

## Change in Chromosome Number Associated With a Double Deletion in the *Neurospora crassa* Mitochondrial Chromosome

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### ABSTRACT

The mitochondrial genome of *Neurospora* is usually found in a single covalently closed circular 62-kbp DNA molecule. We report here that the mitochondrial genome of a phenotypic revertant of a stopper mutant (*stp-ruv*) is contained primarily in two separate, nonoverlapping, autonomously replicating circular chromosomes. The circles, one about 21 kbp and the other somewhat less than 36 kbp are derived from the most frequent classes of recombinant chromosomes (21 and 41 kbp) in the chromosomal population of mitochondria in the original stopper mutant. The new, more stable chromosomal configuration, is associated with the deletion of two sequences (1 kbp and 4 kbp) at the splice junctions of the two circles. The data suggest that both deletions are likely to have originated from a single recombinational event involved in generating the 36-kbp circle. Secondary, spontaneously arising derivatives of *stp-ruv* have been found to yield, at high copy number, short sections of the 21-kbp circle in covalently closed supercoiled circles varying from unit length to very high multimers. The amplified segments span a common segment likely to contain the replication origin of the 21-kbp chromosome.

**I**NTRACHROMOSOMAL recombination is a frequent event during the normal growth and replication of mitochondria in many different plant and fungal species (CUMMINGS, BELCOUR and GRANDCHAMP 1979; PALMER and SHIELDS 1984; GROSS, HSIEH and LEVINE 1984). In *Neurospora*, intramolecular recombination appears to generate a variety of circular derivatives of the normal 62-kbp mitochondrial chromosome (ALMASAN and MISHRA 1988; DEVRIES *et al.* 1986; GROSS, HSIEH and LEVINE 1984). These, usually smaller, circular forms are found among a heterogeneous class of molecules making up only a small fraction of the total DNA of normal mitochondria; however, some of the derivatives are the most frequent molecules found in mitochondria from stopper (*stp*) mutants (GROSS, HSIEH and LEVINE 1984). The characteristic stop-start mode of growth of at least one of these mutants was shown to be correlated with severe reversible fluctuations in the frequency of different classes of recombinant molecules which result in a deficiency of a region shown likely to code for subunits of NADH dehydrogenase (GROSS, HSIEH and LEVINE 1984; DEVRIES *et al.* 1986).

Selection for continuously growing derivatives of the original *stp* mutant yielded a wide variety of reasonably stable mitochondrial genotypes. One of them, *stp-ruv*, was of particular interest because its mitochondrial chromosomal composition seemed to consist primarily of the two most frequent recombinant chromosomes found to fluctuate as a function of the growth state of the original *stp* mutant (GROSS, HSIEH

and LEVINE 1984). As indicated in the following sections, the stability of the  $n = 2$  chromosomal complement is associated with the loss of two extended sequences of the single chromosome of normal mitochondria. One of these deletions includes the coding sequences for the NADH dehydrogenase subunits found deficient in the original *stp* mutant. The deficiency of the subunits cannot be crucial for growth, a fact already pointed out by DEVRIES, DEJONGE and SCHRAGE (1985) with respect to the deletion of the same region in another stopper mutant (E35). We show here, and describe more fully in the accompanying paper (GROSS *et al.* 1989) that the *stp-ruv* strain appears to behave like some derivatives of the senescent strains of *Podospora anserina* which have been found by TURKER, DOMENICO and CUMMINGS (1987) to amplify short sections of mitochondrial DNA.

### MATERIALS AND METHODS

The origin of the parental *stp* mutant, 1Ar155(II)2,107A, as well as a description of its nutritional requirements and mode of growth are described in GROSS, HSIEH and LEVINE (1984). Methods employed for the isolation of mitochondria, the separation of linear and nicked *mt* DNA from supercoiled, covalently closed molecules by CsCl-ethidium bromide density gradient centrifugation, electrophoretic analysis, as well as the procedures employed in isolating and cloning *Hind*III fragments of *mt* DNA in pSCC31 (CHENG and MODRICH 1983) are described in the same paper. In several instances DNA hybridization was determined by the biotinylation method of WAHL, STERN and STARK (1979) as described in the kit supplied by Bethesda Research Laboratories. Otherwise, the standard SOUTHERN (1975) hybrid-

ization procedure was employed using  $^{32}\text{P}$ -labeled nick translated DNA. In each case restriction fragments were generated according to instructions supplied by the vendor of the enzyme.

