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Amplification of the nucleolus organizer region during the sexual phase of *Neurospora crassa*

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Abstract. Previously we have shown that the nucleolus organizer region (NOR) of *Neurospora crassa* displays frequent size changes during crosses. In these initial studies, we observed that decreases in NOR size are far more common than increases. Here, we have investigated the inheritance of NOR size in a strain with an unusually small NOR. We call this strain SNO for *s*mall *n*uclelous *o*rganizer. We found that progeny that inherit their rDNA from SNO receive either an NOR that is larger than that of SNO or, rarely, the same size, but never an NOR that is smaller than that of SNO. The number of progeny that inherit their NOR from SNO is not significantly different from the number that inherit their NOR from the other parent in the cross. This argues against the idea that the failure to find progeny with NORs smaller than that of SNO is due to inviability of spores carrying such an NOR, or that it is due to unconscious bias by the experimenter against isolating such spores. These results can most easily be explained by a combination of unequal sister chromatid exchanges in the rDNA, or sister chromatid conversion, coupled with selection against nuclei harboring small NORs during the premeiotic phase of the *Neurospora* life cycle. Other, less conventional, explanations are also possible, such as "directed" increase in the target NOR without corresponding loss at some other NOR.

Introduction

In *Drosophila melanogaster*, partial deficiencies of rDNA correspond to the bobbed (*bb*) mutation (Ritossa et al. 1966). The phenotypic effects of *bb* mutant alleles include short bristles, abdominal etching, subnormal growth rate and, for some alleles, death. The *bb* phenotype reverts at an unusually high, but variable, frequency in the germlines of *bb* males (Ritossa 1968; Tartof 1973). Reversion events can occur either premeiotically or meiotically. Molecular evidence supports the idea that the phenotypically normal progeny are not rDNA deficient (Ritossa 1968; Tartof 1973). Ritossa (1968) called the reversion process rDNA "magnification".

The rDNA of *Neurospora crassa* is located at a single site in the genome called the nucleolus organizer region (NOR), which forms a terminal segment on the left arm of linkage group (LG) V (Barry and Perkins 1969). The rDNA at the NOR is arranged as a series of 100 to 200 tandem repeat units. The size of the NOR, which is an expression of the number of rDNA repeat units, is subject to a high degree of change during the sexual phase of the life cycle (Butler and Metzenberg 1989, 1990). Segregants of a cross typically have NOR sizes that are different from those of their respective parents. Most of the changes in the size of the NOR occur during the premeiotic portion of the sexual phase, and additional changes occur relatively rarely during meiosis. Decreases in NOR size are seen more frequently than increases. Obviously, *Neurospora* cannot repeatedly diminish the size of its NOR in the sexual phase and remain viable.

Some progeny from a cross described in Butler and Metzenberg (1989) were remarkable in that they had inherited an NOR that was only about half of the size of that of their parent. Surprisingly, they had no apparent abnormal phenotype. We called these progeny SNO, for *s*mall *n*ucleolus *o*rganizer. We wanter to know whether the general tendency to reduce the size of the NOR in the sexual phase was true of an SNO strain or, alternatively, whether an SNO strain exhibited a magnification-like process. In a cross of an SNO strain to a strain with a more typical-sized NOR, we found that progeny that inherit their rDNA from SNO receive either an NOR that is larger than that of SNO, or rarely, the same size, but never an NOR that is smaller than that of SNO. In contrast to the previous crosses, in which changes usually happen premeiotically, changes in the

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size of the NOR occurred frequently (though not exclusively) during meiosis. Changes in the size of a very small (about five repeat units), ectopically located rDNA cluster in SNO occur frequently. However, in contrast to the normally situated NOR of SNO, these changes are roughly equally distributed between increases and decreases.

Materials and methods

Strains, genetic methods and plasmids. SNO-4 is referred to as E4 in Butler and Metzenberg (1989). The parent called NS-1 was obtained from the Fungal Genetics Stock Center (FGSC 945). All crosses were made on minimal synthetic crossing medium at 25° C (Westergaard and Mitchell 1947). For the directional cross in which SNO-4 acted as the female, it was inoculated first and allowed to grow for 6 days, during which time the female reproductive structures (protoperithecia) were formed. These were then fertilized with a conidial suspension of NS-1, which therefore acted as the male in the cross. For the cross in which SNO-4 acted as the male, the order of inoculation was reversed and, with it, the sexual roles of the two parental strains. The spores from complete, unordered asci and individual perithecia were ripened as described by Metzenberg (1988). The plasmid used in this study, pRW528, includes all but approximately 0.2 kb of the 9.3 kb repeat unit. The *Saccharomyces cerevisiae* strain S288C was used as a source of DNA for molecular weight standards.

