A Combination Inversion and Translocation in *Neurospora crassa* With Inviable Deficiency Progeny That Can Be Rescued in Heterokaryons

Edward G. Barry

Department of Biology, University of North Carolina, Chapel Hill, North Carolina 27599

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ABSTRACT

Chromosome rearrangement In(II;IR)(II;IIIR)SLm-1, has a pericentric inversion in linkage group I associated with a reciprocal translocation between I and III. The rearrangement was identified cytologically in pairing with normal sequence chromosomes at pachynema. Rearrangement breakpoints were mapped genetically in II, IR and IIIIR by crosses with normal sequence strains and in crosses with an inversion that partially overlaps the SLm-1 inversion. When rearrangement SLm-1 is crossed to parents with normal sequence chromosomes, one class among the progeny has a small chromosome deficiency and large duplication. The ascospores containing this deficiency/duplication died either before germination or just after, when growth commences. Germ tubes of the deficiency/duplication progeny, which start to grow then stop, resemble the aborted growth of auxotrophic mutants germinated on minimal medium. Efforts to correct the deficiency with nutritional supplements were not successful. However, the defective class can be rescued by fusing the germinating hyphae of the deficiency ascospore with a complementary auxotrophic mutant to form a heterokaryon.

A deficiency/duplication nucleus that is rescued in a heterokaryon can serve as a fertilizing nucleus in crosses with a normal sequence parent. One half of their progeny have the normal chromosome sequence and one half have the chromosome deficiency syndrome and die at germination.

MATERIALS AND METHODS

Mutant strain SLm-1 was obtained from SAMSON R. GROSS (Duke University). The exact designation of the chromosome rearrangement it contains is In(II;IR)(II;IIIR)SLm-1 but it will be abbreviated here as SLm-1.

*Genetics:* Other mutant and marker strains were obtained from the collection of DAVID D. PERKINS or originated in this laboratory. Wild types used were 74-OR23-1A and ORSa.f° a and f°° a are acroconidiate strains that are used as standards for testing mating type and scoring normal, aberration or duplication chromosome sequence (PERKINS 1974; PERKINS et al. 1989). Linkage group I mutants used were ro-10 (AR7, ropy); f° (B110, frost); un-5 (b39, unknown temperature sensitive); A/a (mating types); arg-1 (B369, arginine requiring); his-2 (Y152 M14, histidine); nic-2 (43002, nicotinic acid); al-2 (15360, albino); al-1' (ALS4, albino); and R (35408R, Round Spore). Linkage group III mutants used were cum (P5241, cumulus); acr-2 (HK5, acridine resistant); and leu-1 (SLm-1, leucine). These mutant markers and the procedures for scoring them are described in PERKINS et al. (1982).

Unordered tetrads were obtained following the method described by STRICKLAND (1960) [see also PERKINS (1966b) for details]. Patterns of aborted (deficiency) ascospores in individual tetrads were used to diagnose chromosome rearrangements as described by PERKINS (1966a).

*Cytology:* Meiotic chromosomes were stained with aceto-orcein as described by BARRY (1966). f° and f°° stocks were used as the cytological standards of normal chromosome sequence in crosses to SLm-1. Neurospora intermedia strains P405 and P420, which have the same gross chromosome sequence as *N. crassa* and often make excellent pachytene chromosome preparations (PERKINS, TURNER and BARRY 1976), were also used as normal sequence parents in...
TABLE 1

<table>
<thead>
<tr>
<th>Zygote genotype and recombination %</th>
<th>Crossovers in intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Parental combinations</td>
</tr>
<tr>
<td>a N</td>
<td>1 2 1 and 2</td>
</tr>
<tr>
<td>A Ab leu-1</td>
<td>28 1 2 0</td>
</tr>
<tr>
<td>8.2 3.3</td>
<td></td>
</tr>
</tbody>
</table>

N is normal chromosome sequence. Ab is aberration (or rearrangement) sequence. Intervals are numbered from left to right. The numbers of a mating type progeny for every class are given in the upper line of the table. The numbers of A mating type progeny of the reciprocal classes are in the lower line. Seventy-one percent of isolated ascospores germinated and produced viable cultures. Ten progeny were slow growing and not included in the table.

crosses heterozygous for the SLm-1 rearrangement.

