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Nonrandom Sister Chromatid Segregation and Nuclear Migration in Hyphae of *Aspergillus nidulans*

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Radioactive conidiospores of *Aspergillus nidulans* were prepared by growing a purine-requiring mutant with tritiated adenine. When these spores germinated in a nonradioactive medium, the dispersion of the original chromosome set could be followed by treating the hyphae with ribonuclease and preparing radioautograms. Germinating spores with four or eight nuclei contained two highly labeled nuclei and two or six nuclei with much less or no radioactivity. Successive mitotic divisions thus distributed the deoxyribonucleic acid (DNA) of the eight spore chromosomes among only two of the progeny nuclei. The two nuclei containing the original chromosome set were not dispersed at random along the linear hypha but were usually located near the growing tip. These results are compatible with the view that chromatids containing DNA strands of identical age segregate as a unit during mitosis. They further indicate that the mechanism which disperses newly formed nuclei in the growing hypha can distinguish between nuclei containing DNA strands of different ages.

Lark, Consigli, and Minocha (10) followed the radioactivity of cultured mammalian cells during growth with tritiated thymidine or after labeling for a single generation. They concluded from the distribution of the label among individual cells that sister chromatids did not segregate at random during mitosis. Rather, chromatids containing deoxyribonucleic acid (DNA) strands used as templates two cycles previously segregated into one nucleus and chromatids with template strands from the last division segregated into the other. By examining anaphase or telophase cells, in which the common origin of the two sets of chromosomes is clear, Lark (9) showed that nonrandom segregation of sister chromatids also occurred during mitosis in growing plant roots. Lark explained these segregation patterns on the basis of his model for bacterial chromosome duplication (8).

To explore the generality of these findings among eukaryotic cells, we have examined sister chromatid segregation in a filamentous fungus, the ascomycete *Aspergillus nidulans*. *A. nidulans* is typically haploid, with eight chromosomes, and forms mononucleate conidiospores which can germinate into multinucleate hyphae (6). Individual germinating conidiospores will thus contain all of the progeny of their original nucleus. Techniques for the isotopic labeling and radioautography of *A. nidulans* nuclei have been worked out (7). The dispersion of the original chromosome set can therefore be followed by germinating labeled conidiospores in a nonradioactive medium and by examining radioautograms prepared from them. Should chromatid segregation prove to be nonrandom, such experiments also allow the identification of the two nuclei formed by the first nuclear division. The position of these nuclei in the germ-tube should provide information about the movement of nuclei in relation to hyphal growth and extension.

Our results indicate that sister chromatid segregation at mitosis is nonrandom and follows the pattern described for mammalian and plant cells. In addition, the two nuclei containing the original chromosome set are not positioned at random along the hypha but tend to be close to the growing hyphal tip.

MATERIALS AND METHODS

Strain and growth conditions. Strain 46 NX, a purine-requiring mutant described previously (7), was used throughout this investigation. Conidia with ^{3}H-labeled nucleic acids were obtained by inoculating a

glucose-ammonia-histidine medium (7) solidified with agar and containing 10 μg of adenine per ml and 5 or 10 μc of adenine-8-^{3}H per ml (specific activity, 3 to 8 c/mmole, Schwartz Bio Research Inc., Orangeburg, N.Y.). Tritiated adenine and a purine-requiring strain were used since labeling with thymine or thymidine was not effective (7). After incubation at 37 C, the conidia were harvested and washed as described previously (7, 15). The radioactive conidia were germinated in a liquid glucose-ammonia-histidine-adenine medium in a shaking water bath at 37 C.

Preparation of radioautograms. Samples from cultures of germinating conidiospores were collected, fixed with cold 5% trichloroacetic acid for 30 min, and treated with ribonuclease and NaOH to remove cellular ribonucleic acid (RNA) as described previously (7). In addition, control slides were incubated with deoxyribonuclease (7). After being covered with Ilford K5 emulsion, the slides were held for 8 to 16 days at 4 C; then they were developed and stained with hemotoxylin (7). Grain density in the background and over nuclei in deoxyribonuclease-treated preparations was negligible compared to that over labeled nuclei (Fig. 7), and experimental counts were not corrected.

