between the partial diploid and the haploid translocation sequence progeny, I took advantage of the fact that approximately 10% of the conidia (the asexual spores) in a partial diploid culture are expected to be *nic-2*$^+$ (*i.e.*, only 10% of the nuclei retain the duplication; the remainder of the nuclei do not have the translocated segment and therefore are *nic-2*). In a translocation sequence haploid, 100% of the conidia are expected to be *nic-2*$^+$. From each prototrophic culture, approximately 500 conidia were plated onto minimal medium and also onto minimal plus nicotinic acid medium. Those cultures that gave rise to approximately 10-fold more colonies on the nicotinic acid medium than on minimal medium were classified as partial diploids. Those cultures that gave rise to equal numbers of colonies on both media were classified as translocation sequence haploids.

To examine the NORs of these progeny I took advantage of the fact that type II rDNA repeat units do not have a recognition site for the restriction enzyme *Bcl*I (BUTLER and METZENBERG 1989, 1990). Intact chromosomal DNA was prepared from both classes of progeny (grown on minimal medium), digested with *Bcl*I, fractionated with pulsed-field gel electrophoresis system called CHEF, blotted, and probed with radiolabeled rDNA.

Figure 2 shows the analysis of NOR size in partial diploids and translocation sequence haploids. Figure 2A shows the translocation parent T(I;V)AR190 and eight randomly collected partial diploid progeny. At this level of autoradiographic exposure, which was typical for the work reported in this paper, most of the partial diploids display faint, but numerous bands hybridizing to rDNA. The apparent increase in hybridization around 600 kbp in most lanes is a small

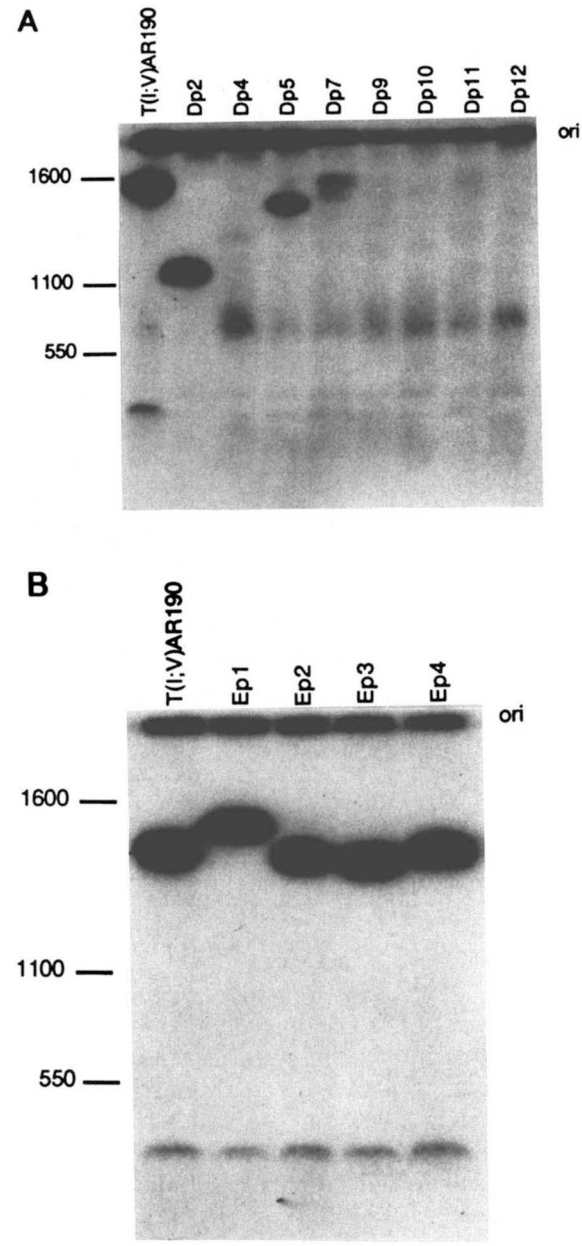

FIGURE 2.—Pulsed-field gel analysis of partial diploid and haploid translocation sequence progeny of T(I;V)AR190. (A) Autoradiogram of *Bcl*I-digested DNA from T(I;V)AR190 and eight randomly collected partial diploid progeny. The Southern blot was probed with radiolabeled rDNA. The CHEF gel electrophoresis conditions were 120 second pulse time at 140 V. Intact *S. cerevisiae* chromosomes were used as size standards. The positions of yeast chromosomes *VII*, *XV* (first mark from the top), *XIV* (middle mark) and *IX* (bottom mark) along with their estimated molecular masses in kbp are indicated at the left of the autoradiogram. The molecular masses of the yeast chromosomes are only approximations (see BUTLER and METZENBERG 1989). (B) Autoradiogram of *Bcl*I-digested DNA from T(I;V)AR190 and four representative haploid translocation sequence progeny. The CHEF gel electrophoresis conditions were 150-sec pulse time at 145 V. The size standards are as in (A).

region of poor resolution, where bands of different size become concentrated (see VOLLRATH and DAVIS 1987). Bands between 600 and 1600 kbp appear much

lighter because, under these electrophoresis conditions, this is the region of optimal resolution. Longer exposure of this blot clearly revealed rDNA hybridization from about 600 kbp to about 1600 kbp for each partial diploid, except Dp2 (data not shown). In terms of rDNA repeat units, the NORs within most partial diploids range in size from approximately 65–175 repeat units.

The NORs of the haploid translocation sequence progeny are in marked contrast to those of the partial diploid progeny. Figure 2B shows T(I;V)AR190 plus four representative examples of haploid translocation sequence progeny. These progeny yield a large band which hybridizes to rDNA and a small rDNA band of about 200 kbp which represents the translocated tip of the NOR. Except for small differences between progeny in the size of the major band, they each appear like the T(I;V)AR190 parent (and like most other strains of Neurospora). I conclude that most partial diploid progeny derived from T(I;V)AR190 have a population of different size NORs and, furthermore, that it is only the partial diploid progeny that display the heterogeneity of NOR size within strains.