RESULTS

N. crassa mutant SLm-1 was isolated as a UV-induced, leucine-requiring, leu-1, mutant following selection for resistance to aminotriazole on medium containing 3-amino-1,2,4-triazole. Kidd and Gross (1984) found that the leu-1 mutation of SLm-1 is linked in the right arm of linkage group I, which is the normal location of leu-1 mutants, and also is linked to mating type in the left arm of linkage group I. This implied the presence of a translocation in SLm-1. I confirmed the presence of the chromosome rearrangement and discovered that the SLm-1 breakpoints are separable from the leu-1 mutation. Data in Table 1 show that the rearrangement is closely linked to mating type in linkage group I and to leu-1 in linkage group III with which it has recombined in about 3% of the progeny.

Tetrad analysis: The ascus patterns of black viable and white aborted ascospores in unordered octads shot from perithecia in crosses of chromosome rearrangements to wild type are used as a general guide to the type of rearrangement under investigation (Perkins 1974; Perkins and Barry 1977). The types and frequencies of ascospore patterns produced by SLm-1 crossed to wild type are listed in Table 2. The occurrence of 6B:2W asci (six black spores and two white spores) is characteristic of rearrangements in which one of the segmental aneuploid classes of progeny is viable and contains a duplication with no complementary deficiency. Such duplication progeny are usually derived from crosses where one parent has either an insertional translocation or an effectively terminal translocation. Some pericentric inversions with one breakpoint at the end of the chromosome beyond all essential genes also make duplication progeny when crossed to normal sequence strains (Newmeier and Taylor 1967; Turner et al. 1969). In Neurospora, duplication progeny from most duplication-producing rearrangements are barren, producing perithecia devoid of ascospores (Perkins and Barry 1977; Raju and Perkins 1978). There were few barren progeny from SLm-1 crosses. Furthermore, no 2:1 allele ratios were observed for genetic markers near breakpoints (see Table 1, for an example); 2:1 ratios are characteristic of crosses heterozygous for rearrangements that produce viable duplication progeny (Perkins 1974). (The 2:1 ratios result from survival of duplication progeny but death of the complementary deficiency progeny.) Thus, progeny analysis did not support the expectation, based on the ascus-pattern analysis, that SLm-1 was one of the types of rearrangements which would produce duplication-bearing progeny.

Only 60–70% of black ascospores from crosses heterozygous for SLm-1 grew up to produce cultures (see Table 1 for example). Microscopic inspection of the nonproductive ascospores revealed that many had formed short germ tubes, then ceased growing. In the typical situation with SLm-1 crosses, 20% of those ascospores that fail to produce cultures have germinated and show clearly the presence of a substantial germ tube. Additional nongrowing spores may show swelling and some disruptions of the spore wall. Genetic evidence presented below confirms the interpretation that most of the ascospores failing to produce a culture are nevertheless viable at the time germination starts.

Meiotic tetrads were isolated to determine if the origin of these black, viable, germinating, nongrowing ascospores were associated with a particular ascus class or pattern. Both ordered and unordered ascospore tetrads were isolated, and their germination was attempted on appropriate media. The germinating, nongrowing, black ascospores are found in asci with
of the complement are in this focal plane).

aborted spores in the opposite half of the ascus. The ascus to the two white spores, which are presumably chromosomes were examined after orcein staining. From ordered tetrads, it was found that the four black, germinating, nongrowing spores are the complement to four white, germinating, non-growing spores. These are in the opposite half of the ascus to the two white spores, which are presumably the complementary products.

Cytological analysis: SLm-1 stocks were crossed to normal sequence fl A or fl a testers and also to A and a strains of N. intermedia (P420 and P405). Meiotic chromosomes were examined after orcein staining.