RESULTS

Distribution of radioactivity among the nuclei of individual hyphae. Samples taken from cultures of germinating conidiospores contained, besides ungerminated spores and older forms, hyphae with two, four, or eight nuclei. Approximately 4% of the hyphae which could be scored contained nuclei in the process of division (14). In hyphae having two nuclei, the radioactivity was, as would be expected, equally distributed between the two sets of chromosomes (Fig. 1). Thus, plotting the number of grains over individual nuclei against the total number of grains over the hypha containing them gave a scatter diagram with almost all of the points between the value for equal distribution and its standard deviation (Poisson distribution). This equal distribution occurred despite the differences in total numbers of grains over individual hyphae. Therefore, our methods do not produce artifacts which make one nucleus appear more radioactive than another.

To analyze the distribution in hyphae having four nuclei, we plotted the number of grains over the two most highly labeled nuclei and over the two remaining nuclei against the sum of grains over the hypha (Fig. 2). For the plot, we randomly chose 50 hyphae from a total of 319 examined. It is clear from the scatter diagram that one pair of nuclei was much more highly labeled than the other. The grain counts for the more radioactive pair were almost entirely between the value for 85% of the total grains and its standard deviation.

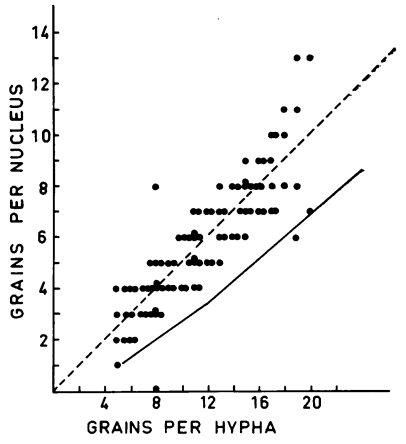

FIG. 1. *Distribution of radioactivity in 50 germinating conidia containing two nuclei. Labeled conidia were germinated in nonradioactive medium, radioautograms were prepared, and the grains over individual nuclei were plotted against the total grains over the hypha containing them. The dashed line is the expectation for equal distribution and the solid line is its standard deviation.*

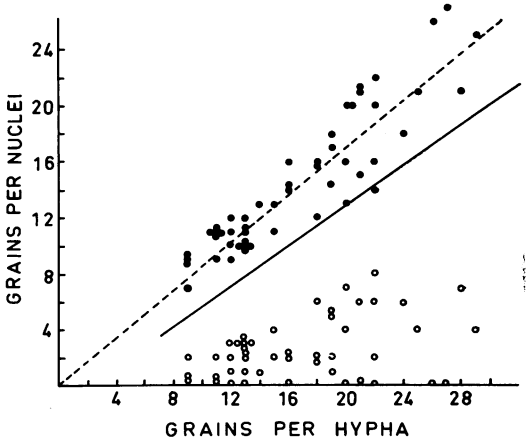

FIG. 2. *Distribution of radioactivity in 50 labeled conidiospores, germinating in cold medium and containing 4 nuclei. The sum of grains over the two most radioactive nuclei and over the two remaining nuclei is plotted against the total grains over the hypha. Symbols: ●, more radioactive pair; ○, less radioactive pair; dashed line, expectation for 85% of total radioactivity in one pair of nuclei; solid line, its standard deviation.*

Figure 3 is a scatter diagram of the same type for hyphae containing eight nuclei. The data represent 50 hyphae chosen at random from a total of 106 examined. The number of grains over the two most highly labeled nuclei and over the remaining six nuclei is plotted against the total radioactivity of the hypha. Clearly, two popula-

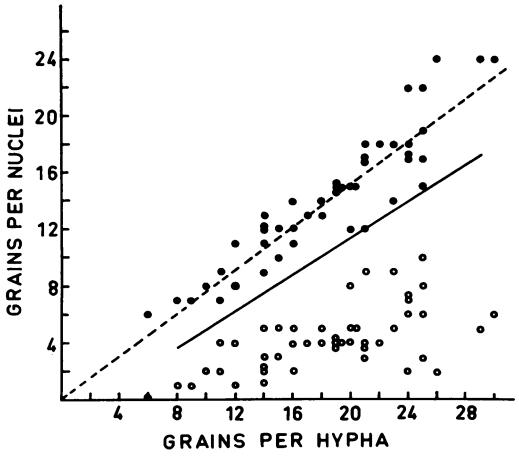

FIG. 3. *Distribution of radioactivity in 50 labeled conidiospores, germinating in cold medium and containing 8 nuclei. The sum of grains over the two most radioactive nuclei and over the six remaining nuclei is plotted against the total grains over the hypha. Symbols;* ●, *radioactive pair;* ○, *remaining six nuclei; dashed line, expectation for 75% of total radioactivity in radioactive pair; solid line, its standard deviation.*