The *stp-ruv* mutant was obtained by selecting for a continuously growing derivative of the original *stp* after irradiation of *stp* conidia with ultraviolet light in the following way: About  $1 \times 10^7$ , 7-day-old conidia were irradiated with ultraviolet light to about 10% survival, then used as an inoculum for growth tubes prepared as described by RYAN, BEADLE and TATUM (1943). A derivative which grew continuously was obtained during the first growth tube passage. Upon subsequent growth tube passages and purification by conidial reisolation the growth of the isolated strain, *stp-ruv*, though continuous, was at a highly variable rate, averaging about half that of wild type. Derivatives of *stp-ruv* which yield amplified small circular DNA molecules arose spontaneously upon standing at room temperature. In addition to a requirement for methionine, *stp-ruv* requires inositol for growth. As a consequence, it does not survive when the concentration of available inositol is low (LESTER and GROSS 1959). All cultures were routinely started from conidia stored in silica gel at  $-20^\circ$ .

## RESULTS

The organization of the mitochondrial genome of a derivative of the original stopper (*stp*) mutant, IA155(II)2,107A capable of continuous growth, *stp-ruv*, was found to be quite different from that of its parent which, in the growth phase, consisted largely of 21- and 41-kbp circular molecules. These chromosomes were shown to be derived from the normal single 62-kbp chromosome by intramolecular recombination within the directly repeated tRNA<sup>met</sup> sequences illustrated in Figure 1. The entire nucleotide sequence appeared represented in the two circular forms which were capable of reversion to something resembling wild type, albeit at very low frequency (GROSS, HSIEH and LEVINE 1984). Figure 1, which is based on the electrophoretograms of Figure 2, illustrates that the genome of the *stp-ruv* contains two large deletions, one a 4–5-kbp segment extending from a point within the tRNA<sup>met</sup> sequence of *Hind*III, 9, to some point in *Hind*III, 3, and another of about 1 kbp extending from the tRNA<sup>met</sup> sequence of *Hind*III, 1, to some point in *Eco*RI, 6. The breakpoints of the two deletions appear to form the splice junctions of the two derivative circular molecules. The salient feature of the *stp-ruv* mitochondrial genome is that it consists primarily of two independently replicating nonhomologous chromosomes along with recombinant derivatives of the two at low frequency.

The data of Figure 2 are consistent with the notion that the smaller of the two chromosomes of *stp-ruv* is identical to the 21-kbp circular chromosome of its parent. They are also consistent with the idea that the deletions arose as a consequence of a single intramolecular recombination event either within a 41-kbp circular chromosome or some larger component of the chromosomal population in the mitochondria of

the *stp* parent. The loss of one of the tRNA<sup>met</sup> sequences in *stp-ruv*, along with the deletion of a segment of the chromosome common to *Eco*RI, 6, *Bam*HI, 3, and *Hinc*II, 8, was established previously by hybridization analysis with the homologous tRNA<sup>met</sup> sequence of *Hind*III, 9 (GROSS, HSIEH and LEVINE 1984). Confinement of the deleted sequences to the region of the 41-kbp circle is reflected further by retention of the 7.2-kbp *Hinc*II junction fragment of the 21-kbp circle, which joins *Hinc*II, 8 to 9, and the replacement of the 1.1-kbp *Hinc*II junction fragment of the *stp*'s 41-kbp circle with a 2.9-kbp fragment, joining a segment of *Hinc*II, 8, within *Eco*RI, 6, to some point within *Hinc*II, 6, and *Hind*III, 3. The deleted segment, indicated in Figure 1, which extends from a point in *Hind*III, 9, beyond the tRNA<sup>met</sup> sequence, to a point about 4–5 kbp away in *Hind*III, 3, is included in the 5-kbp deletion of *stp* E35 of DEVRIES *et al.* (1986) which contains two open reading frames specifying two peptides resembling subunits of NADH dehydrogenase (CHOMYN *et al.* 1985). The electrophoretic patterns obtained after subjecting covalently closed circular DNAs to *Sal*I hydrolysis illustrate the relative mobility and yield of the uncut 41- and 36-kbp circular forms and the linearized derivatives if the 21-kbp circle and the 62-kbp wild-type chromosome. Particularly heavily loaded samples were run so that the minor, linearized molecules in the 5–17-kbp range could be seen. These are particularly obvious in the *stp-ruv* lanes and might represent linear derivatives of small circular molecules whose replication were initiated at an *ori* sequence not too distant from the unique *Sal*I site of the mitochondrial chromosome (see below). The relative intensity of the staining of the *Sal*I bands indicate that the 21- and 36-kbp circles of *stp-ruv* are not present in equal amounts. This is indicated clearly by the 1.5–2.0-fold excess of the 7.5-kbp *Hind*III junction fragment of the 21-kbp circle over the 11.0-kbp junction fragment of the 36-kbp circle in DNA derived from circular as well as linear molecules.

