Proc. Natl. Acad. Sci. USA
Vol. 77, No. 10, pp. 6032–6036, October 1980
Genetics

Deletion mutants of *Neurospora crassa* mitochondrial DNA and their relationship to the "stop-start" growth phenotype

(extranuclear mutant/illegitimate recombination/restriction enzyme analysis)

HELMUT BERTRAND*, RICHARD A. COLLINS†, LORI L. STOHL†, ROBERT R. GOEWERT†, AND ALAN M. LAMBOWITZ†‡

*Department of Biology, University of Regina, Regina, Saskatchewan, S4S 0A2, Canada; and †The Edward A. Doisy Department of Biochemistry, Saint Louis University School of Medicine, Saint Louis, Missouri 63104

Communicated by Norman H. Giles, June 20, 1980

ABSTRACT "Stoppers" are a class of *Neurospora crassa* extranuclear mutants characterized by gross deficiencies of cytochromes *b* and aa_3 and an unusual growth phenotype which involves irregular periods of growth and nongrowth. In the present work, mtDNAs from all four stopper mutants were found to contain deletions or insertions detectable by restriction enzyme analysis. [*stp*] mtDNA consists predominantly of defective molecules which retain a 16-megadalton segment (*Eco*RI-1, -4, and -6) of wild-type mtDNA (40 megadaltons). The other stopper mutants show smaller alterations: [*stp A18t*]-618, a 0.35-kilobase deletion in *Eco*RI-7b; [*stp B2*]-651, a 4-kilobase insertion in *Eco*RI-2; and [*stp A*]-574, a 5-kilobase deletion in *Eco*RI-2 and -10. Based on these results, we propose that "stop-start" growth results from competition between certain defective mtDNAs which have a tendency to predominate and low concentrations of less defective mtDNA species which must be retained to sustain growth. Three additional extranuclear mutants ("nonstoppers") have also been found to contain deletions in mtDNA. Remarkably, the defective mtDNA species in two of these mutants ([*poky*] H1-10 and [*SG-3*]-551) retain different sizes (18 and 13 megadaltons, respectively) of the same region retained in [*stp*] mtDNA (i.e., *Eco*RI-1, -4, and -6). The findings suggest that production of defective mtDNAs in *Neurospora* is nonrandom with a preferred mechanism leading to retention of this segment. It may be significant that the retained segment contains both mitochondrial rRNA genes and most mitochondrial tRNA genes. These deletion mutants may provide a tool for genetic mapping of *Neurospora* mtDNA.

"Stoppers" are a class of *Neurospora crassa* extranuclear mutants distinguished by an unusual growth pattern on solid media: in contrast to wild-type strains which grow by continuous extension of hyphal tips, stopper mutants alternate between periods of growth and nongrowth, either of which may last for a few hours or several days (1–5). Stopper mutants are also characterized by gross alterations in mitochondrial phenotype including severe deficiencies of cytochromes *b* and aa_3 (1–5). These findings suggest that the stopper phenotype results from a mutation in mtDNA, but the relationship between the mitochondrial defect and the growth characteristics has remained obscure.

Recently, in the course of studying variant *Neurospora* mtDNAs which contain tandem reiterations, Mannella *et al.* (6) identified a unique derivative of the extranuclear [*poky*] mutant ([*poky*] H1-10) in which the predominant mtDNA is a defective species containing an 18-megadalton (MDal) segment (*Eco*RI-1, -4, and -6) of wild-type mtDNA (40 MDal). The [*poky*] H1-10 strain also contains a lower level of intact mtDNA (6). Although defective mtDNAs are well known in yeast petite mutants, these had been considered a special case because yeast is a facultative aerobe which can survive by fermentation in the complete absence of mitochondrial function (7). The retention of low levels of intact mtDNA in the [*poky*] H1-10 strain was assumed to reflect the fact that *Neurospora* is an obligate aerobe which requires at least some functional mitochondria for growth.

The above findings suggest that defective mtDNAs might also be relevant to the stopper mutants. If we assume that some defective mtDNA species have a selective advantage in heteroplasmons with wild-type mtDNA, then growth stoppage could be accounted for by accumulation of defective mtDNAs at the hyphal tips. Resumption of growth would then require either "catch-up" replication by less defective mtDNAs or extension of new hyphal tips from more distal sectors of the culture. In the present work, we sought to test this hypothesis as a first step in the systematic identification and isolation of additional deletion mutants of *Neurospora* mtDNA. The predominant mtDNA species in all four stopper mutants were found to contain deletions or insertions detectable by restriction enzyme analysis. mtDNAs from two additional extranuclear mutants, 540 and [*SG-3*]-551, were also found to contain deletions. The results provide insight into the consequences of defective mtDNAs in obligately aerobic organisms and may be relevant to the development of genetic mapping methods for *Neurospora* mtDNA.