A very small aberrant chromosome was observed. This is shown at metaphase I in Figure 1, where the small chromosome is not paired with a homologous chromosome region and has moved to the spindle pole prematurely. The small chromosome sometimes pairs partially at pachynema with one end of chromosome 1, and sometimes it pairs partially with the end of chromosome 3 (Figure 2). My interpretation is that this small chromosome consists of the short arm and centromere of chromosome 3 combined with a small tip of the short arm of chromosome 1.

In addition to the small rearrangement chromosome, a complementary very long chromosome was observed which paired at pachynema both with chromosomes 3 and 1. Pairing of the long rearrangement chromosome with part of normal chromosome 1 was often in a loop, which is characteristic of an inversion. A clear figure of the inversion pairing with chromosome 1 and the translocation pairing with chromosomes 1 and 3 is shown in Figure 3.

To determine if the inversion is paracentric or pericentric, crosses heterozygous for SLm-1 were examined for acentric chromosome fragments and dicentric chromosome bridges at anaphase and later meiotic stages. The frequency of bridges and fragments was not far above the usual background frequency of 10% in some crosses between normal sequence Neurospora strains. Thus, the inversion of the SLm-1 rearrangement is pericentric, because the bridge and fragment production would be very high if such a long inversion were paracentric.

Genetic analysis: Crosses to strains with genetic markers in linkage groups I and III confirmed the cytological evidence of a combined translocation and pericentric inversion in SLm-1.

Mapping of breakpoints: The breakpoint in linkage group III lies between acr-2 and leu-1. Data supporting this order are given in Table 3.

SLm-1 breakpoints in linkage group I were first determined in a cross using markers covering most of the length of the chromosome (Table 4). One rearrangement breakpoint is near or at fr and the second is proximal to al-1. Crossing over between fr and al-1 was much reduced from the normal rate, and double crossover progeny were recovered with almost the same frequency as apparent single crossovers. Thus, the genetic data provides these two lines of evidence for a pericentric inversion: (1) widely separated breakpoints in one chromosome with the centromere known to lie between them and (2) suppression of single crossovers between the two breakpoints.

SLm-1 was crossed to ro-10, the leftmost known marker in linkage group I (Table 5). Some of the progeny were mosaic morphologically for ro-10+ and ro-10. This was most apparent when a culture was transferred to minimal medium to test for leu-1. A culture scored ro-10+ by morphology in the original isolation tube might be quite clearly ro-10+ in the transfer tube. Isolates which were uncertain for ro-10 or leu-1 scoring were progeny-tested, and their revised scoring is entered in the second and fourth lines in Table 5.

The most likely explanation of mosaicism is that progeny are disomic as a result of chromosome nondisjunction. The disomics then break down during vegetative growth, making mixed cultures of disomic and euploid nuclei. Euploid nuclei have a selective advantage in growth, and subsequent transfers of the culture show the phenotype of the euploid majority nuclear type. Furthermore, only euploid nuclei will enter the sexual cycle, and a progeny test thus shows the stable, surviving euploid chromosome type. Disomics consist of two types. If normal linkage groups I and III accompanied the small translocation chromosome, the culture is a ro-10 mosaic in which the translocation chromosome (ro-10+) can be eliminated. Or, if the translocation complement is accompanied by a normal linkage group III, the culture is at first heterozygous for leu-1 but becomes leu-1+ with loss of normal III. No other chromosomes of these two disomic types can be lost because inviable deficiencies would result.

The linkage group II breakpoint lies between ro-
TABLE 3
Progeny from the cross SLm-1 leu-1 a × acr-2 A

<table>
<thead>
<tr>
<th>Zygote genotype and recombination %</th>
<th>Parental combinations</th>
<th>Crossovers in intervals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>acr f</td>
<td>N</td>
<td>27</td>
</tr>
<tr>
<td>acr s Ab leu-1</td>
<td></td>
<td>28</td>
</tr>
</tbody>
</table>

Conventions of the table are the same as for Table 1. acr' progeny of all classes are on the upper line. Seventy-three percent of ascospores isolated produced viable cultures. There were nine other progeny, not included in the table, with slow growing phenotypes that caused difficulty in scoring acr and leu.