In view of the genetic behavior of the translocated segment in partial diploids, I can envision three testable models to explain the NOR size heterogeneity within partial diploids. In model 1, the translocated segment is lost by chromosome breakage in the NOR. To account for the NOR size heterogeneity within each partial diploid, I propose that there are many independent breakage events and that breakage in any particular NOR in each culture occurs at a random site in the NOR. Following breakage, the chromosome is promptly stabilized (*i.e.*, the new end is prevented from fusing) by addition of telomeric DNA sequences (see Figure 3A). In model 2, I propose that the translocated segment is lost by chromosome breakage at one point in the NOR, for example, the original translocation breakpoint. However, to account for the NOR size heterogeneity, I propose that the new rDNA ends remain unhealed and initiate fusion-bridge-breakage cycles with sister chromatids (see Figure 3B; see also McCLINTOCK 1939, 1941). In model 3, I postulate that there is also chromosome breakage and healing of the broken ends, as in model 1. However, between the time of chromosome breakage and the time of healing, the broken ends engage in cycles of fusion-bridge-breakage, as in model 2.

CHEF analysis of haploid normal sequence strains derived from partial diploids: As a first step in deciding between these models, I obtained single conidial isolates (called SCIs) derived from partial diploids and examined their NORs. The partial diploid cultures are phenotypically prototrophic, but the nuclei that have lost the translocated segment will give rise to nicotinic acid auxotrophs if they become homokaryotic (see Figure 1). Therefore, any SCIs that are *nic-2* will be haploid, but will have been derived from partial diploid nuclei. If model 1 or model 3 is correct, I would expect the individually derived *nic-2* SCIs to show no NOR size heterogeneity, but I would expect much heterogeneity between SCI strains. If model 2 is correct, I would expect any given *nic-2* SCI to show extensive within-strain NOR size heterogeneity, similar to that of the original partial diploid strains.

Dilute suspensions of conidia from the prototrophic partial diploid cultures were plated onto nicotinic acid supplemented medium and individual colonies were transferred to tubes containing nicotinic acid supplemented medium. The SCI colonies were scored for nicotinic acid auxotrophy by spotting a dense suspension of conidia onto minimal medium. Those SCIs that did not grow on minimal medium after 3 days were classified as *nic-2*. Intact chromosomes were prepared from the *nic-2* SCIs and the NORs were analyzed on CHEF gels as above. Figure 4A shows the NORs from T(I;V)AR190, partial diploid Dp7 and four typical *nic-2* SCIs derived from Dp7, called Dp7-a, Dp7-b, Dp7-c and Dp7-d. Clearly, the *nic-2* SCIs do not display the kind of NOR size heterogeneity seen in Dp7. Each *nic-2* SCI has only one or, at most, two bands that hybridize to rDNA. It is not surprising that an occasional isolate has two bands, because conidia often contain more than one nucleus (see PERKINS and BARRY 1977). The *nic-2* SCIs from the other partial diploids gave similar results. This result is consistent with models 1 and 3, but rules out model 2.

Telomeric DNA sequences are associated with the NORs of *nic-2* SCIs: Regardless of the mechanism of NOR size heterogeneity, chromosome breakage in the NOR and stabilization of the broken chromosomes requires the NORs of the *nic-2* SCIs to have acquired telomeres. Tandem repeats of the hexamer, TTAGGG, have been found at the termini of Neurospora chromosomes (SCHECHTMAN 1987, 1990). Therefore, to test the hypothesis, I completely stripped the rDNA probe from the blot shown in Figure 4A and reprobed it with a radiolabeled oligonucleotide (called TEL1) of the following sequence: $(TTAGGG)_4$. Figure 4B shows the result of this experiment. For the *nic-2* SCIs, it is clear that TEL1 hybridizes to the same large bands as did the rDNA probe. The *nic-2* SCIs from other partial diploids show similar results (data not shown). This indicates that telomeric DNA is associated with the NORs of the *nic-2* SCIs. As expected, TEL1 does not hybridize to the NOR of T(I;V)AR190, since this NOR is interstitial.

Faint hybridization can also be seen in the Dp7 lane. Reprobing of the blot in Figure 2A with TEL1 revealed faint, thinly dispersed hybridization in all lanes,

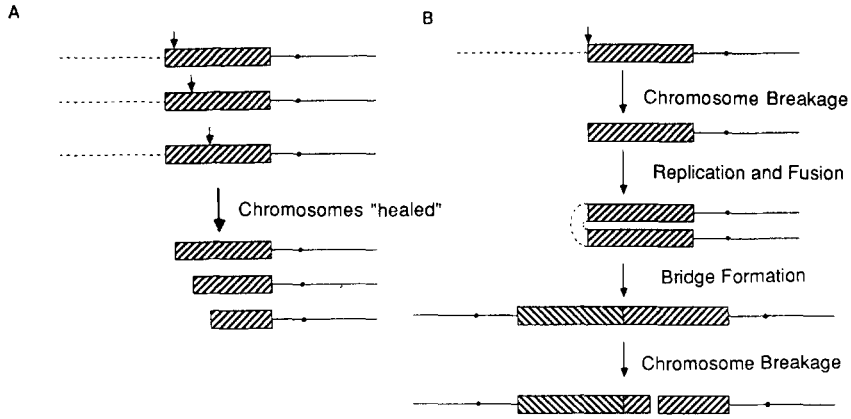

FIGURE 3.—Models for generating NOR size heterogeneity. (A) Schematic representation of model 1: LG V;I is broken at some point in the NOR (the position is indicated by the arrow). The acentric chromosomal fragment containing the translocated segment of LG I plus some rDNA is lost. Within a partial diploid, chromosome breakage in NORs of different nuclei usually occurs at different points in the NOR. The new ends are prevented from fusion by healing. The NOR size heterogeneity results from variability in the position of breaks in the NOR. All symbols are as in Figure 1. (B) Schematic representation of a breakage-fusion-bridge cycle involving the NOR. This process is taken from MCCLINTOCK (1941). LG V;I is broken in the NOR (in the figure, breakage is shown as occurring at the original translocation breakpoint; however, there is no reason to exclude the possibility that breakage occurs at another preferred site in the NOR). The acentric chromosomal fragment containing the translocated segment of LG I and some rDNA is lost. The broken rDNA end is not healed. Following replication of the broken chromosome, the unhealed rDNA ends of each sister chromatid fuse, forming a dicentric chromosome. Note also that the fusion of sister chromatids generates an NOR that is a giant palindrome. During mitosis, if the centromeres are pulled to opposite poles, a chromatin bridge is formed. To complete the segregation of centromeres, the dicentric chromosome must rupture at a point between the two centromeres. The diagram shows a rupture in the NOR. If dicentric chromosomes break at random points between the two centromeres, then repeated cycles of breakage-fusion-bridge will quickly generate NOR size heterogeneity within a culture. All symbols are as in (A).

except for that of Dp2, which showed hybridization only at the position of the major rDNA hybridizing band (data not shown). Thus, telomeric DNA is associated with many, or possibly all, of the NORs in partial diploids.