MATERIALS AND METHODS

Strains of Neurospora. Strains having wild-type mtDNA were 74-OR-1*A* (abbreviated 74*A*) and *nic*-1 *al-2 A* [+]. Extranuclear mutants were: [*stp*] (subisolates 596, 606, and 610; each having slightly different growth characteristics), [*stp A*]-574, [*stp A18t*]-618, [*stp B2*]-651, 540, and [*SG-3*]-551, a unique subculture of the [*SG-3*] group I extranuclear mutant (1–5).

The stopper mutants studied were subcultures recovered after storage for 11 years on silica gel. In each case, slow growth and deficiencies of cytochromes *b* and aa_3 were confirmed. *Bona fide* stopper growth was confirmed for [*stp*] (596, 606, and 610), [*stp A*]-574, and [*stp B2*]-651. [*stp A18t*]-618 showed a growth pattern similar to that of the [*poky*] mutant, characterized by a slow initial growth phase. Mutant 540 is an extranuclear mutant deficient in cytochromes *b* and aa_3, discovered in Bertrand's culture collection. The strain was originally thought to be a subculture of [*stp C*] (3) but was subsequently found to have characteristics not expected for the [*stp C*] subculture.

Isolation and Restriction Enzyme Analysis of mtDNA. Methods used for isolation of *Neurospora* mtDNA and restriction enzyme analysis have been described (6). Preparations of *Neurospora* mtDNA ordinarily consist of linear molecules

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U. S. C. §1734 solely to indicate this fact.

Abbreviations: kb, kilobase(s); MDal, megadalton.

‡ To whom reprint requests should be addressed.

with molecular mass distributed around 20 MDal, reflecting two or more double-strand breaks per molecule (6). Some preparations show decreased yields of high molecular weight restriction fragments. *Eco*RI, *Hin*dIII, and *Hin*cII digests were analyzed by electrophoresis on horizontal slab gels containing 0.8% agarose; *Hpa* II, *Hae* III, and *Msp* I digests were analyzed on vertical slab gels containing a linear gradient of 4–9% polyacrylamide (6).

Assay of Mitochondrial Protein Synthesis. Rates of mitochondrial and whole-cell protein synthesis were measured by incorporation of L-[4,5-^{3}H]leucine into trichloroacetic acid-precipitable material in the presence or absence of cycloheximide (8). Mitochondrial translation products were identified on NaDodSO$_4$/polyacrylamide gradient gels by a modification of the procedure of Douglas and Butow (9) to be described elsewhere.

RESULTS

mtDNA from [*stp*]. Figs. 1–3 show restriction enzyme digests of mtDNAs from wild-type and several mutant strains. The digest patterns for the three [*stp*] subisolates (596, 606, and 610) were distinguished by a few bands strikingly amplified with respect to the remaining bands (e.g., *Eco*RI-1, -4, and -6 in Fig. 1). Such patterns are assumed to reflect mixtures of defective mtDNAs (amplified fragments) and lower levels of more intact mtDNAs (nonamplified fragments). It should be noted that the nonamplified fragments show somewhat different patterns for the different [*stp*] subisolates, that they include "novel" fragments which do not comigrate with wild-type fragments, and that some wild-type fragments (e.g., *Eco*RI-3) cannot be detected (see *Discussion*). The structure of the predominant mtDNA can be deduced from the digest patterns. Fig. 4 shows that the amplified *Eco*RI, *Hin*cII, and *Hin*dIII fragments define a continuous 16-MDal segment of wild-type mtDNA. Wild-type and [*stp*] mtDNA sequences were compared in more detail by polyacrylamide gel analysis of *Hpa*II digests to display a large number of small fragments. As expected, [*stp*] mtDNA showed amplified and nonamplified *Hpa* II fragments, and all but one of the amplified fragments comigrated with a wild-type fragment (Fig. 3). Together, the results suggest that the predominant mtDNA in [*stp*] consists of a continuous 16-MDal segment of wild-type mtDNA that has not undergone substantial internal rearrangement. The single anomalous *Hpa* II fragment (upper arrow, Fig. 3) could be derived from a junction or an end or could represent an internal deletion.