10 and mating type (Table 5). fr must be proximal to the IL break point since progeny in Table 4 show no mosaic morphology for this marker, unlike the distal marker ro-10, which showed mosaicism.

Since the presumptive inversion breakpoints of SLm-1 were thought to be close to the breakpoints of another pericentric inversion, In(IL;IR)OY323 in linkage group I, SLm-1 was crossed to OY323. If the breakpoints of two intercrossed inversions are alternating in a partially overlapping pattern, crossing over in their common inverted sequence will produce viable progeny with duplications of the two separated, inverted regions in which the inversions do not overlap (Sturtevant and Beadle 1936; see Barry and Leslie 1982 and Turner and Perkins 1982 for examples of the technique in Neurospora). Neurospora strains which contain a duplication have a barren phenotype (Raju and Perkins 1978).

SLm-1 was crossed to In(IL;IR)OY323 strains with markers around the breakpoints. The progeny from these crosses are recorded in Tables 6 and 7. The results show that the SLm-1 inversion overlaps the OY323 inversion. The genotypes of some progeny in
Combined Inversion-Translocation

TABLE 4
Progeny from a cross of SLm-1 a × fr A al-I R

Recombinants in intervals

<table>
<thead>
<tr>
<th>Zygote genotype</th>
<th>Parentals</th>
<th>Singles</th>
<th>Doubles</th>
<th>Triples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>2 and 3</td>
<td>4 and 5</td>
<td>2, 3, 5</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2, 3, 5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The solid line represents linkage group I. The dashed line is linkage group III. Circles on the chromosome lines represent centromeres. fr progeny of all classes are on the upper line. Sixty-one percent of ascospores isolated produced viable cultures.

TABLE 5
Disomic identification and ro-10 linkage. Progeny from a cross of SLm-1 leu-1 a × ro-10 A

Recombinants in intervals

<table>
<thead>
<tr>
<th>Zygote genotype</th>
<th>Parentals</th>
<th>Singles</th>
<th>Doubles</th>
<th>Triples</th>
<th>Quadruples</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1, 2, 3</td>
<td>2, 3, 4</td>
<td>1, 2, 3</td>
<td>1, 2, 3</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1, 2, 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers in parentheses on lines 1 and 3 are based on the original phenotypic scoring of each isolate. Numbers on lines 2 and 4 are the revised tabulation after doubtfully classified isolates were progeny tested and their genotype determined for the stable euploid chromosome complement. ro-10 progeny of all classes are on lines 1 or 2.

Interval 1, between ro-10 and left breakpoint; interval 2, between left breakpoint and mt (A, a); interval 3, between mt and right breakpoint; interval 4, between right breakpoint and leu-1.

Table 6 are questionable because the duplications which are generated by exchanges in the common overlapping inverted region tend to break down and make scoring of markers uncertain. However, the main observation is that progeny of the expected barren duplication types do occur. Furthermore, the allele ratios of markers in the region inverted in common to both inversions are 1:1. Mating type, arg-1, his-2, and nic-2 show this ratio. In contrast, a marker (e.g., un-5) inverted in SLm-1 but not in OY323, or any marker (e.g., al-2) inverted in OY323 but not in SLm-1, is expected to show a 2+:1− phenotypic ratio because it will be duplicated and heterozygous in the progeny produced by a crossover in the long common inverted region. Observation of 2+:1− ratios for un-5 and al-2, as well as the un-5+ and al-2+ phenotypes of the duplication progeny, confirm the cytological and mapping results that show the pericentric inversion in SLm-1.