DNA preparations and restriction digestions. Preparation of intact *Neurospora* and *S. cerevisiae* chromosomes was as described in Butler and Metzenberg (1989). Restriction digestions of intact chromosomes were carried out as described (Butler and Metzenberg 1989). The restriction enzymes *Hind*III and *Bcl*I were purchased from the Promega Corporation.

Contour clamped homogeneous electric field (CHEF) gel electrophoresis. The apparatus used was described by Chu et al. (1986). Pulsed-field CHEF gel electrophoresis was performed for 24 h in 0.5 × TBE at 10°–11° C, with 1% agarose gels cast in 0.5 × TBE (Chu et al. 1986) unless otherwise stated in the figure legend. The pulse times and voltages were varied from experiment to experiment to optimize resolution of pertinent fragments (Vollrath and Davis 1987) and are given in the appropriate figure legends.

Results

Expansion of the NOR

The sexual phase of *Neurospora* begins when cells of opposite mating type fuse (see Fig. 1 for a schematic representation). A dikaryotic tissue is formed in which the haploid nuclei undergo seven to ten mitotic divisions. In the last two or three divisions, the nuclei of opposite mating type divide in synchrony. This is the premeiotic portion of the sexual phase. Following the mitotic divisions, nuclei of opposite mating type fuse, forming the only true diploid cell in the life cycle, and the diploid nucleus immediately enters meiosis. The products of meiosis undergo one mitotic division before spores are delineated (see Perkins and Barry 1977 for details).

NORs frequently change size during the premeiotic portion of the sexual phase. In crosses between strains carrying NORs of average size, decreases were much more common than increases (Butler and Metzenberg 1989). Four progeny of such a cross (called SNO) were remarkable, in that they contained only approximately half as much rDNA as their parent. In spite of this, they had no obviously abnormal phenotype. We wanted to know whether the tendency to reduce the size of the NOR in the sexual phase continued with additional outcrosses of an SNO strain, or whether the NOR remained stable at this size, or, finally whether the NOR of an SNO strain underwent a magnification-like process. We therefore backcrossed one of the SNO strains, called SNO-4, to one of its parents, called NS-1 (NS-1 is the parent of SNO-4 that did *not* pass on its NOR to SNO-4) and examined the size of the NOR in the progeny that inherited their NOR from SNO-4.

