Chromosome Rearrangements Recovered Following Transformation of *Neurospora crassa*

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ABSTRACT

New chromosome rearrangements were found in 10% or more of mitotically stable transformants. This was shown for transformations involving a variety of different markers, vectors and recipient strains. Breakpoints were randomly distributed among the seven linkage groups. Controls using untransformed protoplasts of the same strains contained almost no rearrangements. A study of molecularly characterized Am$^+$ transformants showed that rearrangements are frequent when multiple ectopic integration events have occurred. In contrast, rearrangements are absent or infrequent when only the resident locus is restored to *am*$^+$ by a homologous event. Sequences of the transforming vector were genetically linked to breakpoints in 6 of 10 translocations that were examined using Southern hybridization or colony blots.

TRANSFORMATION events in eukaryotes usually involve integration of transforming DNA at nonhomologous chromosomal sites [reviewed by FINCHAM (1989) and ROTH and WILSON (1988)] (*Saccharomyces cerevisiae* and trypanosomes are known exceptions). Entry of exogenous DNA into the cell and its heterologous insertion into the chromosome might be expected to induce mutations and chromosome rearrangements. Although inactivation of genes has been observed to result from heterologous insertion of transforming DNA [*e.g.*, by TILBURN, ROUSSEL and SCAZZACHIO (1990)], to our knowledge there has been no systematic quantitative examination of mutation or rearrangement frequencies among transformants.

New translocations and other gross rearrangements can readily be detected in Neurospora, where their presence is signalled visibly by the presence of unpigmented deficiency-duplication ascospores in test crosses. We report here a heightened frequency of new translocations following transformation in *Neurospora crassa*. Segments of transforming DNA are closely linked to breakpoints in many of the rearrangements.

MATERIALS AND METHODS

General methods and media were as described by DAVIS and DE SERRES (1970) and PERKINS (1959, 1986). Crosses were carried out on synthetic crossing medium (SC) containing 2% agar and 1% sucrose.

Transformation protocols involved modifications of the method of CASE *et al.* (1979) as applied by H. ZALKIN (1980 unpublished results) for *qa-2*, by LEGERTON and YANOFSKY (1985) for *his-3*, by VOLLMER and YANOFSKY (1986) for *pan-1*, and by KINNAIRD *et al.* (1982) and ASCH *et al.* (1992) for *am*. DNA isolation, DNA blots and hybridizations were as described by ASCH *et al.* (1992). Colony blots were as described by KINSEY (1989).

Vectors and strains used as recipients for the various transformations are identified in tables or text. Linkage testers are listed in the Fungal Genetics Stock Center (1992) stock list. Improved *fluffy* testers, *fl*(RL) *A* and *a* (PERKINS and POLLARD 1989), were used both for initial screening and for routine scoring of transformants.

In the experiments with *am*, the mutant recipient strains were all of mixed genetic background. The frequency of new rearrangements was determined using primary transformants that had not been put through a cross prior to testing. The newly isolated Am$^+$ transformants that bear UK numbers were purified on selective media either by repeatedly streaking and isolating single conidia or by transferring serially with very small conidial inocula, monitored visually by watching conidia float down from an inoculating needle through a beam of light onto the agar surface of a slant. Selection for Am$^+$ was imposed at each transfer by supplementing minimal medium with 1.5 mg glycine per ml, which inhibits growth of the otherwise leaky *am* mutant. The procedure was intended to decrease the frequency of mixtures or heterokaryons, but did not guarantee purity. Stocks of each verified rearrangement were established using progeny or descendants of the primary transformant.

Detection and analysis of rearrangements: Strains that contain new rearrangements were identified by the production of inviable unpigmented ascospores in characteristic patterns when transformed strains were crossed × normal-sequence testers. For general principles and methodology, see PERKINS (1974) or PERKINS and BARRY (1977). Initial identification of rearrangements was accomplished by fertilizing 5-day-old *fluffy* testers in individual 10 × 75-mm tubes of SC at 25° (PERKINS *et al.* 1989; PERKINS and POLLARD 1989). Cultures were scored tentatively as aberrant when fewer than 95% of the ascospores shot from perithecia of

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these test crosses were black. About 75% of ascospores are black and 25% are white (defective) when duplication-producing rearrangements are heterozygous, and 50% black, 50% white is typical of reciprocal translocations; about 95% of ascospores are black in crosses between strains that are isosequential, either both normal sequence or both rearranged (PERKINS 1974). When sexually compatible strains containing a segmental duplication are crossed, they typically result in barren perithecia which produce few or no ascospores.