Two subclones of the *stp-ruv* mutant strain were found to produce, at high copy number, small circular derivatives of a short region of the 21-kbp circle. The first of these produced circular multimers of a 1.9-kbp segment including some or all of *Hind*III, 19a, and extending into *Hind*III, 14. As indicated in Figure 3, the segment includes the chromosome's single *Sal*I site. It yields a 1.9-kbp linear molecule after cutting with *Sal*I that hybridizes with *Hind*III, 15 and 19a. Unfortunately, this *stp-ruv* derivative died before the analysis could be extended much further. A search among isolates from the original strain, however, yielded one which produced, at high copy number, circular derivatives of two short sequences, 286 and 291 bp (GROSS *et al.* 1989), which hybridized with

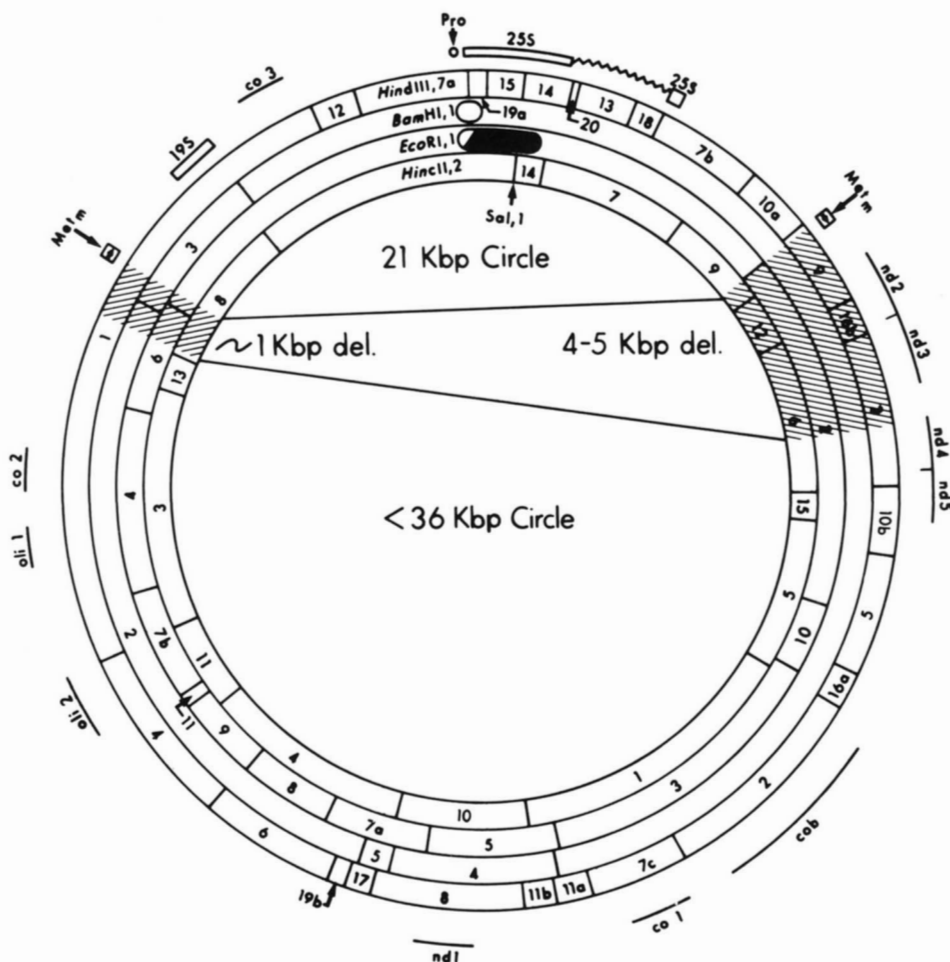


FIGURE 1.—A composite restriction map of the *Neurospora* mitochondrial chromosome showing the deletions and major circular chromosomal derivatives present in the *stp-ruv* mutant strain. The map of type II mitochondrial DNA is derived largely from those of TAYLOR and SMOLICH (1985), COLLINS (1986), DEVRIES *et al.* (1986) and NELSON and MACINO (1987). Ribosomal RNA sequences are noted by open boxes and the intron within the 25S rRNA region by a jagged line. The two tRNA<sup>met</sup> sequences are noted by boxes with arrows indicating sequence orientation. The deletions of *stp-ruv* are cross hatched and the junctions of the 21- and <36-kbp circular derivatives are noted by lines drawn across the circle. The 286-bp and 1.9-kbp circular derivatives are drawn to include the *Hind*III, 7a-19a, junction. This region has not been shown definitively to be included within the 1.9-kbp circle. It is left unshaded.