Two discrepancies in the digest patterns should be noted here: (*a*) *Hin*cII-3 should be amplified in [*stp*] mtDNA (Fig. 4) but does not appear amplified in the gel pattern (Fig. 2); and (*b*) *Hin*cII-13 is amplified in the gel pattern (Fig. 2) but is not continuous with the retained 16-MDal segment according to Fig. 4. The first discrepancy can be resolved by assuming that *Hin*cII-3 is part of a larger composite fragment that comigrates

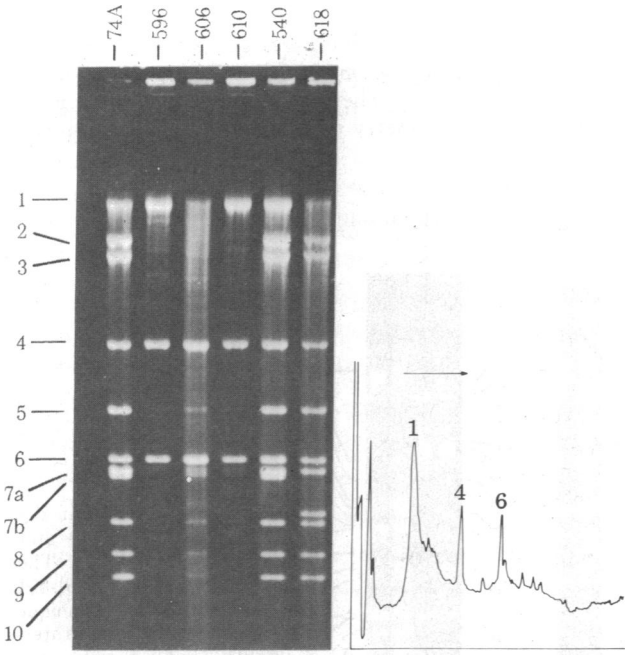

FIG 1. (*Left*) *Eco*RI digests of mtDNA from wild-type 74A, [*stp*] (subisolates 596, 606, and 610), 540, and [*stp A18t*]-618. (*Right*) Scan of the digestion pattern for [*stp*]-610 obtained from a negative of the gel photograph. Some light bands visible in the original photograph have been lost here.

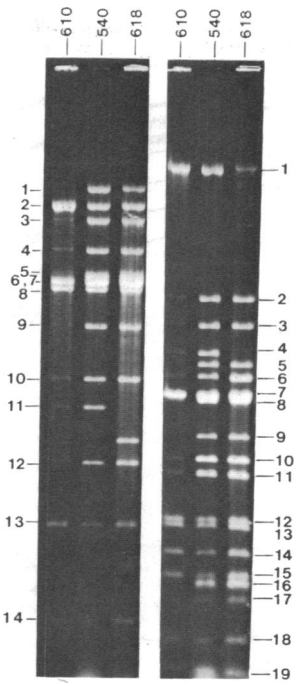

FIG. 2. *Hin*cII (*Left*) and *Hin*dIII (*Right*) digests of mtDNA from [*stp*]-610, 540, and [*stp A18t*]-618. The gel patterns for wild type 74A are indistinguishable from those for 540 (data not shown).

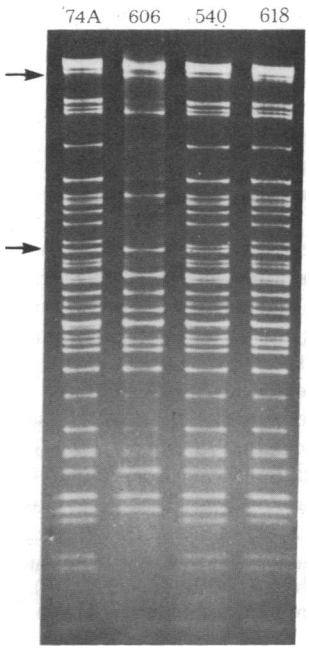

FIG. 3. *Hpa* II digests of mtDNA from wild-type 74A, [*stp*]-606, 540, and [*stp A18t*]-618. The upper arrow indicates the altered fragment in [*stp*]-606; the lower arrow indicates the altered fragment in 540. Two alterations in the digest of [*stp A18t*]-618 are also detectable but not indicated. Digest patterns for [*stp*]-596 and 610 show the same amplified bands as in the pattern for [*stp*]-606 (data not shown). *Hpa* II cleaves the sequence C-C-G-G only if the internal cytosine is unmethylated whereas *Msp* I cleaves the same sequence irrespective of methylation of the internal cytosine (10). *Msp* I and *Hpa* II digest patterns were found to be identical for wild-type 74A, 540, and [*stp A18t*]-618 mtDNA (data not shown).