To confirm that defective ascospores were due to heterozygosity for a chromosome rearrangement rather than to another cause such as mutation of a gene affecting ascospore pigmentation, patterns of aborted ascospores were examined in individual asci as described by PERKINS (1974). Rearrangement progeny of opposite mating type were obtained from crosses of rearrangement × wild type. Rearrangement strains derived from the same transformant were intercrossed to confirm that ascospore progeny were black and viable as expected for parents that are isosequential rearrangement sequence. Mapping of rearrangement breakpoints to linkage groups was accomplished using either *alcoy; csp-2* (PERKINS *et al.* 1969) or *multicent-4* (PERKINS 1990), depending on whether ascus patterns indicated that the breakpoints were centromere-linked. Subsequent crosses employed *alcoy* follow-up strains with four linkage groups marked (Fungal Genetics Stock Center, 1992), or other multiply marked testers.

RESULTS

Preliminary observations: An opportunity to examine transformants for the presence of new rearrangements was first provided in 1980 by experiments with which HOWARD ZALKIN sought to increase transformation efficiency of *qa-2* (quinate utilization). Regeneration plates containing transformants were given to us by ZALKIN, and 438 colonies were isolated to slants of medium unsupplemented for *qa-2*. (For reasons not relevant here, the double mutant *qa-2; aro-9* was used.) The cultures were examined for vegetative stability after successive transfers using very small conidial inocula. Eighty isolates tested positive (Qa^+) on the first transfer but negative (Qa^-) on the second. Of the strains that remained Qa^+ after two transfers, a sample of 100 was carried further. Ninety-six of these remained stable through a series of 11 transfers; all but three of the successive transfers were to medium selective for Qa^+. Ten percent of the 96 stable transformants but only 1% of 124 unstable transformants proved to contain new translocations (Table 1). Genetic mapping revealed that breakpoints of the translocations were distributed in all seven linkage groups, apparently at random (Table 2). (Transformation in Neurospora usually involves ectopic integration to apparently random positions in the genome.)

When certain other transformation systems were examined, similar results were obtained. Of 100 His-3^+ transformants provided by TIMOTHY L. LEGERTON, at least four were shown to contain new rearrangements. Breakpoints mapped in six of the seven linkage groups. Of 28 Pan-1^+ transformants obtained from STEVEN J. VOLLMER, one contained a new translocation (Tables 1 and 2). (The values are probably minimal because isolates were not rigorously purified before being examined for rearrangement in either of these experiments.) Several of the rearrangements were recovered from areas in the test-cross tubes where 50% of the ascospores were white in an otherwise normal background of black spores. These probably came from mixed cultures.

No rearrangement was detected among 111 Arg-12^+ primary transformants from LEGERTON, 12 Trp-1^+ transformants from MICHAEL G. SCHECHTMAN, or 19 prototrophic progeny of Am^+ (glutamate dehydrogenase) transformants tested by KINSEY and RAMBOSEK (1984). These negative results are of limited significance. The Arg^+ transformants were transferred only once before testing, and it is likely that most of them were still heterokaryons or mixtures in which any rearrangements in the transformed nuclei would be obscured by the presence of Normal sequence untransformed nuclei. The 1980 experiment with *am* differed from all the others in that f_1 progeny rather than primary transformants were tested; this would decrease the probability of detecting a new rearrangement.

Taken together, these preliminary experiments suggested that the frequency of new rearrangements is increased among vegetatively stable transformants. Aside from the comparison of unstable *vs.* stable Qa^+ transformants, no direct controls were done to determine rearrangement frequencies in untransformed protoplasts or unprotoplasted conidia. Long experience with monitoring and scoring the presence of rearrangements in progeny of test crosses had shown that new rearrangements are not present in most untreated cultures in frequencies greater than 1% of nuclei, and that their frequency is probably much less. For example, no rearrangements had been found among 300 single-conidial isolates of *rg-1 cr-1* (Table 4 of PERKINS 1974). Nor were any new rearrangements detected in 714 single-ascospore isolates from crosses of QNS-6 *nit-2 leu-3 caf-1 at a* (FGSC 5381) with several normal-sequence strains (V. C. POLLARD and D. D. PERKINS, unpublished). Additional evidence that new rearrangements are rare in untransformed and unmutagenized cultures is provided by controls in the experiments with Am^+ transformants described below.