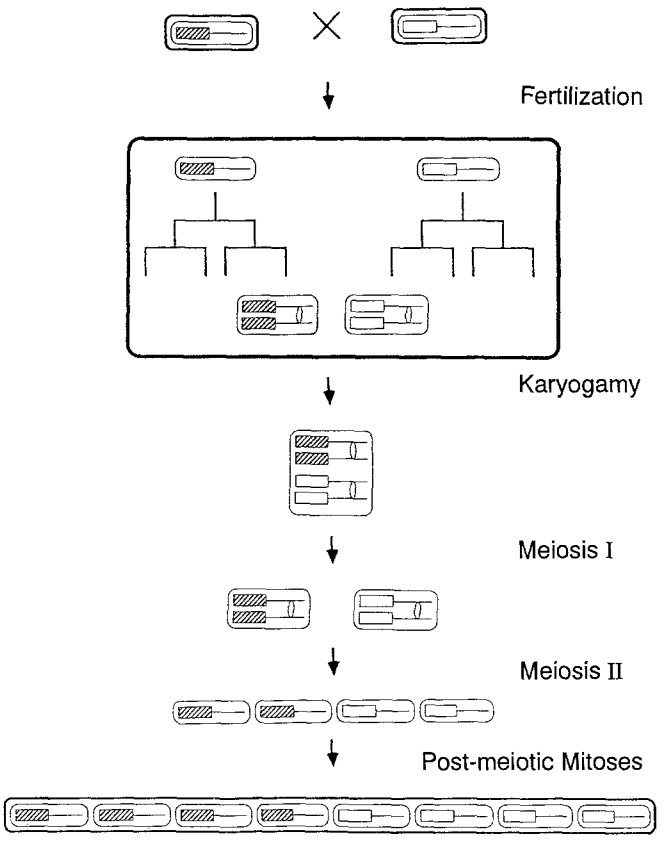

Fig. 1. Schematic representation of the sexual phase of *Neurospora crassa*. Two haploid cells of opposite mating type are shown at the top. Depicted within each cell is the chromosome bearing the nucleolus organizing region (NOR). Fertilization results in the dikaryotic ascogenous hypha. The nuclei within the ascogenous hypha undergo seven to ten premeiotic conjugate mitotic divisions (only two divisions are shown). The final round of premeiotic DNA synthesis occurs in the haploid nuclei. Karyogamy forms diploid nuclei with replicated chromosomes. All pairs of nuclei fuse and undergo the following events, though only one is shown in the diagram. Meioses I and II generate four haploid nuclei. The haploid nuclei undergo one postmeiotic mitosis before ascospore delineation and ascus formation. All of the asci and ascospores within a single perithecium are the result of a single fertilization event. Chromosome symbols: the *hatched rectangular box* represents an NOR composed of type II rDNA repeat units; the *open rectangular box* represents an NOR composed of type I rDNA repeat units (see text for definition of rDNA type); the *solid lines* represent non-rDNA sequences; the *open circles* represent centromeres. The symbols are not drawn to scale

The NORs derived from NS-1 and SNO-4 can be distinguished on the basis of a *Hin*dIII restriction fragment length polymorphism (RFLP) in the rDNA. NS-1 has only type I rDNA repeat units at the NOR and SNO-4 has only type II rDNA repeat units at the NOR (data not shown). Type I rDNA repeat units have one *Hin*dIII site in the non-transcribed spacer region, while type II rDNA repeat units have two *Hin*dIII sites in the non-transcribed spacer region. Even though the number of rDNA repeat units in the NOR changes during a cross, the *type* of rDNA repeat units in the NOR does not (Russell et al. 1988; Butler and Metzenberg 1989). Thus, the *Hin*dIII RFLP can be used to identify those progeny that inherit their NOR from SNO-4.

A total of 119 progeny were isolated and scored for rDNA type. Those progeny with type II rDNA were analyzed for NOR size. To determine the size of the NOR, we took advantage of the fact that type II rDNA repeat units do not have a recognition site for the restriction enzyme *Bcl*I (Butler and Metzenberg 1989). Intact chromosomal DNA was prepared from SNO-4 and from 56 type II rDNA progeny, digested with *Bcl*I, fractionated by CHEF gel electrophoresis, blotted, and probed with radiolabeled rDNA.

Based on saturation hybridization experiments, the size of the NOR in a commonly used wild-type strain has been estimated to be about 185–200 rDNA repeat units (Krumlauf and Marzluff 1980). With the CHEF system, the same strain yields a single rDNA-containing *Bcl*I fragment of 1630 kb (Butler and Metzenberg 1989). Given that the size of a single rDNA repeat unit is 9.3 kb, the maximum number of repeat units that can be contained in the 1630 kb *Bcl*I fragment is 175. Thus, the size of the NOR as measured by CHEF gel electrophoresis agrees well with another method of size determination.

Figure 2 shows SNO-4 and nine representative type II rDNA progeny. It is immediately obvious that all of the progeny contain an NOR larger than that of SNO-4. The molecular size of the NOR in SNO-4 is about 800 kb, which corresponds to about 85 rDNA repeat units. Among the progeny shown in Fig. 2, the NORs range in size from approximately 1000 kb to approximately 1600 kb. In total, 49 out of 56 type II rDNA progeny examined had an NOR larger than that of SNO-4; the remainder had the same size NOR as SNO-4. We did not recover any type II rDNA progeny with an NOR smaller than that of SNO-4. However, the resolution of our gels is such that we probably could not see a difference between the NOR of SNO-4 and an NOR that was one to a few repeat units smaller. The same general result was seen in crosses of defined direction, in which SNO-4 acted as the female only or the male only in the cross (data not shown). Table 1 presents a summary of the data. A trivial explanation for these results is that the *Bcl*I restriction enzyme is not cutting at the same *Bcl*I sites relative to the rDNA in the parents and the progeny. This general possibility has been ruled out in our previous work by comparing the digestion pattern of *Bcl*I with that of *Not*I, a second enzyme that does not cut within rDNA. In the cases examined, the differences in *Bcl*I rDNA fragment sizes between the parents and progeny is arithmetically related to those seen with *Not*I (Butler and Metzenberg 1989).