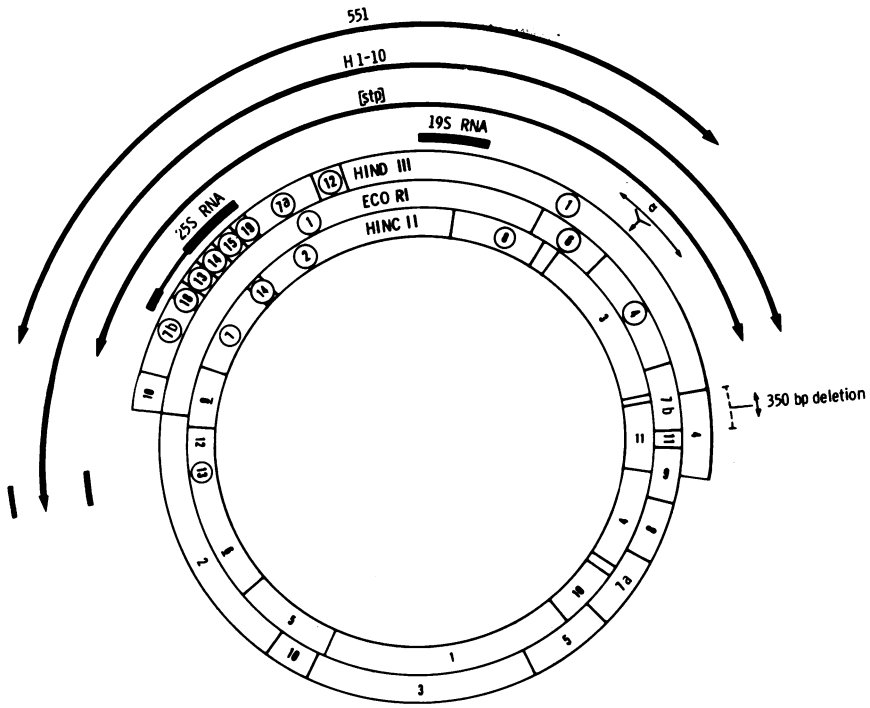

FIG. 4. Restriction enzyme cleavage maps of *Neurospora* mtDNA: the *Eco*RI map is from refs. 11 and 12; the *Hin*cII map is that recently described by de Vries *et al.* (13); the *Hin*dIII map is that described in refs. 13–15. α, 1.4-MDal sequence at the junction of *Eco*RI-4 and -6, amplified by tandem reiterations in some mutant strains (6). Solid lines indicate continuous mtDNA segments which appear to be retained in [*stp*], [*poky*] H1-10, and [*SG-3*]-551. The circled numbers indicate restriction fragments amplified in [*stp*] mtDNA. The 0.35-kb deletion in [*stp A18t*] is indicated.

with *Hin*cII-2 (see Fig. 2). Such a fragment, formed by illegitimate recombination, would be expected if the retained sequences were organized as multimers (see *Discussion*). The discrepancy involving *Hin*cII-13 has three possible explanations: (*i*) the published *Hin*cII map is incorrect, (*ii*) *Hin*cII-13 has been joined to the retained 16-MDal segment by illegitimate recombination, or (*iii*) *Hin*cII-13 is present on a separate class of defective mtDNA molecules.§

mtDNAs from [*stp A18t*], [*stp A*], and [*stpB2*]. The remaining stopper mutants contained smaller deletions or insertions. For [*stp A18t*]-618, the restriction patterns showed a 0.350-kb deletion that appeared in *Eco*RI-7b, *Hin*cII-11, and *Hin*dIII-4 (Figs. 1 and 2). Fig. 4 shows that the deletion can be mapped unambiguously from the affected fragments. mtDNAs from two other stopper mutants were characterized by *Eco*RI-digest patterns only. mtDNA from [*stp B2*]-651 had a 4-kb insertion in *Eco*RI-2 and the predominant mtDNA in [*stp A*]-574 had a 5-kb deletion in *Eco*RI-2 and -10 (data not shown).

mtDNA from Extranuclear Mutant 540. Extranuclear mutant 540 was found to have defects in mitochondrial ribosome assembly similar to those in [*poky*] and the other group I extranuclear mutants (ref. 16; unpublished data). mtDNA from 540 showed no detectable alteration in digest patterns with *Eco*RI, *Hin*cII, *Hin*dIII (Figs. 1 and 2), or *Hae* III (data not shown). However, *Hpa* II or *Msp* I digests of 540 showed a single band of increased mobility corresponding to a deletion of less than 10 base pairs (Fig. 3). The affected *Hpa* II fragment was not amplified in [*stp*] mtDNA and migrated normally in digests of [*stp A18t*]-618 mtDNA (Fig. 3). These findings indicate that the deletion in 540 is outside of the region retained in [*stp*] and does not overlap the deletion in [*stp A18t*].