Experiments using well characterized Am^+ transformants: Cultures were available of Am^+ transformants that had been obtained in a variety of ways and that had been characterized molecularly as to whether integration of transforming sequences was homologous or ectopic, and whether copies were integrated at single or multiple ectopic sites (FREDERICK, ASCH

TABLE 1

Confirmed chromosome rearrangements from early experiments

Experimental series	Recipient strain	Transforming vector	No. transformants tested	No. rearrangements confirmed
HZ, stable transformants	*qa-2; aro-9*	pES55 (*qa-2*$^+$)	96	10 (10%)
HZ, unstable transformants	*qa-2; aro-9*	pES55 (*qa-2*$^+$)	124	1 (1%)
TL	*his-3*	pHN60 (*his-3*$^+$)[a]	100	4 (4%)
SV	*pan-1*	pSV50 (*pan-1*$^+$)[b]	28	1 (4%)

[a] And derivatives with partial deletions of *his-3*.
[b] In cosmid pool.

TABLE 2

Diagnosis of rearrangements from preliminary experiments

Qa-2$^+$ stable transformants		
Reciprocal translocations (8):	*T(I;VR)Z3*	*T(I;III)Z119*
	T(IR;IVR)Z10	*T(I;IV)Z135*
	T(IIIR;VR)Z52	*T(VR;VII)Z175*
	T(II;VI)Z99	*T(IIL;VI)Z194*
Quasiterminal translocation (1):	*T(VIIR → IR)Z88*	
Qa-2$^+$ unstable transformant		
Reciprocal translocation (1):	*T(I;VR)Z252*	
His-3$^+$ transformants		
Reciprocal translocations (3):	*T(VI;VII)TLd5-7*	*T(IV;V)TLd9-6*
	T(I;IV)TLd9-2	
Complex translocation (1):	*T(III;IV;VI)TLd4-4*	
Pan-1$^+$ transformant		
Reciprocal translocation (1):	*T(VR;VII)SV1*	

Fifteen rearrangements were identified and confirmed in a total of 224 stable transformants. Among the more rigorously purified Qa-2$^+$ stable transformants, the frequency was 9 in 96. *qa-2* is in linkage group VII, *his-3* in I, and *pan-1* in IV.

and KINSEY 1989; ASCH and KINSEY 1990; FREDERICK and KINSEY 1990a,b; D. ASCH, G. FREDERICK and J. KINSEY, unpublished). Vegetatively purified primary transformants from the various series were used, that had not been put through a cross. Control series were included that had been obtained by picking colonies from nontransformed regenerated protoplasts. (These were subjected to the same purification procedure.) The coded cultures were tested for rearrangements by one of us who had no knowledge of their transformation history or molecular classification. The results are summarized in Tables 3 and 4.

The overall frequency of translocations among 255 tested strains is 11%. There is a significant excess of translocations in strains having transforming DNA integrated at multiple ectopic sites, and translocations were completely absent from the 51 strains that had undergone only homologous transformation or conversion, without ectopic integration. As in the earlier experiments with *qa-2* and *his-3*, rearrangement breakpoints seem to be distributed at random throughout the chromosome complement. The rearrangements include all the types most commonly encountered–reciprocal, insertional, and quasiterminal translocations. Two complex translocations involving more than two linkage groups were also obtained, and one pericentric inversion. Six of the 28 rearrangements were insertional or quasiterminal.

As a control, 143 colonies regenerated from protoplasts of *am*$_{132}$ *inl A* that had not been exposed to DNA were isolated to slants, purified, and tested. This is the strain used in transformation experiments UK2 and UK3, where 11 rearrangements were found in 62 cultures tested. No rearrangement was detected in the controls. As a second control, 122 nontransformed protoplasts of *am*$_6$ *al*^{s} *a* were regenerated, purified and tested. Five experiments with this strain had produced 13 translocations in 161 transformants tested. In contrast, only one rearrangement was found in the untransformed control series. Clearly, the rearrangements found among transformants cannot be attributed to protoplast formation and regeneration.