Telomeric DNA sequences associated with the NORs of *nic-2* SCIs represent new chromosomal termini: The above experiment indicates only that telomeric DNA is present at the NOR. It does not indicate whether the telomeric DNA is present at new chromosomal termini. To address this issue, I identifed telomeric restriction fragments specific to the NOR and determined whether they were sensitive to the exonuclease Bal-31 (sensitivity to Bal-31 is definitive evidence for terminal DNA–see BLACKBURN and SZOSTAK 1984; ZAKIAN 1989).

Digestion of low molecular weight genomic DNA with various restriction enzymes reveals 10–14 restriction fragments that hybridize to a telomere oligonucleotide (SCHECHTMAN 1989, 1990). A few of these restriction fragments have been assigned to specific termini of the seven chromosomes by RFLP mapping (SCHECHTMAN 1989). The large number of telomeric restriction fragments complicates the RFLP mapping approach to identifying telomeric restriction fragments specific to the NOR. Therefore I used a different method. Intact chromosomal DNA samples from *nic-2* SCIs were digested with *Bcl*I and the resulting digests were subjected to CHEF electrophoresis on a low melting temperature gel. The conditions were such that all rDNA (and only rDNA) migrated as a single band near the top of the gel. Agarose blocks containing the bands (referred to as norDNA) were excised, destained, and incubated with the restriction enzyme *Bam*HI. Then the digested norDNA and a control sample of the corresponding total genomic DNA (also digested with *Bam*HI), were fractionated by conventional gel electrophoresis, blotted, and probed with TEL1. For each SCI strain, the total genomic DNA and the norDNA were run side-by-side. Figure 5A shows the result of such an experiment. It is clear that the norDNA lanes contain one or, at most, two bands that comigrate with a band (or two bands) in the total genomic DNA lane (the relevant band in the Dp7-a lanes is very small; less than 500 bp). I consider these bands to be telomeric restriction fragments associated with the NOR (the fact that each *nic-2* SCI has a novel restriction fragment size is discussed below).

Next, I sought to determine if telomeric restriction fragments associated with the NOR represent true chromosomal termini. Aliquots of total genomic DNA from some of the *nic-2* SCIs were digested with Bal-31 for varying lengths of time. Following this, the DNA samples were digested with *Bam*HI, fractionated, blotted and probed with TEL1. Figure 5B shows a typical result. The telomeric restriction fragments associated with the NORs of Dp7-b and Dp7-c are marked by the arrows in Figure 5B. These restriction fragments are clearly sensitive to Bal-31. The kinetics

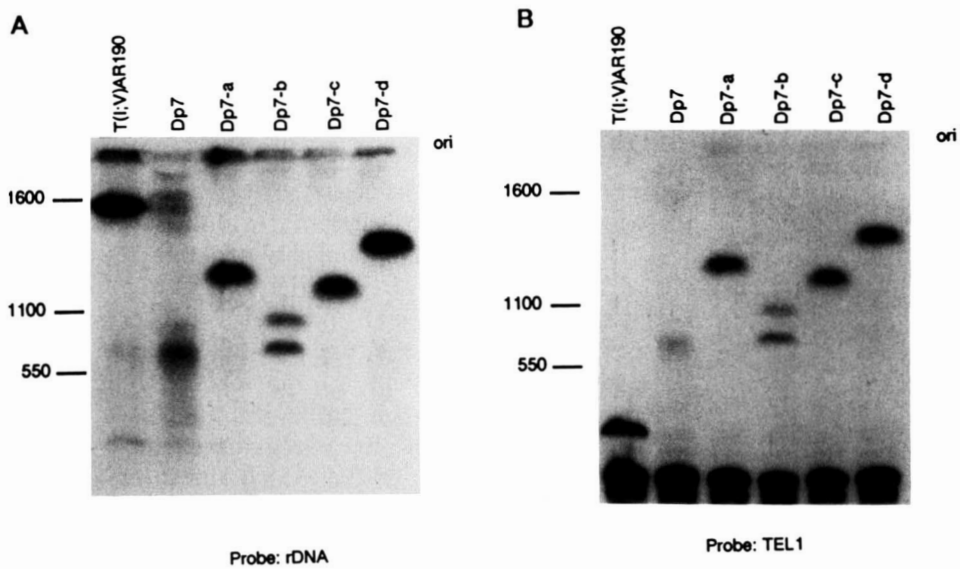

FIGURE 4.—Analysis of haploid normal sequence derivatives of a partial diploid. (A) Autoradiogram of *Bcl*I-digested DNA from T(I;V)AR190, Dp7 and four *nic-2* SCIs derived from Dp7, called Dp7-a, Dp7-b, Dp7-c and Dp7-d. The Southern blot was probed with radiolabeled rDNA. The CHEF gel electrophoresis conditions were 120-sec pulse time at 140 V. Intact *S. cerevisiae* chromosomes were used as size standards. The positions yeast chromosomes *VII*, *XV* (first mark from the top), *XIV* (middle mark) and *IX* (bottom mark) along with their estimated molecular masses in kbp are indicated at the left of the autoradiogram. (B) The Southern blot in (A) was stripped of the rDNA probe and reprobed with radiolabeled telomere probe.

of their disappearance is similar to that of other chromosome ends. The NOR-associated telomeric restriction fragment of Dp2 is also sensitive to Bal-31 (data not shown). As a control, the blot shown in Figure 5B was stripped and reprobed with radiolabeled DNA known to originate at a nonterminal site. The resulting bands showed no sign of Bal-31 sensitivity (data not shown). I conclude that the telomeric DNA sequences associated with the NOR represent chromosomal termini.

In spite of the fact that only one NOR size is apparent in Dp7-b (see Figure 4A), this strain has two *Bam*HI telomeric restriction fragments associated with the NOR (Figure 5A). There are two possible explanations for this. One is that there is a telomeric tract at a site within the NOR. The second is that there are actually two NORs of similar size in Dp7-b, but that they are not resolved under the CHEF electrophoresis conditions used in Figure 4A. Since both of the NOR-specific fragments of Dp7-b are sensitive to Bal-31 (Figure 5B), the second explanation seems more likely.