mtDNA from [SG-3]-551. Defective mtDNAs were also identified in [*SG-3*]-551, a specific subculture of the [*SG-3*] group I extranuclear mutant. The digest patterns with *Eco*RI, *Hin*cII, and *Hin*d III suggest at least two classes of molecules which contain deletions and possibly other alterations (Fig. 5). The predominant species (amplified fragments) retained a 13-MDal segment defined by *Eco*RI-1 and -6. (Note that *Hin*cII-13 is also amplified, creating the same discrepancy discussed previously for [*stp*] mtDNA.) The less defective mtDNA species (nonamplified fragments) retained most wild-type mtDNA restriction fragments but had lost *Eco*RI-2, -3, and -10. Novel fragments also appear to be present (e.g., above wild-type *Eco*RI-2; see Fig. 5).

Mitochondrial Protein Synthesis in Mutant Strains. The mutants studied here are characterized by slow growth rates and gross deficiencies of cytochromes *b* and aa_3 (1–5). The cytochrome deficiency could be correlated with strongly decreased rates of mitochondrial protein synthesis in all mutants examined (Table 1). Fig. 6 compares gel patterns of mitochondrially synthesized polypeptides in wild-type, [*stp*]-606, [*stp A18t*]-618, and 540. The mutants showed no obvious changes in the stoichiometry of major translation products

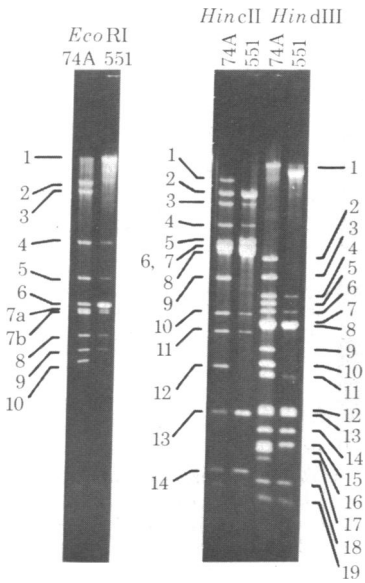

FIG. 5. Restriction digests of wild-type 74A and [*SG-3*]-551 mtDNAs. The *Hin*cII digest of [*SG-3*]-551 showed an apparently unique fragment that migrated slightly behind wild-type *Hin*cII-5 and that could be derived from a junction (if the retained sequences were organized as multimers) or an end.

§ Recent mapping studies have shown that *Hin*cII-13 is a subfragment of *Eco*RI-6 and not of *Eco*RI-2 as published by de Vries *et al.* (13). The revised map position is consistent with amplification of *Hin*cII-13 in [*stp*] and [*SG-3*]-551 mtDNA.

Table 1. Rates of whole-cell and mitochondrial protein synthesis

Strain	Rate* Whole cell	Rate* Mitochondrial	Ratio†
74A	10,650	993	0.093
606	4,922	179	0.036
610	3,271	36	0.011
618	5,116	112	0.023
540	5,870	92	0.016
651	2,158	62	0.028

* Expressed as ^{3}H cpm incorporated per mg of whole cell or mitochondrial protein per min of incorporation.
† Ratio of the rates of mitochondrial to whole-cell protein synthesis.

compared to wild type. However, all three mutants did show additional polypeptides, these being particularly prominent in [*stp A18t*]-618 and 540. The additional polypeptides could be directly related to the mutations or could merely reflect increased proteolytic activity in the mutant strains. Preliminary results for [*SG-3*]-551 showed a deficiency of one major polypeptide, probably ATPase subunit 13, but with no obvious changes in the stoichiometry of other translation products (data not shown).

DISCUSSION

The present work supports the hypothesis that stopper growth characteristics result from accumulation of defective mtDNA molecules. mtDNAs from all four stopper mutants were found to contain deletions or insertions detectable by restriction enzyme analysis whereas such large mtDNA alterations are not characteristic of other classes of *Neurospora* extranuclear mutants. In principle, the stopper phenotype could be caused by any "tight" mutation that results in loss of mitochondrial function and also confers some selective advantage on the mutant mtDNA. The results here suggest that a substantial proportion of such mutations involve deletions or insertions in mtDNA.