The control experiments just described employed the same identical strains that served as recipients for transformation in the experimental series. Additional experiments were also carried out using untransformed protoplasts from three other strains that were not used as recipients (PERKINS and KINSEY 1993). Results were negative with two of these strains, ORS-6a (FGSC 2490) and QNS-6 (FGSC 5381); no rearrangement was found among 255 isolates. Unexpectedly, however, 14 different translocations were found in 278 isolates with the third strain, OR23-1VA (FGSC 2489). Wild type OR23-1VA was used

TABLE 3

Confirmed chromosome rearrangements among Am$^+$ transformants

Series No.	Recipient strain	Transforming vector	Transformants tested	Rearrangements confirmed	Fraction rearranged when integration was:[a] Homologous	Single ectopic[b]	Multiple ectopic	Undetermined
Transformants:								
UK-1	*am$_4$ure-2; cot-1 A*	pJR1[c]	32	4	0/11	0/8	4/13	0
UK-2	*am$_{132}$inl A*	pJR1[c]	24	7		1/5	6/19	0
UK-3	*am$_{132}$inl A*	pJR1[c]	38	4		2/16	2/22	0
UK-4	*am$_6$; al^s a*	pJR1 (*Hin*dIII)[d]	31	3	0/3	1/6	2/20	0/2
UK-5	*am$_6$; al^s a*	pJR1 (*Sma*I)[d]	32	4	0/5	0/10	4/13	0/4
UK-6	*am$_6$; al^s a*	pJR1.1[e]	25	1	0/1	0/4	1/19	0/1
UK-9	*am$_6$; al^s a*	Various[f]	42	5			5/42	
UK-10	*am$_6$; al^s a*	Various[f]	31	0	0/31			
Experimental totals			255	28 (11%)	0/51	4/49	24/148	0/7
Untransformed controls:								
UK-17	*am$_6$; al^{s}a*		122[g]	1[h]				
UK-18	*am$_{132}$ inl A*		143[g]	0				
Control totals			265	1 (0.3%)				

Tests were done "blind," without knowledge of the treatment, in the transformants series but not in the two controls.

[a] If the probability of rearrangement is independent of the type of integration, expected numbers of rearrangements are 6:5:16 based on 21% of transformants with homologous transformation, 20% with single ectopic integration, and 60% with multiple ectopic. P from $\chi^2 < 0.01$.

[b] Translocations UK2-29, UK3-2, UK3-11 and UK4-17 appeared to involve single ectopic integration.

[c] See KINSEY and RAMBOSEK (1984) for structure of plasmids.

[d] Transformants were obtained with pJR1 that had been cut to completion with *Hin*dIII or *Sma*I.

[e] See FREDERICK, ASCH and KINSEY (1989) for structure of plasmids.

[f] Transformants in series 9 and 10 were selected from a large collection of transformants obtained with a family of related plasmids described by FREDERICK and KINSEY (1990a,b). In series 9, selection was for transformants with multiple ectopic integration events. In series 10, selection from the same collection of transformants was for homologous integration.

[g] Untransformed protoplasts were regenerated for the controls, using strains identical to the experimentals.

[h] *T(I;VI)UK17-51.*

TABLE 4

Diagnosis of rearrangements among Am$^+$ transformants

Reciprocal translocations (22):			
T(III;IVR)UK1-15G	*T(IL;VII)UK2-29*	*T(III;VII)UK5-20*	*T(I;III)UK9-26, UK9-25, UK9-15*[a]
T(IVR;VR)UK1-27	*T(I;II)UK3-2*	*T(IR;VI)UK5-27*	
T(II;IV)UK1-29	*T(I;II)UK3-11*	*T(II;V)UK5-31*	*T(II;V)UK9-17*
T(II;IVR)UK2-3	*T(III;VI)UK3-36*	*T(VI;VII)UK5-32*[b]	*T(VR;VIL)UK9-18*
T(IR;VI)UK2-17	*T(IL;VIIR)UK4-17*	*T(IL;VR)UK6-43*	*T(I;IV)UK9-30*
T(V;VI)UK2-22	*T(II;IV)UK4-33*	*T(IR;VIL)UK9-13d*	
Quasiterminal or insertional rearrangements[c] (6):			
*T(IR → VL)UK*1-35, *UK*1-33[a]	*T(IVR → VL)UK*2-32	*T(VR → VIL)UK*3-41[d]	
*T(IR → VII)UK*2-26[d]	*In(VR → VL)UK2-y*	*T(IIR → VL)UK*4-22	