Figure 4 shows the normal genetic sequence of linkage groups I and III and the rearranged sequences of SLm-1. Figure 5 is an interpretation of how the normal chromosomes and SLm-1 chromosomes pair in meiosis. If the small translocation chromosome fails to pair with its homologous segments of III and IL, it will assort independently of them to give rise to disomics. Also, the random assortment of normal III centromere with either normal I or aberrant I centro-
TABLE 6
Progeny from a cross of Slm-1 A x In(IL;IR)OY323 al-2 nic-2 his-2 a

<table>
<thead>
<tr>
<th>Zygote genotype</th>
<th>Phenotype of progeny which have inversion sequence</th>
<th>Apparent crossover position</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>nic + his + mt + al</td>
<td>None (parental)</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>+ + A +</td>
<td>None (parental)</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>- - a -</td>
<td>1 and 2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>+ + A -</td>
<td>1 and 4</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>- - a +</td>
<td>1 and 5</td>
<td>3</td>
<td></td>
</tr>
</tbody>
</table>

Phenotype of progeny which are duplications

<table>
<thead>
<tr>
<th>nic + his + mt + al</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ + A +</td>
<td>1</td>
</tr>
<tr>
<td>- + A +</td>
<td>2</td>
</tr>
<tr>
<td>- - A +</td>
<td>3</td>
</tr>
<tr>
<td>- - a +</td>
<td>4</td>
</tr>
</tbody>
</table>

For several isolates it was doubtful whether they should be scored as duplication or as inversion because of a probable breakdown from duplication to inversion sequence. Markers in parentheses on the chromosome are not present in the cross but are included for orientation purposes.

TABLE 7
Progeny from a cross of Slm-1 a x In(IL;IR)OY323 arg-1 un-5 al-2 A

<table>
<thead>
<tr>
<th>Zygote genotype</th>
<th>Phenotype of progeny which have inversion sequence</th>
<th>Apparent crossover position</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>arg + mt + un + al</td>
<td>None (parental)</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>- A - -</td>
<td>None (parental)</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>+ A - -</td>
<td>1 and 2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>- A + +</td>
<td>1 and 3</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>+ A + +</td>
<td>2 and 3</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

Phenotype of progeny which are duplications

<table>
<thead>
<tr>
<th>arg + mt + un + al</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>+ a + +</td>
<td>1</td>
</tr>
<tr>
<td>- a + +</td>
<td>2</td>
</tr>
<tr>
<td>- A + +</td>
<td>3</td>
</tr>
</tbody>
</table>

a Two isolates were normal sequence because of crossovers within the inverted loops, shown unpaired in the diagram.

meres is predicted from Figure 5 if paired homologous centromeres regularly disjoin while nonhomologous centromeres segregate independently. This accounts for the second disomic class which consists of normal III in addition to aberrant I and aberrant III.

The Slm-1 deficiency/duplication chromosomes:
As described earlier, approximately 30% of the progeny spores from crosses of Slm-1 by wild type are black and normal-looking but fail to give rise to normal cultures. About 20% of these spores have produced short germ tubes. Ascospores which die on germination are encountered erratically in various other types of crosses and have been observed by many Neurospora workers. They are usually quite rare and reasonably can be attributed to exceptional events such as spontaneous lethal mutations.

In the present case, the germinated, nongrowing spores appeared to be a regular recombinant product of meioses involving Slm-1. The ordered tetrad observations, described earlier in the section on tetrad analysis, confirmed this. The dying spores were predicted to represent a class of nuclei which contain a nearly complete chromosome set. Either of two meiotic events produce duplication/deficiency chromosome complements: (1) A translocation dyad chromosome I

III which segregates with normal III produces a deficiency of the IL tip (marked by ro-10) and a duplication of IIIR (marked by leu-1) (see Figure 5).
This develops as an ascus with four black, early dying, duplication/deficiency spores and four white aborted spores. (2) A recombinant chromosome with a crossover in the inverted region of linkage group I which segregates with normal III produces a deficiency of the IL tip (ro-10) and a duplication of IR distal to the IR breakpoint (marked by al-2 and R). This develops variously as a 6B:2W or a 4B:4W or a 2B:6W ascus, depending on the assortment pattern of centromeres I and III and whether the crossover is left or right of the I centromere.