*Hind*III, 19a and 7a (Figure 4). The electrophoretograms and hybridizations of Figure 4 (using DNA from the cloned 286-bp sequence) indicate that only a very small fraction of the minicircles are in monomeric form. The extent and complexity of the ladder of the two circular derivatives suggest the involvement of a large number of polymeric forms, some of them, perhaps, unresolved knotted circles. Of special note is the curious 5.3-kbp *Hind*III fragment that hybridizes strongly with the 286-bp sequence but appears to be only a minor constituent as judged by ethidium bromide staining. Since it has only a single *Hind*III site and is very much larger than either *Hind*III, 7a or 19a, it is likely to contain a highly iterated sequence derived from *Hind*III, 19a. The 5.3-kbp *Hind*III fragment was transient, appearing only in DNA from a few subcultures of *stp-ruv* 286/291.

The base sequences of the 286 and 291 subunits are presented in GROSS *et al.* (1989) along with sequences of other plasmids derived from the same strain and an analysis of the recombination and replication mechanisms involved in generating the plasmids of *stp-ruv*.

#### DISCUSSION

Two properties of *stp-ruv* distinguish it from its *stp* parent; (1) a switch from a fluctuating population of

recombinant chromosomes to a genome consisting primarily of two complementary, separately replicating chromosomes, and (2) a proclivity to amplify derivatives of a short section of one of the chromosomes. A discussion of the relation of base sequence to mechanisms of recombination and replication of the nested set of plasmid-like elements derived from the 21-kbp chromosome can be found in GROSS *et al.* (1989). We reserve the discussion here to the overall change in the chondriotype from the  $n = 1$  of the wild type to  $n = 2$  of *stp-ruv* and its relation to replication initiation from a replication origin within the 21-kbp chromosome.

The genotypic analysis of the chromosomal composition of mitochondria in the original stopper mutant, IAr155(II)2,107A and normal mitochondria from its inositol requiring parent, 89601, revealed that intramolecular and intermolecular recombination were normal events during the life history of a mitochondrial chromosomal population. However, the main difference between normal and stopper chromosomal populations was found confined largely to frequency rather than kind of chromosomes present. We were able to show that the 21- and 41-kbp circular chromosomes, the major fluctuating