One obvious hypothesis that could explain the above finding is that progeny ascospores that inherit an NOR smaller than 800 kb are inviable owing to an insufficient number of rRNA genes and are therefore not recovered. If this were true, significantly fewer progeny should inherit their NOR from SNO-4 than progeny inheriting their NOR from NS-1. Since SNO-4 has only type II

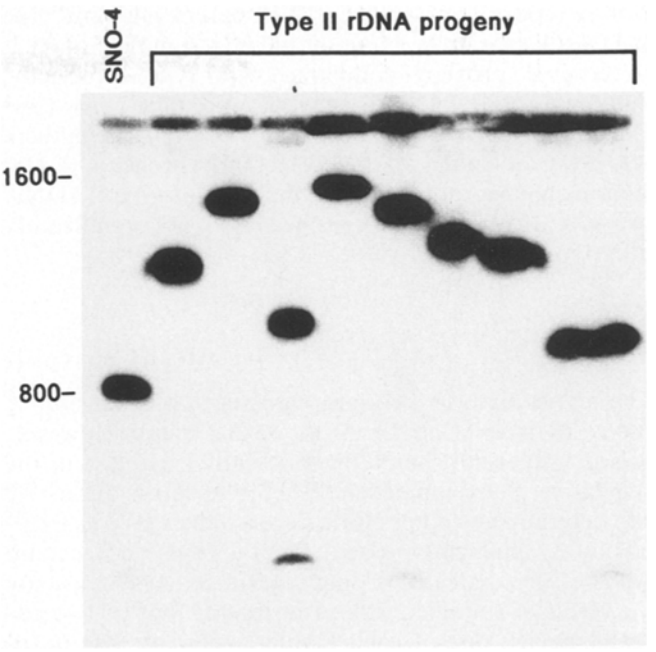

Fig. 2. Autoradiogram of *Bcl*I-digested DNA from SNO-4 and nine randomly collected type II rDNA progeny. The Southern blot was probed with radiolabeled rDNA. The rDNA band at approximately 50 kb in SNO-4 and in some of the progeny represents the small cluster of rDNA repeat units on LG I (see text). The contour clamped homogeneous electric field (CHEF) gel electrophoresis conditions were 120 s pulse time at 145 V. Intact *Saccharomyces cerevisiae* chromosomes were used as size standards. The positions of yeast chromosomes VII, XV (*first mark from the top*) and XIV (*bottom mark*) along with their estimated molecular weights in kilobase pairs are indicated at the left of the autoradiogram. The molecular weights of the yeast chromosomes are only approximations (see Butler and Metzenberg 1989)

Table 1. Distribution of NOR sizes among the type II rDNA progeny, the type I rDNA progeny and the progeny that bear the interstitial NOR

rDNA type	Total progeny	NOR size classes		
		Parental	Larger	Smaller
Type II	56[a]	7	49	0
Type I[b]	63	2	11	4
Interstitial[c]		6	3	4

[a] This number includes progeny from the single perithecium and from isolated asci
[b] Only 17 type I progeny were examined for NOR size
[c] rDNA cluster at the interstitial locus on LG I

rDNA repeat units and the NS-1 parent has only type I rDNA repeat units, there should be a significant deficit of type II progeny compared with type I progeny. Among a total of 119 progeny, 56 were type II for their rDNA and 63 were type I for their rDNA. Though there was a small deficit of type II rDNA progeny, a chi-square analysis indicated that the ratio of type II rDNA progeny to type I rDNA progeny was not significantly different from 1:1 (P value > 0.1).

The timing of NOR expansion

The above analysis strongly suggests that expansion of the NOR is associated with the sexual phase. However, it is possible that the NOR is actually expanded in the vegetative phase, immediately prior to fertilization. We can determine whether NOR size can change after fertilization by analyzing progeny from a single perithecium. All the ascospores of a single perithecium are usually the result of a single fertilization event; that is, two parental nuclei, one of each mating type, give rise to all the ascospores of a perithecium (Perkins and Barry 1977). If the NOR can only expand before fertilization, then all of the ascospores with type II rDNA from the same perithecium will have the same size NOR. In contrast, if the NOR expands after fertilization, then various ascospores from a single perithecium with type II rDNA will harbor different sizes of NOR.