Other specific chromosome rearrangements of Neurospora also produce a lethal combination of chromosomes, a deficiency class, which results in spores which appear to mature and to darken and become black, but which do not germinate (see for example aberrations T(II;V)AR30 or T(V;VII)AR45 listed in Perkins and Barry 1977). In some of these rearrangements, the breakpoints are known to map near the ends of the chromosomes or in linkage group V left arm. The assumption is that one pattern of adjacent segregation from the translocation complex produces chromosome sets with segmental duplications and small deletions. The genes deleted are not required for the spore-autonomous steps of normal wall formation and darkening but are essential for germination. These examples with deficiency ascospores that blacken are in contrast to most Neurospora rearrangements, which typically produce complementary deficiency/duplication genomes that result in early aborting white (or hyaline) ascospores.

Since large numbers of SLm-1 progeny spores germinated and grew somewhat before dying, they might have stopped growing because they had depleted stored supplies of some essential substance from the ascospore. The deficiency of a small segment of chromosome could mean the absence of a gene which was active in the pathway of synthesis of the substance. Auxotrophic spores will germinate on medium lacking the substances they cannot make. They produce short germ tubes but stop growth when they run out of stored pools of the necessary nutrient. Germinating, nongrowing progeny spores from SLm-1 crosses have that same appearance.

Ascospores from heterozygous SLm-1 crosses were germinated on two variations of a complete medium modified from medium 2 of Tatum et al. (1950). The germinating, nongrowing spores did not grow further on the supplemented media.

**Rescue of the deficiency ascospores in heterokaryons:** An attempt to rescue the defective germinating spores by complementation in a heterokaryon proved successful. In theory, if the germinating SLm-1 deficiency segregant is made heterokaryotic with a complementary auxotroph, the two nuclear types should complement each other resulting in a phenotypically wild-type heterokaryon which will grow on minimal medium. Two auxotrophic mutant types, ad-3B and met-1, were tested separately. Both were successful, but the first proved more informative in tests designed to show that the SLm-1 deficiency was recovered. ad-3B cyh-1 is an adenine-requiring strain carrying a defective a mating type gene and resistant to cycloheximide (Griffiths and Délange 1978; Perkins 1984). The adenine requirement was used as the forcing marker in heterokaryon formation. The defective a mating type allows complementing heterokaryons to form with strains of either mating type, A or a, provided that the alleles at all other heterokaryon incompatible loci are alike.

Germinating conidia of the rescuing strain (ad-3B) were added to 10 × 75-mm culture tubes containing minimal medium and individual ascospores from heterozygous SLm-1 crosses. Because it is probable that germinating, nongrowing ascospores die rather
quickly, \(ad-3B\) conidia were added to the tubes just after the heat shock activation of the ascospore. Octads of the 6B:2W type were used in some experiments because they can be analyzed to identify which ascospores actually are the SLm-1 deficiency types. By this procedure, several vigorous heterokaryons were recovered which were composed of nuclei of \(ad-3B\) and presumptive SLm-1 deficiency types.

Conidia from some of these heterokaryons were plated out on sorbose medium supplemented with adenine. Two classes of colonies were obtained. One was the \(ad-3\) type, the rescuing type; and the other was an adenine-independent, \(ad-3^+\), culture. However, the \(ad-3^+\) colonies when grown up and plated again gave rise to the two colony types: \(ad-3\) and \(ad-3^+\). Further successive platings of \(ad-3^+\) colonies produced the same result. In short, it was not possible to recover a homokaryotic \(ad-3^+\) colony. Thus, while a pure \(ad-3\) type could be separated from the heterokaryon, the second component could not be isolated. The conclusion is that the SLm-1 deficiency chromosome has lost some genetic information essential for vegetative growth, but that otherwise the genetic complement is sufficient for ascospore maturation and germination.