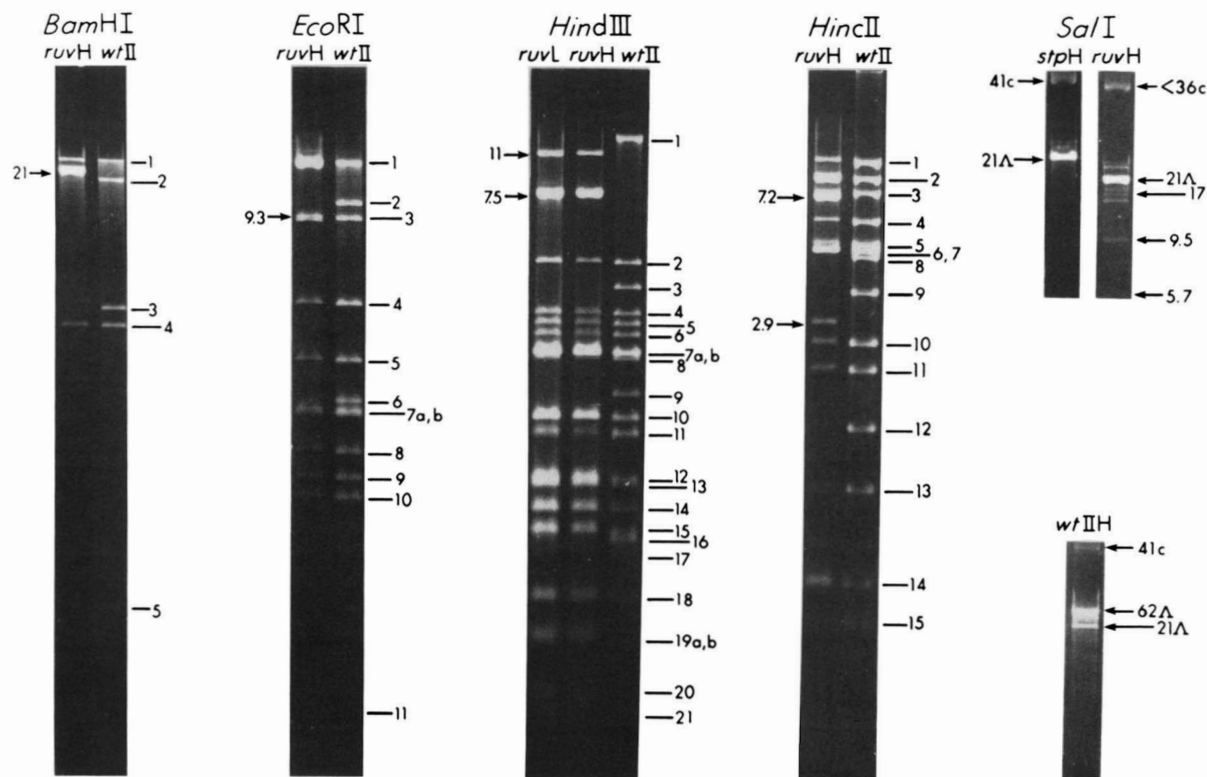


FIGURE 2.—Restriction fragment patterns of mitochondria *stp-ruv* and wild-type II DNA. The electrophoretic patterns of *Bam*HI, *Eco*RI, *Hinc*II, *Hind*III and *Sal*I restriction patterns of covalently closed circular DNA from *stp-ruv mt (ruv)* are compared with the corresponding patterns obtained from total wild-type II *mt* DNA. Restriction patterns of linear and nicked *stp-ruv* DNA were found, in each case, to be similar to those obtained for covalently closed molecules. This is illustrated by the patterns obtained after cutting covalently closed circular (H) and nicked circular plus linear (L) DNA molecules with *Hind*III. The fragments are identified by the numbers listed to the right of the type II channels, and the sizes of each of the junction fragments, in kbp, are listed to the left of the *stp-ruv* channels. The three *Sal*I patterns on the right are presented to illustrate the classes of DNA molecules, found at low frequency, in preparations of closed circular *stp-ruv* DNA. Nothing was detectable on these gels beyond the smallest listed fragment. Uncut covalently closed circular (c) DNA is at the top of the gel and the remaining bands are derived from circular forms linearized by *Sal*I ( $\Delta$ ). Electrophoresis of uncut samples (not presented) yield only broad bands near the top of the gel.

classes of the *stp* mutant, were present, albeit at low frequency, in normal mitochondria (GROSS, HSIEH and LEVINE 1984) and that the two chromosomes were generated by homologous, reciprocal recombination within the directly repeated tRNA<sup>met</sup> sequences located about 20 kbp apart on the *Eco*RI, 1 fragment (Figure 1). No specific explanation for the difference between the chondriotypes of the normal or *stp* mutants was obtained, but because reversion to wild type was very rare it was assumed that a reasonably stable, rarely reversible sequence alteration was responsible for the *stp* phenotype. It was clear from the studies of E35 mitochondria (DEVRIES, DEJONGE and SCHRAGE 1985), that the region of the mitochondrial chromosome containing *nd2* and *nd3*, subunits of NADH dehydrogenase, that was poorly represented in *stp* mutant mitochondria was dispensable since it was deleted from the E35 genome. (It has been shown recently that *stp-ruv*, which also is missing the two *nd* coding units respire by a CN insensitive pathway (J. SIEDOW, personal communication).

While E35 and *stp* grow and support the replication of recombinant chromosomes, neither strain gener-

ates the kinds of plasmid-like objects produced by *stp-ruv*. Furthermore, several continuous growing derivatives of the *stp* mutant have been obtained in addition to *stp-ruv*. The most pertinent of these has a chondriotype consisting of a single chromosome with a deficiency in the same region and about the same size as that in E35 (our unpublished observation). This strain does not generate plasmids. In fact, the generation of polymeric derivatives of short sections of the *Hind*III, 7a and 19a, region have only been found under two circumstances, growth of mutant and normal strains on inhibiting levels of acriflavin (our unpublished observation) and spontaneously, during the growth of *stp-ruv*.

There are two major manifestations of the differences between the chondriotype of *stp-ruv* and the strain from which it was derived, both perhaps directly related to the short (about 1 kbp) deletion of the *Eco*RI, 1–6, junction (Figure 1). The first of these is the fact that only a single tRNA<sup>met</sup> is present per genome. Its absence from the 36-kbp circle may account for failure to generate a single large, 57-kbp chromosome. Such a role for the tRNA sequence is