We chose a perithecium from a cross of SNO-4 × NS-1 and analyzed six randomly collected type II rDNA progeny. Figure 3 shows the result of this experiment. Clearly, the NORs of the type II rDNA progeny are of several different sizes, all of them larger than that of SNO-4. We conclude that expansion of the NOR occurs after fertilization.

Previous work has shown that reduction of NOR size is most frequently after fertilization but prior to meiosis

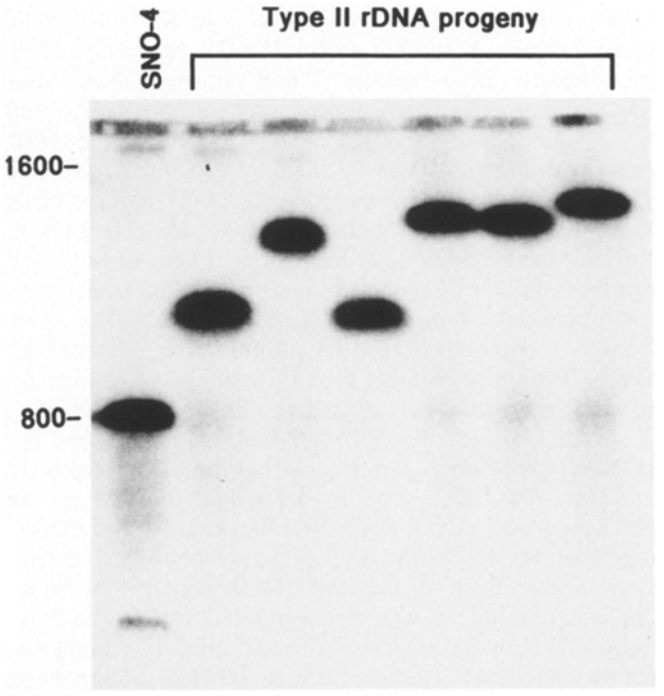

Fig. 3. Autoradiogram of *Bcl*I-digested DNA from SNO-4 and six type II rDNA progeny from the same perithecium. The Southern blot was probed with radiolabeled rDNA. The CHEF gel electrophoresis conditions were as in Fig. 2. The positions of yeast chromosomes VII, XV (*first mark from the top*) and XIV (*bottom mark*) along with their estimated molecular weights in kilobase pairs are indicated at the left of the autoradiogram

(Butler and Metzenberg 1989). We wanted to know whether expansion of the NOR occurs primarily at this time as well. If an NOR expands during the sexual phase, the stage at which expansion occurs will determine the segregation pattern of NOR size in individual asci. If the NORs can only expand before the final round of pre-meiotic DNA synthesis (i.e., during the premeiotic