[a] UK9-15, UK9-25 and UK9-26 appear to have identical breakpoints and have been counted as one. Likewise, UK1-35 and UK1-33 appear to be identical.

[b] May be more complex.

[c] When crossed × normal sequence, these rearrangements generate a recombinant class of viable duplication progeny.

[d] Insertional translocation.

only peripherally in this study and several lines of evidence show unequivocally that it cannot be responsible for the increased frequency of chromosome rearrangements found among Am$^+$ transformants that have undergone ectopic integration (see DISCUSSION).

During analysis of the Am$^+$ rearrangements, some were found to produce progeny that were Am$^-$. Those seen in early generations may have had a trivial explanation, simply the uncovering of the native *am$^-$* gene from the original recipient when it segregated from an ectopic *am$^+$* sequence or sequences. Alternatively, and in later generations more likely, Am$^-$ progeny from Am$^+$ parents could result from repeat induced point mutation (RIP) triggered by the presence of extra, ectopic copies of the gene (SELKER 1990). With several translocation strains that arose by multiple integration, Am$^-$ progeny have been obtained repeatedly from crosses in which both parents were Am$^+$.

In translocation UK9-18, one of the breakpoints is at the *am* locus, as indicated by linkage in crosses and confirmed by Southern analysis (data not shown). The original Am$^+$ UK9-18 transformant also contained multiple ectopic integrations. If an *am*$^+$ insertion occurred at the *am* locus, it must then have been rendered *am*$^-$ by RIP during a subsequent cross. Four of the transformation-associated rearrangements and one translocation in the controls involve one breakpoint in or near the nucleolus organizer, at the left end of linkage group V.

Genetic linkage of transforming DNA sequences to translocation breakpoints: In a previous report, ASCH *et al.* (1992) recloned and sequenced the junctions between chromosomal and transforming DNA for two ectopic integration events in Neurospora. In one instance transforming DNA was found to bridge a translocation junction between linkage group I and linkage group VI. This result suggested that plasmid DNA might routinely be found associated with translocation breakpoints in the translocations recovered following transformation.

To test this possibility, f_1 translocation progeny from 10 different transformants were crossed to normal sequence testers. Ascospore colonies from these 10 crosses were examined for the presence of normal, translocation, or duplication progeny by crossing to testers as described above. For four of the crosses, DNA was isolated from sample progeny of each type and was analyzed by Southern blot analysis using pUC8 (VIEIRA and MESSING 1982) DNA as a probe. Sample data from progeny from a cross of UK5–31 × normal sequence are shown in Figure 1A. For the other six crosses as well as for additional progeny from two of the crosses tested by Southern analysis, colony blots were performed directly on progeny colonies as described by KINSEY (1989). Although pUC8 DNA does not hybridize to Neurospora genomic Southern blots under stringent hybridization conditions, some part or parts of pUC8 (and related plasmids) cause false positives in the colony blot procedure (J. A. KINSEY, unpublished). Therefore the 359-bp *Sca*I/*Bgl*I fragment from the *amp*^{r} gene of pUC8 was used as a probe. This probe does not give false positives in the colony blot procedure, but could lead to underestimates of progeny with plasmid DNA if this portion of the plasmid was not integrated. Sample data for 20 progeny from a cross of UK2-y × normal sequence are shown in Figure 1B. The data for all 10 crosses are summarized in Table 5.