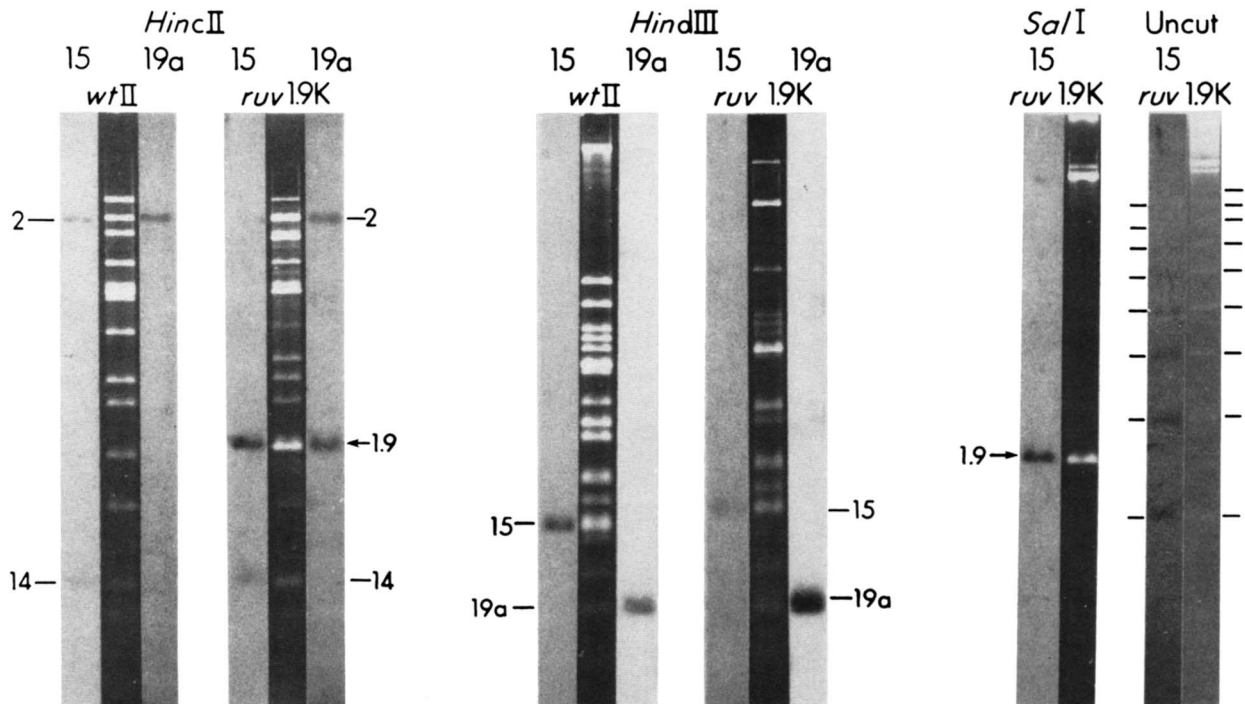


FIGURE 3.—Electrophoretic and hybridization patterns of *stp-ruv mt* DNA containing 1.9-kbp circular derivatives. Biotinylated, cloned *HindIII*, 15 and 19a, fragments were hybridized with *HindIII* and *HincII* cut total DNA as well as *SalI* cut and uncut covalently closed circular DNA from *stp-ruv* 1.9-kbp mitochondria. The linearized 1.9-kbp fragment (marked with arrow) is clearly visible after cutting with *SalI*. It, as well as the ladder of multimers derived from it, hybridizes with *HindIII*, 15. To help visualize these very light blots each of the polymeric forms is marked by a line beside each channel. The size of the monomeric unit, 1.9 kbp, is no shorter after cutting with *HincII* than *SalI*. Hence the unit does not extend significantly beyond the *HincII*, 14 interval. The restriction fragments containing homologous sequences to each of the probes is identified in each case either by number or size.

implicit in the reversible, reciprocal recombination mechanism proposed for generating the population of chromosomes of the original *stp* mutant (GROSS, HSIEH and LEVINE 1984). The second and somewhat surprising consequence is that the single chromosome configuration has not been found to be regenerated by recombination at the many other highly repeated sequences distributed throughout the chromosome. These repeated sequences have been found to be sites for the legitimate and illegitimate recombination events involved in the generation of the multimeric plasmid subunits and their resolution after replication (YIN *et al.* 1982; DEVRIES *et al.* 1986; GROSS *et al.* 1989). Repeated sequences are not confined to the 21-kbp region spanned by the smaller of the two circular chromosomes but many, associated with tandem *PstI* site repeats, are distributed widely in the 36-kbp chromosome as well (NELSON and MACINO 1987; ALMASAN and MISHRA 1988). It seems a possibility, at least, that recombination does occur but that the replication of composite chromosomes is faulty. This implies that there is some kind of replicative function for the region near *EcoRI*, 1–6, which, when missing, may occasionally cause premature termination of replication from an *ori* sequence within the *HindIII*, 7a–19a region. Sequences containing an origin of replication, once synthesized, might then undergo exten-

sive independent replication. It is of some interest to note that the *EcoRI*, 4–6, region has been proposed previously to be involved in some replication function because of the generation of tandem reiterations within the region (MANNELLA, GOEWERT and LAMBOWITZ 1979). However, the essential point to be made from the existence of the two independently replicating chromosomes of *stp-ruv* is that the *Neurospora* mitochondrial chromosome has, at the very least, two replication initiation sites and if yeast and *Podospora* are indicative models, the true number of *ori* sequences in *Neurospora* is likely to be greater than two.