Early studies by Pittenger (17) and Diacumakos *et al.* (18) showed that some *Neurospora* extranuclear mutants predominate over wild type in heteroplasmons formed by heterokaryosis or by microinjection of mutant mitochondria. The mechanism underlying this phenomenon is unknown but could involve either some replicative advantage of mutant mtDNA (19, 20) or asymmetric gene conversion (21). Consistent with our hypothesis, all stopper mutants studied here have been found to predominate over wild type in heteroplasmons (2).

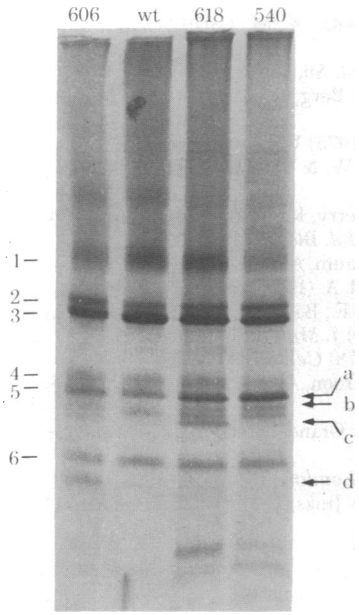

FIG. 6. Gel patterns of mitochondrial translation products from wild type (*nic-1 al-2 A* [+]), [*stp*]-606, [*stp A18t*]-618, and 540. Major translation products were identified by gel mobility: 1, cytochrome oxidase subunit I; 2, cytochrome *b*; 3, cytochrome oxidase subunit II; 4, cytochrome oxidase subunit III; 5, ATPase subunit 9; 6, unknown. Arrows indicate novel polypeptides in the mutants.

Previous work showed that stopper mutants may change growth characteristics during subculturing. The [*stp*] mutant, for example, gave rise to three subcultures, 596, 606, and 610, which have distinguishable growth characteristics. In the present work, *bona fide* stopper growth was confirmed for the three [*stp*] subisolates, for [*stp A*]-574, and for [*stp B2*]-651 whereas [*stp A18t*]-618 showed a slow but continuous initial growth phase similar to that of [*poky*]. In terms of our hypothesis, variable growth characteristics could be accounted for by changes in factors that influence the competition between defective mtDNAs and more intact mitochondrial genomes. For example, cells in the "stop" phase should show strong selection for nuclear or extranuclear mutations that permit resumption of growth. We have also identified three strains, 540, [*SG-3*]-551, and [*poky*] H1-10 (6), in which mtDNA deletions do not result in stop-start growth. There are two possible explanations for such strains: (*a*) the deletion involves a nonessential region or gives rise to a "leaky" phenotype or (*b*) the competition between the defective and more intact mtDNAs results in a stable ratio of these DNAs in heteroplasmons. The first possibility may be relevant to mutant 540 which had a deletion of less than 10 base pairs. We suspect that [*SG-3*]-551 and [*poky*] H1-10 (unique derivatives of the [*SG-3*] and [*poky*] mutants) may represent the second situation in which the defective mtDNA must compete with a group I mutant mtDNA which is itself suppressive (20).

Because *Neurospora* is an obligate aerobe, it seems likely *a priori* that strains that contain defective mtDNAs must retain a low level of more intact mitochondrial genomes to support growth. This idea was supported by the previous results (6) for [*poky*] H1-10. However, the present data are equivocal on this point. Although the three [*stp*] subisolates and [*SG-3*]-551 appear to contain low levels of less defective mtDNAs (nonamplified fragments), some wild-type fragments cannot be detected at all in the digest patterns [e.g., *Eco*RI-3 in the case of [*stp*] and *Eco*RI-2, -3, and -10 in the case of [*SG-3*]-551 (Figs. 1, 2, and 5)]. Likewise, in [*stp A18t*]-618, the gel patterns do not show a "light" fragment corresponding to wild-type *Eco*RI-7b which is the site of a 0.350-kb deletion in the predominant mtDNA (Fig. 1). It is possible, if not likely, that all essential wild-type mtDNA sequences are present and that sequences that appear to be missing are nonessential, are present below the limit of detection, or are incorporated into other fragments. An interesting observation is that the digest patterns for the three [*stp*] subisolates show somewhat different patterns of nonamplified fragments. It is conceivable that novel mtDNAs continue to be generated in these strains by illegimate recombination and that the predominant mtDNA, containing *Eco*RI-1, -4, and -6, retains some selective advantage with respect to the new combinations.