It is clear from these data that pUC8 sequences are linked to the translocation breakpoint in six of the ten translocation strains tested, with no recombinant in any of the six crosses in a total of 141 progeny tested. This shows that transforming DNA is frequently, but not always, integrated at or near the translocation junction in translocations that are recovered following transformation. Since the original transformants had multiple ectopic integrations (with the exception of UK3–2 and UK4–17), we were surprised to find that in those cases where linkage was detected *all* ectopic copies appeared to be linked to the translocation as, for example, in Figure 1A. This was also observed by ASCH *et al.* (1992) for translocation UK-T12. The linkage may imply that one or more copies of transforming DNA are integrated at each of the translocation junctions. There was no evidence of direct tandem repeats when parents or progeny of crosses involving the five multiple ectopic rearrangements were examined by Southern blot analysis. (For example, individual bands were still resolved when digests were made with an enzyme having no target sites in the plasmid, and plasmid-sized bands were not obtained when digests were made with an enzyme that cuts only once in the plasmid.)

DISCUSSION

In Neurospora, transformation occurs primarily by integration of transforming DNA at ectopic sites (FINCHAM 1989; ASCH and KINSEY 1990). ASCH and KINSEY (1990) and G. D. FREDERICK and J. A. KINSEY (unpublished data) have observed that many of the transformants obtained with linear vectors have intact or nearly intact vector sequences integrated at apparently random ectopic sites. ASCH *et al.* (1992) analyzed the junctions of two ectopic integration events as well as the empty sites from a wild-type strain. The junctions appeared to have resulted from the end joining of truncated vector sequences to breaks in chromosomes, without the loss of chromosomal DNA. The recombination did not appear to have been driven by homology. In one of the two cases the integrated DNA bridged sequences from linkage groups I and VI and the transformant strain was shown by genetic analysis to involve translocation of a linkage group I segment to the right end of VI.

In the present study we have examined the association of transformation with chromosome rearrangements using a variety of recipient strains and transforming vectors (Tables 1 and 3). We consistently observed an elevated level of rearrangements among stable transformants as compared with mock-transformed regenerated spheroplasts. For the large collection of molecularly characterized Am$^+$ transformants the average frequency of rearrangements was 11% compared to 0.3% for the controls. Among transformants with multiple ectopic integrations the frequency was nearly 16% (Table 3). In marked contrast, there were no chromosome rearrangements among the 51 transformants which were the result of a single homologous replacement event. (Five or six