At this point, too much evolutionary significance should not be attributed to the change in chromosome number in the *Neurospora* mitochondrion. It is not known how the *Neurospora* mitochondrial chromosome was derived; whether it is a recombinant composite of several chromosomes or whether multiple origins arose by duplication over time. What is clear is that most fungal and mammalian mitochondrial chromosomes have multiple origins and except for the two *ori* sequences in mammals, one for each of the DNA strands (CREWS *et al.* 1979), the function and regulation of the multiple origins are, for the most part, unknown. The information currently available about mitochondria in *Podospora*, *Neurospora* and



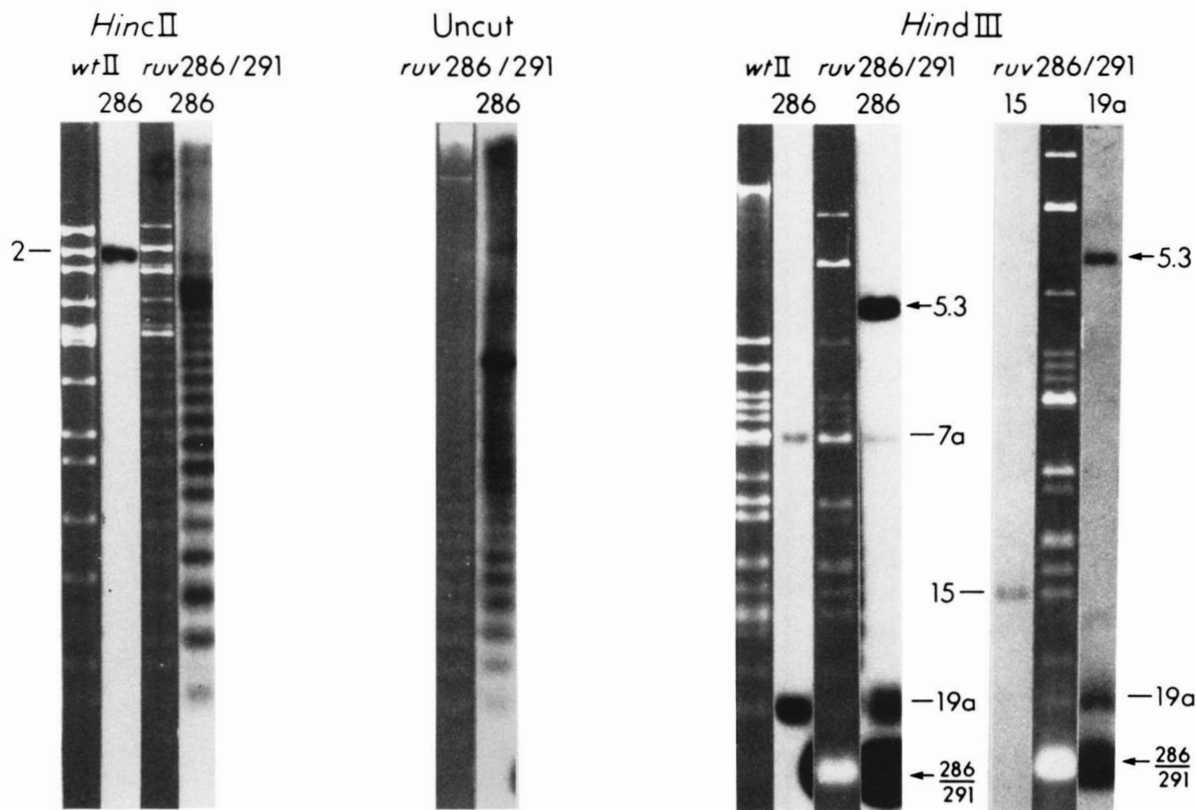


FIGURE 4.—Electrophoretic and hybridization patterns of *stp-ruv* 286/291 DNA.  $^{32}\text{P}$  nick translated cloned 286-bp sequence and biotinylated cloned *Hind*III, 15 and 19a, DNA were hybridized with *Hinc*II, and *Hind*III, cut and uncut covalently closed DNA from *stp-ruv* (*ruv*) mitochondria containing 286- and 291-bp subunit circles. The hybridization ladders were matched to the gel photographs as well as practicable in view of the compression distortions resulting from the blotting procedure itself. Note that very few of the circular forms migrate as monomers and note especially the highly reactive circular form which hybridizes with a very faint 5.3-kbp *Hind*III band. Although not cut by *Hinc*II, the migration of the circular forms is altered after exposure to the enzyme. The hybridized restriction fragments are marked by number and the novel linear derivatives of the circles by size (arrow).

yeast, outlines situations where different *ori* sequences are exploited in the amplification of specific sections of the mitochondrial chromosome. It is yet to be shown whether this amplification process is physiologically significant.