Polarity of rDNA repeat units at new NOR termini: Restriction mapping of genomic rDNA and of rDNA cloned into cosmid vectors indicates that all of the rDNA repeat units in the wild-type Neurospora NOR have the same polarity (D. K. BUTLER and R. L. METZENBERG, unpublished). However, models 1 and 3 make different predictions about the polarity of rDNA repeat units with respect to the telomere at the new NOR termini. If the NOR size heterogeneity is primarily the result of chromosome breakage at different points in the NOR (model 1), then at all new NOR termini, the polarity of rDNA repeat units will the same. Conversely, if the NOR size heterogeneity is primarily the result of breakage-fusion-bridge formation followed by the addition of telomeres (model 3), then approximately half of the new NOR termini will have rDNA repeat units in one polarity and the other half will have rDNA repeat units in the opposite polarity (see Figure 3B). To decide between models 1 and 3, I used restriction mapping to determined the polarity of the terminal rDNA repeat units in eight NORs that have undergone breakage and healing. For this experiment, norDNA was digested with restriction enzymes that cut either once or twice within rDNA repeat units. The fragments were fractionated, blotted, and probed with TEL1. This approach allowed me to make a map of restriction sites directly proximal to the NOR telomere. Figure 6 shows the the restriction maps of the eight NOR termini compared to that of two rDNA repeat units in tandem. In all eight NORs, the order and spatial relationship of restriction sites immediately proximal to the telomere corresponded to that of cloned type II rDNA from wild type. Moreover, the polarity of the terminal rDNA repeat units with respect to the telomere is the same for all eight of the NORs examined. From this experiment, I conclude that the NOR size heterogeneity in partial diploids is primarily the result of breakage at different points in the NOR as the translocated segments are lost.

The rDNA repeat unit is susceptible to breakage at many sites: The restriction mapping experiment also indicates that NORs terminate at several different points in the rDNA repeat unit. I wanted to know if this was true for termini generated within other partial diploids. To answer this question, I isolated norDNA from each of the partial diploids, digested the norDNA with *Bam*HI and probed a blot of the fractionated DNA with TEL1. Figure 7 shows the result of this experiment. There are several different sizes of the NOR-specific telomeric fragments in most par-

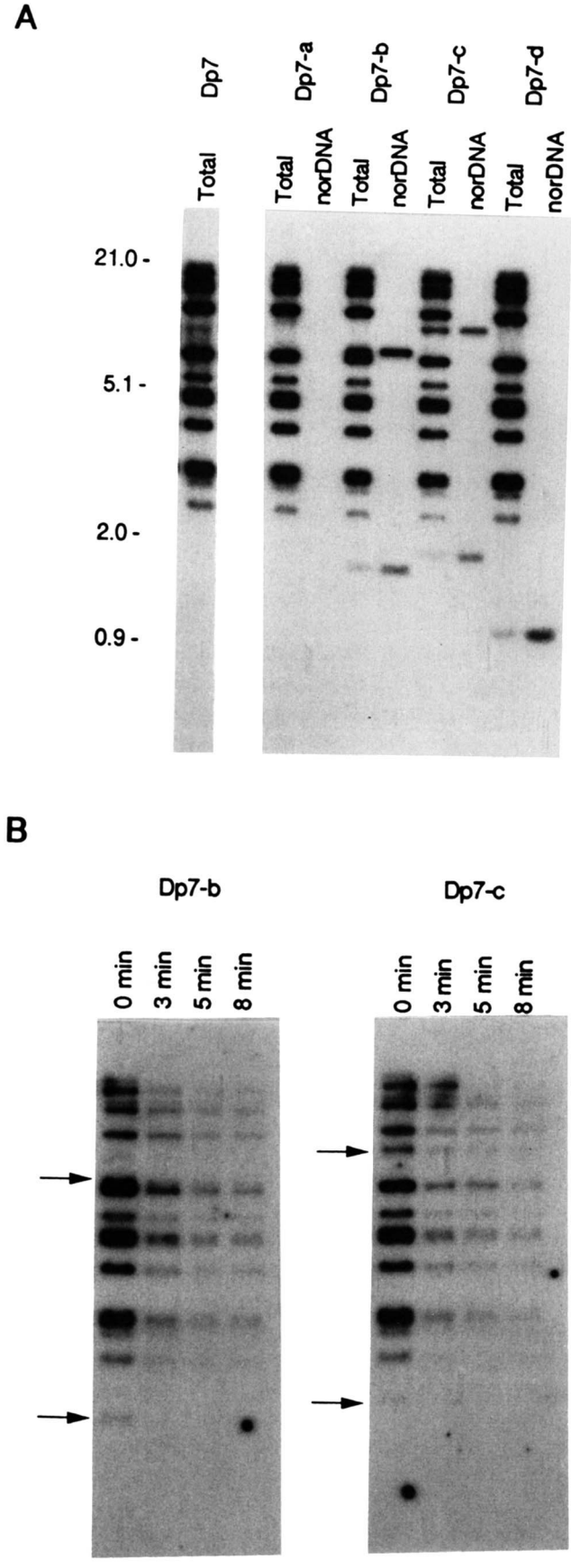

FIGURE 5.—Evidence for new chromosomal termini in the NOR. (A) Autoradiogram of *Bam*HI-digested total genomic DNA from Dp7 (left panel), and total genomic DNA and norDNA from four *nic-2* SCIs derived from Dp7 (right panel). Both panels are from the same gel. Conventional one-dimensional gel electrophoresis was used to fractionate the digested DNA. The Southern blot was probed with radiolabeled TEL1. The position of four *Eco*RI-*Hin*dIII lambda restriction fragments along with their molecular mass in kbp are indicated at the left of the gel. (B) Nuclease Bal-31 experiment with the *nic-2* SCIs, Dp7-b (left panel) and Dp7-c (right panel). Both panels are from the same gel. The digestion times, indicated above each lane, are 0, 3, 5 and 8 minutes. Following Bal-31 digestion, the samples were digested with *Bam*HI and fractionated by one-dimensional gel electrophoresis (see MATERIALS AND METHODS for details). The Southern blot was probed with radiolabeled TEL1. The arrows at the left of each panel mark the *Bam*HI telomeric restriction fragments associated with the NOR.

tial diploids. The visible fragments range in size from about 1.5 to 9 kbp, and, generalizing from the results with strain Dp7-a, there are probably *Bam*HI fragments smaller than 1.5 kbp in most strains. This experiment indicates that the rDNA termini generated within most partial diploids are located at many different points in the rDNA repeat unit. Not surprisingly, Dp2, the partial diploid containing only one size of the NOR, does not show any variability in the size of the NOR terminal restriction fragment.