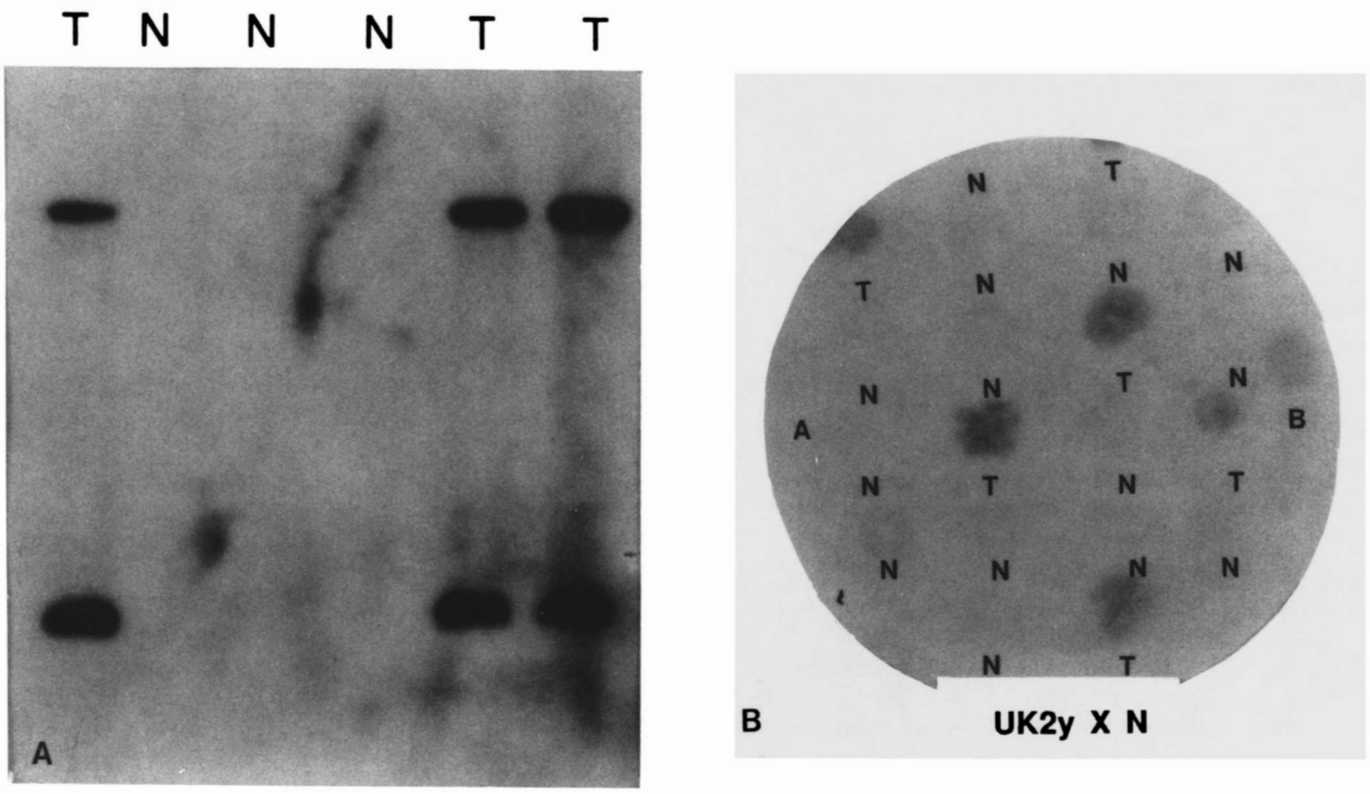

FIGURE 1.—Genetic linkage of integrated plasmid DNA with translocations. (A) Genomic Southern blot of DNA from three translocations (T) and three normal sequence (N) progeny from a cross of translocation UK5-31 × normal sequence. DNA was digested with *Bam*HI and probed with labeled pUC8 DNA. (B) Colony blot of 20 progeny from a cross of inversion UK2-y × normal sequence. Normal sequence progeny are identified by the letter N below the colony, translocation progeny by a T. A normal sequence control is indicated by the letter A and the pUC8-containing control by the letter B.

TABLE 5

Association of plasmid DNA with translocation breakpoints

Transformant tested	Screening method[a]	Parental combination		Recombinant	
		Translocation with pUC8	Normal sequence without pUC8	Translocation without pUC8	Normal sequence with pUC8
UK1-15	Colony blot	2	7	12	4
UK1-29	Colony blot	11	14	0	0
UK2-26	Southern	6[b]	3	0	0
UK2-y	Colony blot	14	25	0	0
UK3-2	Southern/Colony blot	9	5	0	0
UK4-17	Southern	0	3	3	0
UK4-33	Colony blot	0	13	11	0
UK5-31	Southern/Colony blot	10	9	0	0
UK9-18	Colony blot	15	20	0	0
UK9-26	Colony blot	0	20	17	0

[a] The 359-bp *Sca*I/*Bgl*I fragment of plasmid pUC8 was used as a probe. This gives no false positives with Neurospora genomic DNA using either screening method.

[b] Three of the progeny were translocations and three were duplications, which retain one rearranged chromosome (VII) but not the other (I).

rearrangements might have been expected, based upon the overall frequency.)

Our data do not address the reason for the apparent excess of translocations in the class of transformants with multiple ectopic integrations. It was not possible from the available data to make an accurate estimate of the average number of integrations in this class of transformants; possibly the frequency of translocations per integration event could be similar in both single ectopic and multiple ectopic integration classes. Our data also do not rule out the possibility that some or all of the translocations observed in strains with multiple ectopic integrations are the result of mitotic recombination between dispersed copies of transform-

ing DNA subsequent to the transformation events.

The paucity of rearrangements in the controls argues that transformation itself is somehow responsible for the production of these rearrangements. The lack of rearrangements in those transformants with only homologous recombination events argues that the simple presence of transforming DNA in a recipient nucleus is not sufficient to induce rearrangements. Our data do not address the question of why there is an elevated level of rearrangements among transformants with ectopic integrations; however, it is possible to speculate that this could be due to either an active or passive role of transforming DNA in these events. In an active model one would expect that the incoming DNA is end joined to breaks in two different chromosomes. In this case one would expect to see vector sequences associated with at least one of the translocation junctions. ASCH *et al.* (1992) found such an association in the one transformant that had a chromosome rearrangement in their study. We have used genetic analysis of progeny of a sample of 10 rearranged transformants to determine if plasmid sequences are linked to translocation junctions. Our results indicate that the breakpoints of many rearrangements (6 of the 10 tested) are indeed closely associated with such sequences (no recombination between breakpoints and inserted sequences in 141 progeny, overall). Not all rearrangements have vector sequences at the junctions. However, the four negative results with no apparent linkage are somewhat tenuous in this case, since for technical reasons only a part of the vector was actually tested.