Remarkably, the predominant mtDNA species in three different strains—[*stp*] and [*SG-3*]-551 and [*poky*] H1-10 (6)—all retain different lengths of the wild-type mtDNA segment defined by *Eco* RI-1, -4, and -6. This finding suggests that the production of defective mtDNAs in *Neurospora* is nonrandom, with a preferred mechanism leading to retention of *Eco*RI-1, -4, and -6, the segment that contains both rRNA genes and the large majority of tRNA genes (11–15). By contrast, defective mtDNAs in yeast petite mutants have not been found to retain specific regions preferentially (7).

Sequences at the boundary of *Eco*RI-4 and -6 attract particular attention for two reasons: (*i*) all defective mtDNA species

identified so far retain *Eco*RI-6 (and possibly adjoining sequences in *Eco*RI-4) and (*ii*) a 1.4-MDal sequence at the boundary of *Eco*RI-4 and -6 has been found to be amplified by tandem reiterations in some mutant strains (6). The latter may recall λdv plasmids (22, 23) and simian virus 40 or polyoma virus reiteration mutants (24–27) which are characterized by tandem repeats of segments containing the replication origin. Based on these findings, we speculate that the replication origin of *Neurospora* mtDNA will be found near the boundary of *Eco*RI-4 and -6. Some clue about mechanisms for production of defective mtDNAs in *Neurospora* may be provided by information about the location of the replication origin, the mode of replication, and DNA sequences at the boundaries of the defective molecules. Bernardi and coworkers (28) have proposed that defective mtDNAs of spontaneous petite mutants may be generated by nonrandom mechanisms that involve site-specific illegitimate recombination taking advantage of localized homologous sequences (specifically, G+C clusters and A+T tracts). Such a mechanism could also account for nonrandom generation of defective mtDNAs in *Neurospora*. The large size of *Neurospora* defective mtDNAs, compared to petite yeast mtDNAs (cf. ref. 7), may be related to a smaller number of repetitive sequence elements.

The mtDNA of [*stp A18t*]-618 had a 0.350-kb deletion in *Eco*RI-7b. It can be seen in Fig. 1 that the nonamplified fragment in [*stp*]-596 had exactly the same deletion. This situation could be another case of nonrandom generation of deletions. *Eco*RI-2 and -3 are also implicated as "hot spots," alterations in these fragments being noted in several strains.

Some yeast petite mtDNAs have been found to consist of oligomers of retained wild-type mtDNA sequences organized as tandem or inverted repeats (7). The data for the defective [*stp*] and [*SG-3*]-551 mtDNAs give no insight into this level of structure. Indeed, even the inference that the amplified fragments (*Eco*RI-1, -4, and -6) are contiguous on the same mtDNA molecule has not been directly proved. More detailed information could be obtained by isolation of intact circular mtDNA molecules which has proven difficult for yeast and *Neurospora* (29, 30). So far, we have been unable to obtain circular mtDNA species from [*stp*]-610 by CsCl/ethidium bromide centrifugation.

Gel patterns of mitochondrial translation products for [*stp*], [*stp A18t*]-618, 540, and [*SG-3*]-551 show normal stoichiometry of major products, although some patterns do show novel polypeptides which may be related to the mutations (Fig. 6). The failure to find deficiencies of major translation products is unexpected, particularly for [*stp*] and [*SG-3*]-551 in which more than half of the mitochondrial genome is deleted from the predominant mtDNA species. Three explanations come to mind: (*i*) that the structural genes for the major translation products all are present on the predominant mtDNA species, (*ii*) that some or all of the structural genes are deleted and only genes on nondefective genomes are expressed, and (*iii*) that the stoichiometry of the major translation products is regulated so that gene dosage effects are obscured.

An important general implication of the present work is that the occurrence of defective mtDNAs may be more widespread than had been believed previously. In particular, the results suggest that obligately aerobic cells can harbor defective mtDNAs so long as low levels of less defective mitochondrial genomes are retained to sustain growth. Cummings *et al.* (31) recently identified multimeric circular DNA molecules of unit length 2.6 kb in mitochondria from senescent strains of the fungus *Podospora anserina*. The "red" variant of *Aspergillus* also has characteristics similar to those of *Neurospora* stopper mutants: in particular, the responsible factor is lethal in the homoplasmic state but also is suppressive with respect to the normal factor (32, 33). At present, it is not known whether animal cells might also harbor defective mtDNAs. If so, these may have important consequences for cellular pathology.

The authors thank Mr. Kenneth Figlioli, Bro. Paul Galantowicz, and Ms. Ruth Kaufman for excellent technical assistance. This work was supported by National Institutes of Health Grant GM 23961 and a Basil O'Connor Research Grant from the National Foundation–March of Dimes to A.M.L. and by National Sciences and Engineering Council of Canada Grant A-6351 to H.B.