Sequence analysis of three new NOR termini: To characterize the molecular nature of some of the new termini more precisely, I sequenced DNA spanning the rDNA terminus in three strains, Dp4-d, Dp7-a and Dp7-d. Based on the restriction mapping experiment, the location of the rDNA terminus in these strains must be at different points in the 17S coding region. Primers corresponding to the telomere and to the appropriate region of rDNA were used to specifically amplify NOR terminal DNA by the PCR. For each strain, a fragment of the expected size was amplified (see MATERIALS AND METHODS for details of the primers and amplification conditions). These fragments were cloned and sequenced.

Figure 8 shows the nucleotide sequence of three new termini. In all three cases, Neurospora rDNA sequence is directly adjacent to the canonical telomere DNA sequence (the corresponding normal rDNA sequence was obtained from SOGIN, MIOTTO and MILLER 1986). In Dp7-a, the actual terminus is ambiguous (indicated by the box in Figure 8). The GGGT sequence is normally present in the rDNA repeat unit at this position (SOGIN, MIOTTO and MILLER 1986). However, these nucleotides are in register with the telomeric repeats. Thus it is not clear whether some, all or none of these nucleotides were added during telomere formation. This rDNA/telomere junction is unlikely to be due to an artifact of the PCR, because no fragment corresponding to the size of that amplified from Dp7-a genomic DNA was amplified from cloned rDNA or from T(I;V)AR190 genomic DNA (data not shown). Dp7-d has a 5-bp match to the telomere sequence ending four bases

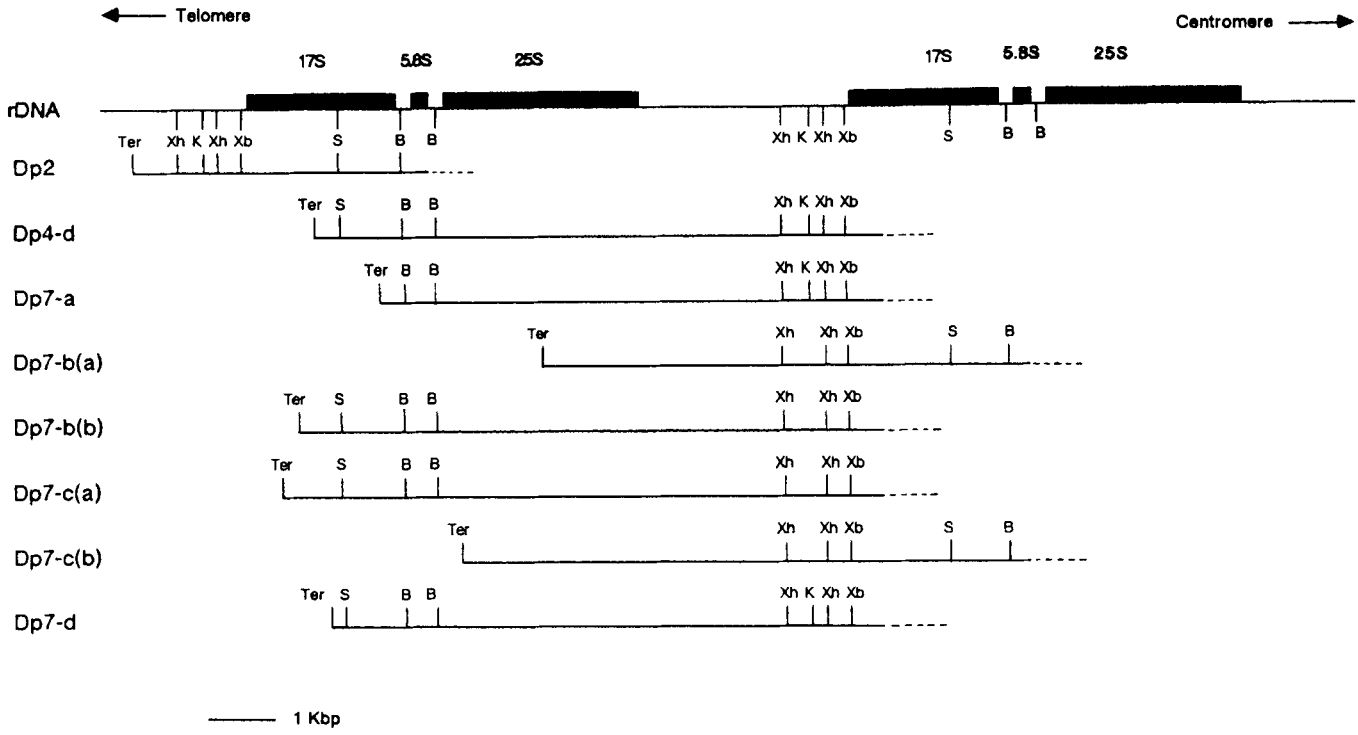

FIGURE 6.—Restriction map of two tandemly repeated type II rDNA repeat units compared to the restriction maps of eight new NOR termini. The rDNA restriction map is from RUSSELL *et al.* (1984). Only those sites used in the mapping of NOR termini are shown. To generate the maps of new NOR termini, Southern blots of norDNA digested with restriction enzymes that cut either once or twice within rDNA repeat units were probed with radiolabeled TEL1. The assignment of penultimate restriction sites, where indicated, was based on partial digestions. *Kpn*I sites were not mapped in the NOR termini of Dp7-b(a), Dp7-b(b), Dp7-c(a) and Dp7-c(b). Even though this figure shows the new termini clustered within the same rDNA repeat unit, the actual termini are separated by, in some cases, hundreds of kilobase pairs of rDNA. Symbols: for the rDNA repeat units, the thick black lines represent rRNA coding regions. The thin horizontal lines represent nontranscribed spacer (upstream of the 17S coding region and downstream of the 25S coding region) and internal-transcribed spacer regions (between the 5.8S and 17S, and the 5.8S and 25S coding regions). The direction of transcription is from left to right in the figure. Abbreviations: B, *Bam*HI; K, *Kpn*I; S, *Sac*I; Xh, *Xho*I; Xb, *Xba*I; Ter, the actual chromosomal terminus.

from the beginning of the telomere (indicated by the underlined sequence in Figure 8). The rDNA within a 100 bp of the Dp4-d terminus does not contain anything more than a 4-bp match to the the telomere sequence.