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#### LITERATURE CITED

- ALMASAN, A., and N. C. MISHRA, 1988 Molecular characterization of the mitochondrial DNA of a new stopper mutant ER-3 of *Neurospora crassa*. *Genetics* **120**: 935–945.
- CHENG, S.-C., and P. MODRICH, 1983 Positive-selection cloning vehicle useful for overproduction of hybrid proteins. *J. Bacteriol.* **154**: 1005–1008.
- CHOMYN, A., P. MARIOTINI, M. W. J. CLEETER, C. I. RAGAN, A. MATSUNO-YAGI, Y. HATEFI, R. F. DOOLITTLE and G. ATTARDI, 1985 Six unidentified reading frames of human mitochondrial DNA encode components of the respiratory chain NADH dehydrogenase. *Nature* **314**: 592–597.
- COLLINS, R. A., 1986 *Neurospora crassa* laboratory strain 74-OR23-1A: mitochondrial genes, pp. 322–324 in *Genetic Maps 1987*, edited by S. J. O'BRIEN. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
- CREWS, S., D. OHALA, J. POSAKONY, J. NISHIGUCHI and G. ATTARDI, 1979 Nucleotide sequence of a region of human mitochondrial DNA containing the precisely identified origin of replication. *Nature* **277**: 192–198.
- CUMMINGS, D. J., L. BELCOUR and C. GRANDCHAMP, 1979 Mitochondrial DNA from *Podospora anserina*. *Mol. Gen. Genet.* **171**: 239–250.
- DEVRIES, H., J. C. DEJONGE and C. SCHRAGE, 1985 The *Neurospora* mitochondrial stopper mutant, [E35], lacks two protein genes indispensable for the formation of complexes I, III, and IV, pp. 285–292 in *Achievements and Perspectives of Mitochondrial Research Volume II: Biogenesis*, edited by E. QUAGLIARIELLO. Elsevier Science Publishers, New York.
- DEVRIES, H., B. ALZNER-DEWEERD, C. A. BREITENBERGER, D. D. CHANG, J. C. DEJONGE and U. L. RAJBHANDARY, 1986 The E35 stopper mutant of *Neurospora crassa*: precise localization of deletion endpoints in mitochondrial DNA and evidence that the deleted DNA codes for a subunit of NADH dehydrogenase. *EMBO J.* **5**: 779–785.
- GROSS, S. R., T. HSIEH and P. H. LEVINE, 1984 Intramolecular recombination as a source of mitochondrial chromosome heteromorphism in *Neurospora*. *Cell* **38**: 233–239.
- GROSS, S. R., P. H. LEVINE, S. METZGER and G. GLASER, 1989 Recombination and replication of plasmid-like derivatives of a short section of the mitochondrial chromosome of *Neurospora crassa*. *Genetics* **121**: 693–701.
- LESTER, H., and S. R. GROSS, 1959 Efficient method for selection of auxotrophic mutants of *Neurospora*. *Science* **129**: 572.
- MANNELLA, C. A., R. GOEWERT and A. M. LAMBOWITZ,

- 1979 Characterization of variant *Neurospora crassa* mitochondrial DNAs which contain tandem reiterations. *Cell* **18**: 1197–1207.
- NELSON, M. A., and G. MACINO, 1987 Three class I introns in the ND4L/ND5 transcriptional unit of *Neurospora crassa* mitochondria. *Mol. Gen. Genet.* **206**: 318–325.
- PALMER, J., and C. SHIELDS, 1984 Tripartite structure of the *Brassica campestris* mitochondrial genome. *Nature* **307**: 437–440.
- RYAN, F. J., G. W. BEADLE and E. L. TATUM, 1943 The tube method of measuring the growth rate of *Neurospora*. *Am. J. Bot.* **30**: 784–799.
- SOUTHERN, E., 1975 Detection of specific sequences among DNA fragments separated by gel electrophoresis. *J. Mol. Biol.* **98**: 503–517.
- TAYLOR, J. W., and B. D. SMOLICH, 1985 Molecular cloning and physical mapping of the *Neurospora crassa* 74-OR23-1A mitochondrial genome. *Curr. Genet.* **9**: 597–603.
- TURKER, M. S., J. M. DOMENICO and D. J. CUMMINGS, 1987 A novel family of mitochondrial plasmids associated with longevity mutants of *Podospora anserina*. *J. Biol. Chem.* **262**: 2250–2255.
- WAHL, G. M., M. STERN and G. R. STARK, 1979 Efficient transfer of large DNA fragments from agarose gels to diazobenzoyloxymethyl-paper and rapid hybridization by using dextran sulfate. *Proc. Natl. Acad. Sci. USA* **76**: 3683–3687.
- YIN, S., J. BURKE, D. CHANG, K. BROWNING, J. HECKMAN, B. ALZNER-DEWEERD, B. POTTER and U. L. RAJBHANDARY, 1982 *Neurospora crassa* mitochondrial tRNAs and rRNAs: structure, gene organization and DNA sequences, pp. 361–373 in *Mitochondrial Genes*, edited by P. SLONIMSKI, P. BORST and G. ATTARDI. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.

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