1. McDougall, K. J. & Pittenger, T. H. (1966) *Genetics* **54**, 551–565.
2. Bertrand, H. & Pittenger, T. H. (1969) *Genetics* **61**, 643–659.
3. Bertrand, H. & Pittenger, T. H. (1972) *Genetics* **71**, 521–533.
4. Bertrand, H. & Pittenger, T. H. (1972) *Mol. Gen. Genet.* **117**, 82–90.
5. Bertrand, H., Szakacs, N. A., Nargang, F. E., Zagozeski, C. A., Collins, R. A. & Harrigan, J. C. (1976) *Can. J. Genet. Cytol.* **18**, 397–409.
6. Mannella, C. A., Goewert, R. R. & Lambowitz, A. M. (1979) *Cell* **18**, 1197–1207.
7. Locker, J., Lewin, A. & Rabinowitz, M. (1979) *Plasmid* **2**, 155–181.
8. Nargang, F. E., Bertrand, H. & Collins, R. A. (1979) *Curr. Genet.* **1**, 1–7.
9. Douglas, M. G. & Butow, R. A. (1976) *Proc. Natl. Acad. Sci. USA* **73**, 1083–1086.
10. Waalwijk, C. & Flavell, R. A. (1978) *Nucleic Acids Res.* **5**, 3231–3236.
11. Bernard, U., Goldthwaite, C. & Kuntzel, H. (1976) *Nucleic Acids Res.* **3**, 3101–3108.
12. Terpstra, P., Holtrop, M. & Kroon, A. M. (1977) *Biochim. Biophys. Acta* **475**, 571–588.
13. de Vries, H., de Jonge, J. C., Bakker, H., Meurs, H. & Kroon, A. (1979) *Nucleic Acids Res.* **6**, 1791–1803.
14. Hahn, U., Lazarus, C. M., Lunsdorf, H. & Kuntzel, H. (1979) *Cell* **17**, 191–200.
15. Heckman, J. E. & RajBhandary, U. L. (1979) *Cell* **17**, 583–595.
16. Collins, R. A., Bertrand, H., LaPolla, R. J. & Lambowitz, A. M. (1979) *Mol. Gen. Genet.* **177**, 73–84.
17. Pittenger, T. H. (1956) *Proc. Natl. Acad. Sci. USA* **42**, 747–752.
18. Diacumakos, E. G., Garnjobst, L. & Tatum, E. L. (1965) *J. Cell Biol.* **26**, 427–443.
19. Tatum, E. L. & Luck, D. J. L. (1967) in *Control Mechanisms in Developmental Processes*, ed. Locke, M. (Academic, New York), pp. 32–42.
20. Mannella, C. A. & Lambowitz, A. (1978) *Biochem. Biophys. Res. Commun.* **80**, 673–679.
21. Mannella, C. A. & Lambowitz, A. M. (1979) *Genetics* **93**, 645–654.
22. Berg, D. E. (1974) *J. Mol. Biol.* **86**, 59–68.
23. Chow, L. T., Davidson, N. & Berg, D. (1974) *J. Mol. Biol.* **86**, 69–89.
24. Folk, W. R. & Fishel, B. R. (1975) *Virology* **64**, 447–453.
25. Lee, T. N. H., Brockman, W. W. & Nathans, D. (1975) *Virology* **66**, 53–69.
26. Khoury, G., Fareed, G. C., Berry, K., Martin, M. A., Lee, T. N. H. & Nathans, D. (1974) *J. Mol. Biol.* **87**, 289–301.
27. Davoli, D., Ganem, D., Nussbaum, A. L., Fareed, G. C., Howley, P. M., Khoury, G. & Martin, M. A. (1977) *Virology* **77**, 110–124.
28. Faugeron-Fonty, G., Culard, F., Baldacci, G., Goursot, R., Prunell, A. & Bernardi, G. (1979) *J. Mol. Biol.* **134**, 493–537.
29. Borst, P. & Grivell, L. A. (1978) *Cell* **15**, 705–723.
30. Terpstra, P., Holtrop, M. & Kroon, A. (1977) *Nucleic Acids Res.* **4**, 129–139.
31. Cummings D. J., Belcour, L. & Grandchamp, C. (1979) *Mol. Gen. Genet.* **171**, 239–250.
32. Arlett, C. F. (1957) *Nature (London)* **179**, 1250–1251.
33. Arlett, C. F., Grindle, M. & Jinks, J. L. (1962) *Heredity* **17**, 197–209.