Ectopic rDNA defines a new site of chromosome breakage in partial diploids: An important issue is whether the rDNA at the NOR is a site of chromosome breakage simply because it is adjacent to a chromosomal region that is targeted for elimination or whether rDNA, regardless of its genomic location, is actually prone to breakage under aneuploid conditions. To address this, I needed a way to locate rDNA at an ectopic position in the genome such that if breakage occurs at the new site, it does not result in a lethal deletion. Therefore, I made partial diploids derived from a translocation strain called T(I;V)OY321 and from strains called QNS. T(I;V)OY321 is similar in genomic structure to T(I;V)AR190, except that most of the left arm of LG I has been translocated to the NOR (PERKINS, RAJU and BARRY 1984). Partial diploids derived from T(I;V)OY321 and normal sequence are reportedly unstable and, when they break down, always lose the translocated segment of LG I, never the normally located segment (PERKINS, RAJU and BARRY 1984). The QNS strains of Neurospora are wild type in genomic structure, except that they harbor a small ectopic block of rDNA precisely at the T(I;V)OY321 translocation breakpoint in LG I (PERKINS *et al.* 1986). Thus the translocated segment of LG I in T(I;V)OY321 and the segment of LG I distal to the ectopic rDNA in QNS represent an exact duplication (see Figure 9). If rDNA is a target for chromosome breakage regardless of its genomic position, partial diploids derived from T(I;V)OY321 and QNS could revert to the haploid state by loss of the chromosomal segment distal to the interstitial block of rDNA on LG I–in other words, by loss of the normally located segment of LG I (see Figure 9).

I made experimental partial diploids harboring LG V;I from T(I;V)OY321 and LG I from either a QNS-1 or QNS-2 derivative. In the QNS-1 derivative, the block of rDNA on LG I is about 150 kbp and, in the

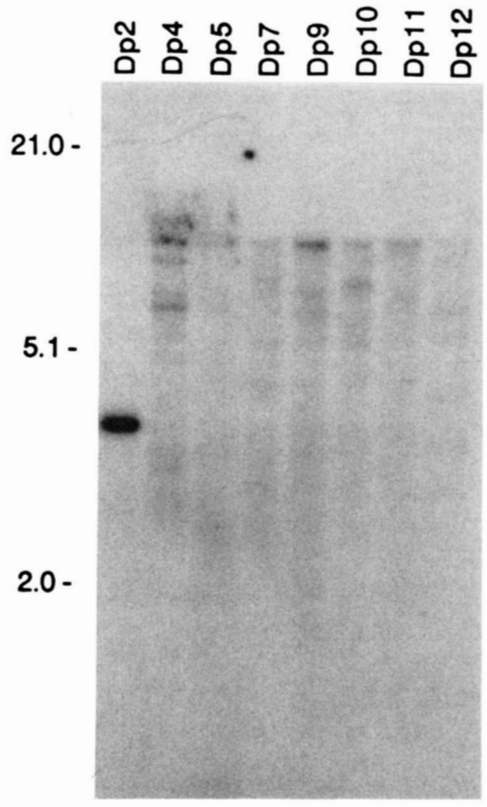

FIGURE 7.—Heterogeneity of *Bam*HI terminal restriction fragments in partial diploids. Autoradiogram of *Bam*HI-digested norDNA from the eight partial diploids. Conventional one-dimensional electrophoresis was used to fractionate the digested DNA. The Southern blot was probed with radiolabeled TEL1. The molecular mass markers are as in Figure 5.

Dp4-d ....TTTAACCTCAACAACTTTAATATACGC**TTAGGGTTAGGGTTAGGG**....

Dp7-a ....GGTTTGGATAGCTTTCCGGCCCT[GGGT]**TAGGGTTAGGGTTAGGG**....

Dp7-d ....TAATCGGCATAGTTTTATGGTTAAGAC**TTAGGGTTAGGGTTAGGG**....

FIGURE 8.—Nucleotide sequence of three new NOR termini. The Neurospora rDNA sequence is shown in plain type and the canonical Neurospora telomere sequence is shown in bold-face type. All sequences are shown in the 5′ to 3′ direction. The boxed sequence indicates the ambiguous terminus of Dp7-a. The underlined sequence in Dp7-d indicates a 5-base match to the telomere sequence. The origin of the first "T" residue of the Dp4-d telomere is uncertain; a "T" normally follows a "G" at this point in Neurospora rDNA (SOGIN, MIOTTO and MILLER 1986). See Figure 6 for the location of these termini in the rDNA repeat unit.

QNS-2 derivative, the ectopic block of rDNA is about 325 kbp (data not shown). I also made control partial diploids harboring LG V;I from T(I;V)OY321 and LG I from a normal sequence strain. In order to follow the fate of the duplicated chromosomal segments, I constructed each partial diploid so that it had a *recessive* allele for methylpurine resistance on the translocated segment of LG I and a *dominant* allele for methylpurine sensitivity (*i.e.*, the wild-type allele) on the normal segment of LG I (see Figure 9). Thus, the control partial diploids should be sensitive to methylpurine due to the recessive nature of the methylpurine resistance allele and the fact that loss is always of the translocated segment that contains the resistance allele. In contrast, if the ectopic block of rDNA on LG I is a sufficient substrate for chromosome breakage, then some experimental partial diploids (or some fraction of conidia thereof) should become methylpurine resistant by chromosome breakage in the ectopic rDNA followed by loss of the normal segment of LG I (see Figure 9).