The deficiency/duplication chromosome complement can be transmitted through the sexual cycle. Two heterokaryons consisting of \(a^{ad-3} ad-3B \text{cyh-1}\) nuclei and SLm-1 deficiency nuclei of a mating type were successfully crossed to a \(fI A\) tester. The \(a^{ad-3}\) nuclei are unable to fertilize or function in meiosis because mutation has inactivated the mating type gene (GRIFFITHS and DELANGE 1978). Thus, only the sheltered deficiency nucleus is able to enter the meiotic cycle with a nucleus of the opposite mating type from \(fI A\). These crosses produced a few black ascospores. When the ascospores germinated, approximately one half produced vigorous cultures, all of \(A\) mating type, and one half of these were \(fI\). Of the remaining ascospores, a few germinated but stopped growing after the production of short germ tubes, just as the presumed parent SLm-1 deficiency/duplication would do. Low ascospore production from these crosses is probably due to a large duplication which accompanies the deletion. The duplication will include either most of the right arm of linkage group III or the distal portion of linkage group I beyond the IR breakpoint of SLm-1.

**DISCUSSION**

The study of SLm-1 reveals a chromosome rearrangement, a pericentric inversion coupled to a translocation, which has not been described before but which may be common. Inviable progeny constitute about 50% of its meiotic products. The chromosome rearrangement found in strain SLm-1 is a variation of a type of rearrangement that has been quite commonly identified in Neurospora. These are three-break rearrangements involving two chromosomes. The ones previously identified are insertional translocations (PERKINS and BARRY 1977). If the events leading to the origin of these two different rearrangements are hypothesized to take place as drawn in Figure 6, their similarity can be seen most clearly. The position of centromeres relative to the breakpoints and the joining pattern are critical for recovery. The positions of breaks which are proposed to have produced SLm-1 would not have produced a viable rearrangement of the insertional type, however, because one chromosome would have been dicentric and the other acentric; a viable insertional translocation would occur if the centromere of the donor chromosome were not included in the translocated chromosome segment.

The very small translocation chromosome of SLm-1 is the smallest rearranged chromosome identified and characterized so far for any Neurospora rearrangement. It includes the centromere, left arm, and telomere of linkage group III and the re-10 gene and IL telomere of linkage group I. This diminutive chromosome is stable and active in the balanced translocation of SLm-1 nuclei. However, disomic progeny of SLm-1 which contain the aberrant chromosome together with a normal chromosome readily lose it, as
shown by the breakdown of ro-10+/ro-10- disomics under nonselective conditions. This small translocation chromosome will be of interest in molecular studies because it disrupts a region of linkage group III connected with the Spore killers of Neurospora (Campbell and Turner 1987).

For the chromosome rearrangement examined in this study, one fourth of the progeny from heterozygous crosses receive an unbalanced complement of genes consisting of a very small deficiency and a large duplication. The extent of the deficiency and the duplication are known by genetic mapping and by chromosome cytology. The deficiency is manifested as a recessive lethal. The deficiency can be compensated by a complementing nucleus, thus showing that the function or functions lost are not nucleus limited. Attempts to rescue the deficiency germings by providing an enriched, complete nutrient medium were unsuccessful.

Recessive lethal mutations have been followed by Atwood and Mukai (1953), who demonstrated their occurrence in Neurospora by making use of complementing nuclei in heterokaryons. De Serres (1968) and de Serres and Miller (1988) also used heterokaryons to obtain mutagen-induced recessive lethals and have shown that in some cases deficiencies of sizeable chromosome segments can be rescued by the complementing nuclei.

Stadler and Crane (1979) suggested that some lethal mutations are nucleus-limited, where the function provided by the normal allele cannot be transferred across the nuclear membrane. Steps in the processing and transport of RNA transcripts might be examples of such activities.

Using translocations in Drosophila, Muller and Settles (1927) and Sturtevant and Dobzhansky (1930) were able to demonstrate that the defective, deficiency/duplication meiotic products of a translocation could be transmitted through a cross if fertilizations involved the complementary chromosome types. An egg receiving the deficiency/duplication product of adjacent segregation from a translocation heterozygote could be fertilized by a sperm with the complementary duplication/deficiency segregation product, thus forming a normal zygote with a balanced though abnormal chromosome complement. Biggoud et al. (1982) have more recently observed the same type of rescue of deficient/duplication gametes in chickens heterozygous for a pericentric inversion.

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