A passive model that might explain the results would suppose that only certain nuclei in the population are susceptible to ectopic integration (perhaps because of the inherent presence of more temporary breaks in chromosomal DNA). Such nuclei might also be more prone to spontaneous rearrangements and the association of translocation with transformation would then be the result of selection (via selection of transformants) of the nuclei most likely to have suffered a spontaneous rearrangement. It is possible that both passive and active forces are at work in this system and it is also possible, as we have speculated previously (ASCH *et al.* 1992), that transforming DNA might indirectly lead to increased chromosome breakage by inducing repair systems in cells with undamaged chromosomal DNA.

The rearrangement frequencies reported here following transformation are comparable to those found following irradiation. New rearrangements have been found in 5–20% of survivors of mild UV irradiation of Neurospora conidia [see Table 5 of PERKINS (1974)]. In all the present experiments the observed frequency of confirmed rearrangements was almost certainly less than the actual frequency of occurrence. Only obvious rearrangements were pursued while questionable candidates were discarded. Incomplete purification of cultures containing transformed nuclei probably obscured the presence of some rearrangements which went undetected. Also, some rearrangement types will be intrinsically difficult to detect using our standard crossing test, which depends on visual recognition of deficiency ascospores (PERKINS and BARRY 1977). With short inversions, for example, deficient meiotic products are rare or absent because they depend on crossing over within the inverted segment; even long paracentric inversions have never been detected in Neurospora, perhaps because dicentric bridges result in death of the entire ascus. Some short insertional translocations [*e.g.*, *T(V → VI)EB4*] might also be overlooked because deficiency ascospores become pigmented even though the spores are inviable.

The transformation-associated rearrangements did not result from the metabolic insult of being plated on selective medium, during phenomic lag before the cells could grow. There was no heightened frequency of chromosome rearrangements either in unstable transformants or in those stable transformants with homologous integration, although both were exposed to the same metabolic stress.

Wild-type strain OR23-1VA, which was used peripherally in this study, has itself been shown to be unstable, producing unique new translocations in 5% of isolates from untransformed protoplasts or conidia (PERKINS and KINSEY 1993). However, few if any of the rearrangements ascribed to transformation (Tables 3, 4 and 5) could have had their origin in OR23-1VA. All the Am^+ rearrangements in primary transformants were initially detected by crossing them to the very stable *fluffy* strains. Putative rearrangement strains were then routinely diagnosed for type of rearrangement by examining patterns of aborted ascospores in asci obtained by crossing them to the same *fluffy* testers. Not until after that were most of the rearrangement strains crossed to wild type to obtain marker-free progeny of both mating types for use as stocks. All but three of the 28 transformation-associated rearrangements (Tables 3 and 4) had already been identified and diagnosed by ascus patterns before they were ever crossed with an Oak Ridge wild type. The exceptions (UK5-20, UK5-31 and UK9-13d) were put through a cross with OR23-1VA after being identified as putative rearrangements but before diagnostic ascus patterns were obtained. Of these three, one was subsequently tested and shown to contain pUC8 DNA sequences linked to the rearrangement. This leaves only two translocations (not tested for vector sequences) that could have originated in OR23-1VA. Conceivably, the white ascospores that led these two to be selected as putative re-

arrangements could have been due to an ascospore-color mutation, and a bona fide translocation could subsequently have been introduced from OR23-1VA. Such a scenario seems highly unlikely. Of the 10 Am^+ rearrangements tested with Southern or colony blots (Table 5), six have pUC8 DNA at or close to one or both breakpoints and thus cannot have been preexisting in OR23-1VA. All told, the fact that strain OR23-1VA is unstable cannot alter our conclusion that transformation involving ectopic integration is associated with a marked increase in the frequency of gross chromosome rearrangements.

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