Thirty control and 66 experimental partial diploids were grown to conidiation on minimal medium and then were scored for methylpurine resistance by spotting a dense suspension of conidia from each isolate onto methylpurine plates. The results were as follows. After 68 hr, 3 of 30 control partial diploids showed partial resistance to methylpurine (in this case, partial resistance manifested itself as weak growth of one or two papillae from the colony). The outgrowth from experimental partial diploids was in marked contrast to the controls. After 68 hr, 22 out of the 37 experimental partial diploids derived from QNS-1 displayed either full or partial resistance to methylpurine (in this case, partial resistance manifested itself as either slow growth of the entire colony or rapid growth of several papillae from the colony), and 28 out of the 29 experimental partial diploids derived from QNS-2 displayed either full or partial resistance to methylpurine. In general, methylpurine resistance among the experimental partial diploids arose earlier and was more robust than the rare methylpurine resistance among the control partial diploids. Clearly, some fraction of nuclei in most experimental partial diploids have lost the normal segment of LG I. I also examined intact chromosomal DNA from five randomly chosen methylpurine resistant experimental partial diploids and from their QNS parent. All of the methylpurine resistant progeny examined displayed within-strain size heterogeneity of the interstitial rDNA cluster on LG I (data not shown). In contrast, the QNS parent harbored a single sharp band corresponding to the interstitial rDNA cluster on LG I. Thus, based on genetic and molecular criteria, I conclude that, in the genetic background of a partial diploid, ectopically located rDNA is a site of chromosome breakage.

DISCUSSION

In this paper I have shown that, under certain genetic conditions, the rDNA of *N. crassa* behaves as a fragile site *in vivo*. In partial diploids derived from the translocation strain T(I;V)AR190, large terminal deletions result from frequent chromosome breakage in the NOR. Within some partial diploids, the NORs generated by breakage can vary in size from about 600 kbp to about 1600 kbp (Figure 2A), indicating that breakage can occur anywhere over the distal two-

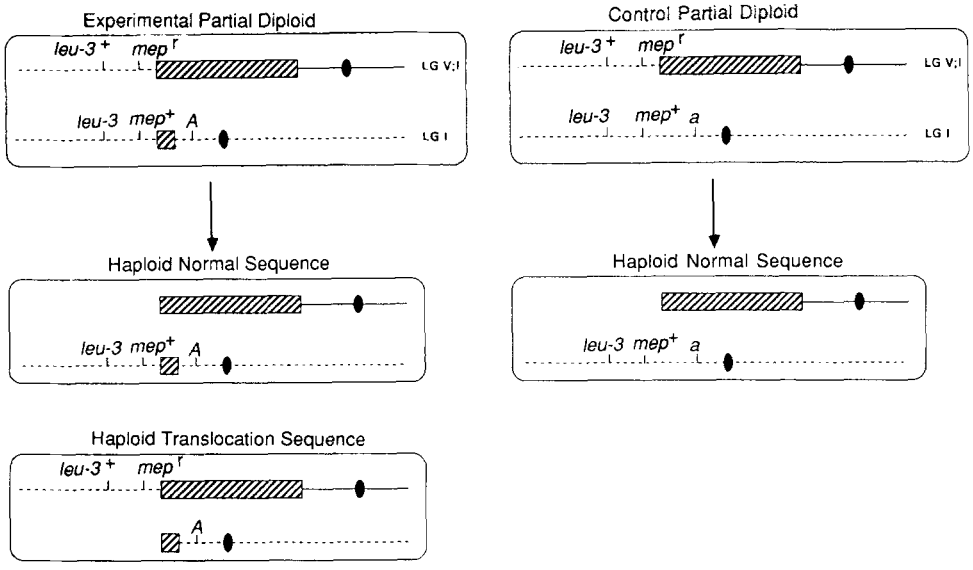

FIGURE 9.—Schematic representation depicting experimental and control partial diploids, and the expected types of haploid revertants. The expected types of haploid revertants are shown below each type of partial diploid. Only LG V;I and LG I are shown. Symbols: The solid lines represent LG V sequences; the dotted lines represent LG I sequences; the hatched rectangular boxes represents NORs composed of type II rDNA repeat units; the filled circles represent centromeres. The symbols are not drawn to scale. Markers: *leu-3*$^+$, leucine prototrophy; *leu-3*, leucine auxotrophy; *mep*$^+$, sensitivity to methylpurine; *mep*^{r}, resistance to methylpurine; *A* and *a* represent the two mating types of *N. crassa*. The *mep* and mating type loci are tightly linked to the T(I;V)OY321 breakpoint on LG I. The *leu-3* locus is moderately linked to the breakpoint, showing perhaps 10% recombination. The *leu-3* and mating type loci were used to identify partial diploids.

thirds of the NOR. The breakage sites are also heterogenous at the level of the rDNA repeat unit. Based on Figure 7, it appears as if breakage sites are located in both the transcribed and nontranscribed regions of the rDNA repeat unit. I have also shown that the rDNA ends resulting from chromosome breakage are healed by the addition of telomeres. Sequence analysis of three new termini demonstrated that canonical telomeric repeats are added directly to the rDNA endpoints. It seems likely that all of the new rDNA termini are healed in this manner. The sequence analysis also revealed that some rDNA termini are located at or near telomere-like sequences normally present in rDNA (Figure 8). However, since this is not an invariant feature of the new rDNA termini, it is clearly not a prerequisite for the formation of a new telomere in the NOR.

Partial diploids derived from the translocation strain T(I;V)OY321 and QNS, apparently undergo chromosome breakage at an ectopically situated block of rDNA. This result implies that rDNA contains a *cis*-acting signal for chromosome breakage. If so, what is the nature of the signal for chromosome breakage in rDNA? One possibility is that a specific sequence in rDNA acts as the signal. In Tetrahymena, a conserved 15-bp sequence, called Cbs (for *C*hromosome *b*reakage *s*equence), is necessary and sufficient for developmentally regulated chromosome breakage (YAO, YAO and MONKS 1990). For Neurospora, however, a strictly sequence-dependent signal is probably not needed, since there is no obvious sequence similarity between the new rDNA termini of Dp4-d, Dp7-a and Dp7-d (see Figure 8). However, I cannot rule out the possibility that breakage occurs in a sequence specific manner followed by a variable amount of exonucleolytic degradation before telomere formation. A second possibility is that the nucleolus itself acts as the signal. There is cytological evidence that the interstitial rDNA of some QNS strains can organize a nucleolus (PERKINS *et al.* 1986). Perhaps a nonspecific endonuclease is targeted to the nucleolus by a localization domain of the endonuclease and once inside the nucleolus, the putative endonuclease cleaves any DNA it encounters. If the nucleolus is the signal, then the ability of rDNA to serve as a substrate for chromosome breakage would very likely depend on its ability to engage in rRNA transcription.