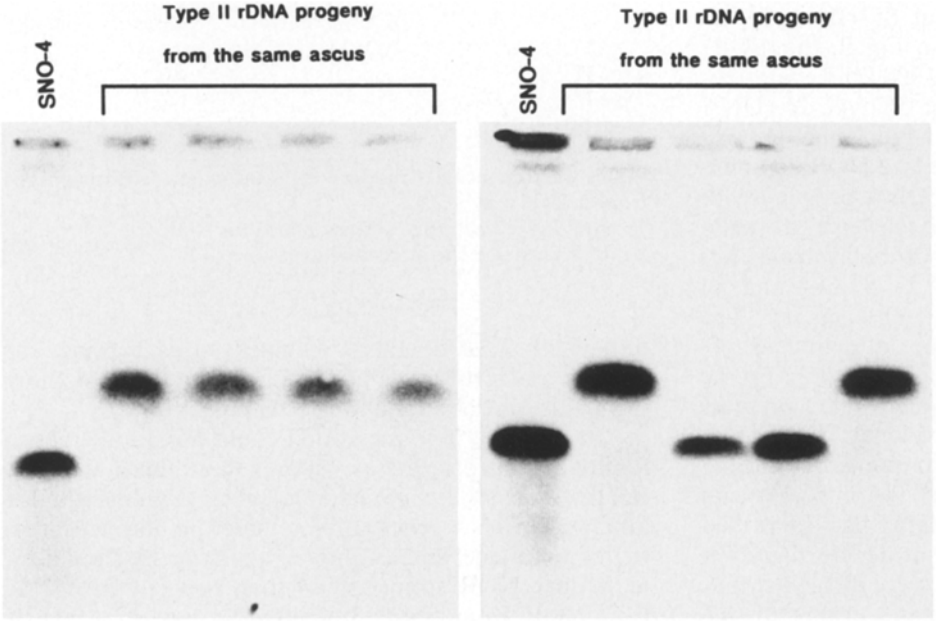

Fig. 4. Autoradiograms of *Bcl*I-digested DNA from SNO-4 and four type II rDNA progeny from the same ascus. The *left panel* shows an example of 4:0 segregation pattern (ascus B) and the right panel shows an example of a 2:2 segregation pattern (ascus C). The Southern blot was probed with radiolabeled rDNA. The CHEF gel electrophoresis conditions were as in Fig. 2

stage; see Fig. 1), then all four type II rDNA progeny of the ascus will have the same size NOR. In contrast,if the NORs expand during meiosis, then there will be two different sizes of the NOR among the four type II rDNA progeny (see Fig. 1). Finally, if the NORs expand postmeiotically, at the mitotic division immediately following meiosis or in the first few divisions after ascospore delimitation, then sister spores of an ascus would harbor NORs of different size (see Fig. 1).

We collected five asci (labelled A through E) from a cross of SNO-4 × NS-1 and analyzed the type II rDNA progeny. In ascus A, all the type II progeny had the same size NOR as SNO-4, approximately 800 kb. In ascus B, the size of the NOR was the same for all type II rDNA progeny, but larger than that of SNO-4 by approximately 200 kb (ascus B is shown in the left panel of Fig. 4). In the remaining three asci (C, D, and E), the segregation pattern of size was consistent with a meiotic event. That is, there were two different sizes of NOR among the type II rDNA progeny of an ascus (ascus C is shown in the right panel of Fig. 4). In ascus C, the larger type II NORs are about 900 kb and other type II NORs are the same size as that from SNO-4, about 800 kb. In ascus D, all of the type II NORs were larger than that of SNO-4. The two non-parental sizes in ascus D were about 850 kb and about 1000 kb. In ascus E, the type II NORs were larger than that of SNO-4. The two non-parental sizes in ascus E were about 875 and about 900 kb. Thus, in the SNO-4 cross, events that change the size of the NOR are frequent during meiosis. This analysis does not indicate that premeiotic events are infrequent. It is quite possible, even likely, that most NORs change size both premeiotically and meiotically.

Size changes at the interstitial rDNA cluster and the NOR of type I rDNA progeny

Some strains of *Neurospora*, called QNS for Quasi Normal Sequence, carry a small cluster of type II rDNA repeat units at an interstitial locus on LG I, in addition to the large cluster of type II rDNA repeat units at the conventionally located NOR (Perkins et al. 1986). SNO-4 is a meiotic offspring of QNS-1 and it carries about five rDNA repeat units at the interstitial locus (data not shown). Thus SNO-4 nuclei carry two rDNA clusters through the sexual phase. Previously, in a cross of QNS-1 to NS-1 (the same type I rDNA strain used in the crosses reported here), we had examined changes in the size of the interstitial rDNA cluster. The interstitial rDNA cluster tended to decrease in size during the sexual phase (Butler and Metzenberg 1989). We wanted to know whether the size of interstitial rDNA cluster in SNO-4 showed any regular tendency to increase or decrease in the SNO-4 × NS-1 crosses. Intact chromosomal DNA was prepared from SNO-4 and from the appropriate progeny, digested with *BclI*, fractionated by CHEF gel electrophoresis, blotted, and probed with radiolabeled rDNA. Table 1 summarizes the data from these experiments. Unlike the situation with the main NOR in SNO-4, both increases and decreases in the size of the interstitial rDNA cluster were common.

We also wanted to know whether the NOR of NS-1 was undergoing amplification during the crosses of NS-1 to SNO-4. NS-1 has a more normal sized NOR than that of SNO-4 (approximately 1350 kb; Butler and Metzenberg 1989). We analyzed NOR size in 17 randomly collected type I rDNA progeny from the cross of NS-1 to SNO-4. Out of the 17 progeny analyzed, 2 type I rDNA progeny had an NOR the same size as that of NS-1, 11 progeny had an NOR larger than that of NS-1 and, significantly, 4 progeny had an NOR *smaller* than that of NS-1 (data not shown). While there is a bias toward increases in the size of the NOR, the behavior of the NOR of NS-1 is clearly different from the behavior of the NOR of SNO-4.

Discussion

We have discovered an rDNA magnification-like phenomenon in the sexual phase of *Neurospora*. The novel finding is that changes in the size of the NOR appear to be exclusively unidirectional; a strain with an unusually small NOR, called SNO-4, virtually always transmits larger NORs to its progeny. We recovered only a few progeny that had an NOR the same size as that of SNO-4 and none that had an NOR smaller than that of SNO-4. Analysis of progeny from a single perithecium clearly indicated that expansion occurs after fertilization. Unlike in other crosses (Butler and Metzenberg 1989), changes in the size of the NOR occur frequently during meiosis.

In the typical *Neurospora* crosses we have examined previously, decreases in NOR size were recovered more frequently than increases. We presented evidence that *intra*-chromatid recombination occurs in rDNA and, to explain the bias toward decreases in NOR size, we suggested that *intra*-chromatid recombination might be the dominant recombination mechanism operating during the premeiotic portion of the *Neurospora* sexual phase (Butler and Metzenberg 1989, 1990). What kind of mechanism could result in the preferential recovery of NORs larger than that of the parent with an atypically small NOR? One possibility, proposed by Tartof (1974) to explain rDNA magnification in *Drosophila*, is unequal sister chromatid exchange, coupled with selection. A second possibility is meiotic conversion to a larger number of repeat units, as was seen at the *CUP1* locus of yeast by Fogel et al. (1984); however, in our case, the conversion would have to be between sister chromatids for the same reason that a crossover exchange would have to be between sister chromatids. Previously we demonstrated that *Neurospora* rDNA is capable of unequal sister chromatid exchange in the sexual phase (Butler and Metzenberg 1990). Unequal sister chromatid exchange is a reciprocal process; a single unequal sister chromatid exchange will generate NORs both larger and smaller than the original NOR. Since we did not recover any type II rDNA progeny with an NOR smaller than that of SNO-4, unequal sister chromatid exchange can

be ruled out unless there is selection against NORs smaller than that of SNO-4. Consistent with the idea that unequal sister chromatid exchange does occur in the SNO-4 crosses, we have observed that changes in the size of a second (ectopic) rDNA cluster in the SNO-4 genome are nearly equally distributed between increases and decreases. This result is to be expected if most changes in the size of the interstitial rDNA cluster are selectively neutral. Considering the extremely small size of the ectopic cluster (approximately five rDNA repeat units in the SNO-4 parent) and the uncertainty that it is even transcribed, this seems likely to be the case.

Surprisingly, we found that the number of progeny that inherit their NOR from SNO-4 is not significantly different from the number of progeny that inherit their NOR from NS-1. This result implies that there is little or no selection against smaller NORs *at the level of ascospores*. At what other point could selection be imposed? The most likely possibility is that unequal sister chromatid exchange and selection operate during the premeiotic phase of the sexual cycle. This part of the sexual cycle is characterized by proliferation of the fertilizing nuclei in a common cytoplasm (see Fig. 1). Thus, this is the only time in the sexual phase when it is possible for the loss of some nuclei (viz those with reduced NORs) to be compensated by extra divisions of other nuclei (viz those with amplified NORs). However, it is not obvious why the nuclei with very small NORs should be at a disadvantage, because they are in a common cytoplasm with nuclei with larger NORs.

Clearly, the existence of one ascus (ascus B) in which all type II rDNA progeny have the same expanded size NOR indicates that expansion of the NOR can occur during the premeiotic phase. However, we found several asci showing meiotic events. In these asci, all of the NORs of the same ascus were either larger than that of SNO-4 or, at most two were the same size as that of SNO-4 and the others larger. Thus, if the meiotic event is unequal sister chromatid exchange, the size of the NOR entering meiosis must be exactly intermediate between the two new sizes generated by the meiotic unequal sister chromatid exchange. It follows, therefore, that the NOR entering meiosis is already larger than that of SNO-4, and the NOR must have undergone at least one premeiotic event that expanded its size. We think that unequal sister chromatid exchange is probably frequent prior to and during meiosis in crosses involving SNO strains. Sister chromatid conversion is, of course, also a possibility.