Could breakage in rDNA play a role in shaping the structure of the chromosome that bears the NOR in Neurospora and, possibly, in other species as well? Neurospora and closely related fungal species have similar chromosome complements, including a terminal location of the NOR (reviewed in PERKINS 1985). Indeed, Neurospora strains harboring translocations involving the NOR have never been recovered from nature, despite the fact that such translocations have been produced and maintained in the laboratory. By cytological means, the chromosomal location of the NOR(s) has been determined in many other plant and animal species. In a large majority of these species, the NOR is located at or near the telomere (see HADJIOLOV 1986). Recently, molecular evidence has

been obtained indicating that, in the human parasite *Giardia lamblia*, the rDNA is located directly adjacent to the telomere of at least two chromosomes (ADAM, NASH and WELLEMS 1991). A plausible explanation for the common occurrence of chromosomes with terminally located NORs is that rDNA has a propensity to break under certain conditions, and organisms having chromosomes with essential genes distal to the NOR are at risk of suffering lethal deletions.

I owe special thanks to ROBERT L. METZENBERG for advice, encouragment and laboratory space throughout the course of this work. I also thank members of the Metzenberg laboratory, past and present, for helpful discussions at various stages in the work, particularly MARY ANNE NELSON and SEOG CHAN KANG. I thank ROBERT L. METZENBERG and MENG-CHAO YAO for critically reading the manuscript. D.K.B. is a Kodak Fellow and was also supported by a grant from the Lucille P. Markey Charitable Trust, Miami, Florida. This research was supported by U.S. Public Health Service grant GM08995 to ROBERT L. METZENBERG.

LITERATURE CITED

ADAM, R. D., T. E. NASH and T. E. WELLEMS, 1991 Telomeric location of Giardia rDNA genes. Mol. Cell. Biol. **11:** 3326–3330.

BLACKBURN, E. H., and J. W. SZOSTAK, 1984 The molecular structure of centromeres and telomeres. Annu. Rev. Biochem. **53:** 163–194.

BUTLER, D. K., and R. L. METZENBERG, 1989 Premeiotic change of nucleolus organizer size in Neurospora. Genetics **122:** 783–791.

BUTLER, D. K., and R. L. METZENBERG, 1990 Expansion and contraction of the nucleolus organizer region of Neurospora: changes originate in both proximal and distal segments. Genetics **126:** 325–333.

CHU, G., D. VOLLRATH and R. W. DAVIS, 1986 Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science **234:** 1582–1584.

HADJIOLOV, A. A., 1985 *The Nucleolus and Ribosome Biogenesis*, pp. 14–15. Springer-Verlag, Heidelberg.

HECHT, F., and G. R. SUTHERLAND, 1984 Fragile sites and cancer breakpoints. Cancer Genet. Cytogenet. **12:** 179–181.

LEBEAU, M. M., and J. D. ROWLEY, 1984 Heritable fragile sites in cancer. Nature **308:** 607–608.

MCCLINTOCK, B., 1939 The behavior in successive nuclear divisions of a chromosome broken at meiosis. Proc. Natl. Acad. Sci. USA **25:** 405–416.

MCCLINTOCK, B., 1941 The stability of broken ends of chromosomes in *Zea mays*. Genetics **26:** 234–282.

NEWMEYER, D., and D. R. GALEAZZI, 1977 The instability of Neuropora duplication *Dp(IL;IR)H4250*, and its genetic control. Genetics **85:** 461–487.

PERKINS, D. D., 1985 Aspects of the Neurospora genome, pp. 277–294 in *Molecular Genetics of Filamentous Fungi*. Alan R. Liss, New York.

PERKINS, D. D., and E. G. BARRY, 1977 The cytogenetics of Neurospora. Adv. Genet. **19:** 133–285.

PERKINS, D. D., N. B. RAJU and E. G. BARRY, 1984 A chromosome rearrangement in Neurospora that produces segmental aneuploid progeny containing only part of the nucleolus organizer. Chromosoma **89:** 8–17.

PERKINS, D. D., R. L. METZENBERG, N. B. RAJU, E. S. SELKER and E. G. BARRY, 1986 Reversal of a Neurospora translocation by crossing over involving displaced rDNA, and methylation of the rDNA segments that result from recombination. Genetics **114:** 791–817.

RUSSELL, P. J., S. WAGNER, K. D. RODLAND, R. L. FEINBAUM, J. P. RUSSELL, M. S. BRET-HARTE, S. J. FREE, P. POWERS and R. L. METZENBERG, 1984 Organization of the ribosomal ribonucleic acid genes in various wild-type strains and wild-collected strains of Neurospora. Mol. Gen. Genet. **196:** 275–282.

SCHECHTMAN, M. G., 1987 Isolation of telomere DNA from *Neurospora crassa*. Mol. Cell. Biol. **7:** 3168–3177.

SCHECHTMAN, M. G., 1989 Segregation patterns of Neurospora chromosome ends: mapping chromosome tips. Fungal Genet. Newsl. **36:** 71–73.

SCHECHTMAN, M. G., 1990 Characterization of telomere DNA from *Neurospora crassa*. Gene **88:** 159–165.

SOGIN, M. L., K. MIOTTO and L. MILLER, 1986 Primary structure of the *Neurospora crassa* small subunit ribosomal RNA coding region. Nucleic Acids Res. **14:** 9540.

TURNER, B. C., 1977 Euploid derivatives of duplications from a translocation in Neurospora. Genetics **85:** 439–460.

VOLLRATH, D., and R. W. DAVIS, 1987 Resolution of DNA molecules greater than 5 megabases by contour-clamped homogeneous electric fields. Nucleic Acids Res. **15:** 7865–7876.

WETERGAARD, M., and H. K. MITCHELL, 1947 Neurospora. V. A synthetic medium favoring sexual reproduction. Am. J. Bot. **34:** 573–577.

YAO, M.-C., C.-H. YAO and B. MONKS, 1990 The controlling sequence for site-specific chromosome breakage in Tetrahymena. Cell **63:** 763–772.

YAO, M.-C., K. ZHENG and C.-H. YAO, 1987 A conserved nucleotide sequence at the sites of developmentally regulated chromosomal breakage in Tetrahymena. Cell **48:** 779–788.

YUNIS, J. J., 1983 The chromosomal basis of human neoplasia. Science **221:** 227–236.

ZAKIAN, V. A., 1989 Structure and function of telomeres. Annu. Rev. Genet. **23:** 579–604.

Communicating editor: R. H. DAVIS