Another alternative mechanism that could expand the NOR without a reciprocal loss of rDNA was originally invoked to explain rDNA magnification (Ritossa 1972). Ritossa proposed that rDNA magnification occurs when extra-chromosomal rDNA circles, amplified by over-replication, integrate into the rDNA-deficient NOR by homologous recombination. A slight modification of this mechanism can explain the bias in NOR size changes during the *Neurospora* sexual phase. That is, rDNA circles could be excised by *intra*-chromatid recombination premeiotically and, depending on genetic or environmental cues related to the adequacy of the size of the NOR, could either be lost (leading to the preferential recovery of smaller NORs) or amplified and reintegrated (leading to the preferential recovery of larger NORs). We must emphasize, however, that this model is purely speculative. It does not seem possible that the reintegration could occur after karyogamy, because that would lead to the formation of hybrid NORs, contrary to observation.

Another explanation for the amplification of the NOR is that the interstitial rDNA cluster is furnishing repeat units to the SNO-4 NOR. While it is not possible definitively to rule this out, since both the NOR and the interstitial rDNA cluster are composed of type II rDNA repeat units, it seems unlikely based on other considerations. One is that the interstitial rDNA cluster can get bigger during the sexual phase of the crosses reported here. This would not be expected if there were a flow of rDNA repeat units from the interstitial rDNA cluster to the NOR. Moreover, NOR size instability during the sexual phase, including increases in NOR size, is not dependent on there being an interstitial rDNA cluster in the genome of the parents (Butler and Metzenberg, unpublished data).

Differences in the size of individual rDNA repeat units within the same organism have been observed (Long and Dawid 1980). This is primarily due to differences in the number of small subrepeats located within the nontranscribed spacer region of the rDNA repeat unit. Is it possible that increases in the *size* of rDNA repeat units and not increases in the *number* of rDNA repeat units can explain our results? To determine rDNA type we must digest genomic DNA with *Hin*dIII (see Results). This restriction enzyme has recognition sites within the rDNA repeat unit, thus *Hin*dIII digestion provides a measure of rDNA repeat unit size. Our results clearly show that the sizes of the *Hin*dIII fragments in type II progeny are not different from those of the SNO-4 parent, indicating that rDNA repeat unit size has not changed during the sexual phase (data not shown).

Two pieces of evidence support the idea that the control of amplification is a property of the NOR itself and, in turn, depends on the size of the NOR. If SNO-4 nuclei, or the ascogenous hyphae, were receiving some sort of signal that they should exclusively increase the size of their NORs, then that signal should also be "heard" by the interstitial rDNA cluster and the NOR of NS-1. Clearly this is not the case, since we recovered progeny with interstitial clusters smaller than that of SNO-4 and type I NORs smaller than that of NS-1. Additionally, from crosses of NS-1 to QNS-1 (a type II rDNA strain with largely the same genetic background as SNO-4, except that its NOR is of a more normal size), we frequently recovered type II rDNA progeny with NORs smaller than that of QNS-1 (Butler and Metzenberg 1989). We should also add that control of amplification is unlikely to reside at a locus tightly linked to the NOR but distinct from rDNA. SNO-4 is a meiotic offspring of NS-1 and QNS-1, thus its NOR must have either NS-1 or QNS-1 flanking sequences. Both NS-1 and QNS-1 have been shown to yield frequent decreases in NOR size during crosses (Butler and

Metzenberg 1989). However, to understand better the mechanism of NOR size change and its regulation, it will be important to find mutations that affect this process. In *Drosophila*, several mutations that affect recombination and repair have been found to inhibit rDNA magnification. Interestingly, some mutations have different effects on the magnification process. Mutations in the *mus-108* gene block both premeiotic and meiotic magnification, while mutations in the *mei-41* gene block only premeiotic magnification (Hawley and Tartof 1983; Hawley et al. 1985). Many mutations that affect recombination and repair functions are known in *Neurospora* (see Perkins et al. 1982). It would be interesting to test whether these mutations can inhibit or block NOR expansion and/or contraction and, if so, whether such mutations have different effects on premeiotic and meiotic events.

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