TABLE 1. *Distribution of radioactivity among nuclei in hyphae with four or eight nuclei*

Nuclei per hypha	No. of hyphae examined	No. of hyphae with given per cent of total label in two nuclei			
		> 75	66.6–75	50–66.6	< 50
4	319	276	31	12	0
8	106	71	21	11	3

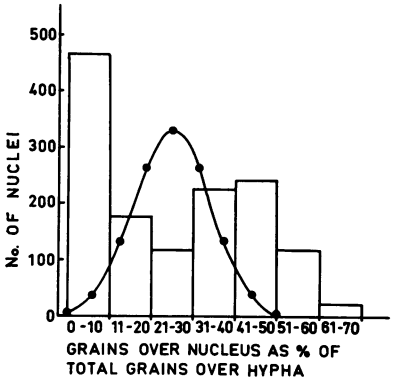

FIG. 4. *Distribution of grains in germinating conidiospores with four nuclei, expressed as the per cent of total hyphal radioactivity in individual nuclei. The solid line shows the distribution expected from the random segregation of eight chromosomes and is calculated from the terms of a binomial expansion. The blocks show the distribution found experimentally.*

tions of nuclei were present, with the radioactivity of the highly labeled pair lying between 75% of the total label and its standard deviation and the sum of grains over the remaining six nuclei falling far below.

Table 1 summarizes the results for all of the hyphae with four or eight nuclei which we have examined. With very few exceptions, two of the nuclei contained 75% or more of the radioactivity.

Distribution of radioactivity expected from a random segregation of sister chromatids. Nuclei of *A. nidulans* contain eight chromosomes (6). Assuming that all the chromosomes contain an equal amount of DNA and radioactivity and that sister chromatids segregate at random into the two products of mitosis, the expected labeling in hyphae containing four nuclei can be calculated from a binomial distribution (Fig. 4). This distribution is very different from that found experimentally and indicates that segregation was not random.

Location of highly labeled nuclei along the hypha. It seemed likely, as discussed below, that the two highly labeled nuclei were the products of the first nuclear division after germination. Their position along the hypha should thus indicate how nuclei move in relation to the linear extension of the growing hypha. For this analysis, we used data from 276 hyphae with 4 nuclei (86% of total examined, Table 1), in which 2 nuclei accounted for 75% or more of the radioactivity, and data from 92 hyphae with 8 nuclei (85% of total examined), in which 2 nuclei accounted for 66.6% or more of the radioactivity. The two highly labeled nuclei clearly tended to aggregate near the growing tip (Fig. 5).

In germinating conidia, the nuclei are usually arranged linearly along the hypha (Fig. 7) and two labeled nuclei can be grouped among a total of four in six different ways. The frequency with which these arrangements occurred is shown in Fig. 6. We have demonstrated that, under the conditions used in these experiments, the nuclei in individual hyphae replicate and divide in very strict synchrony (7, 14). Note then that the simplest way to obtain arrangement 6 (Fig. 6), accounting for 51% of the hyphae with four nuclei, would be for at least one of the nuclei formed in the second nuclear division to move past the highly labeled nuclei in the direction of the conidiospore. In 64% of hyphae with eight nuclei, both of the labeled nuclei were among the four nearest the tip; the arrangement of these four tip nuclei is given in Fig. 6. Note again that these arrangements can be most easily explained by stating that the products of the second nuclear

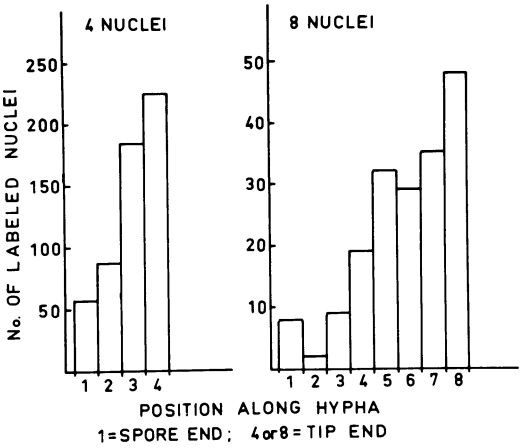

FIG. 5. *Location of the two radioactive nuclei along hyphae containing four or eight nuclei. The positions are numbered from the spore end (position 1), and the frequency with which labeled nuclei occupy them is plotted.*

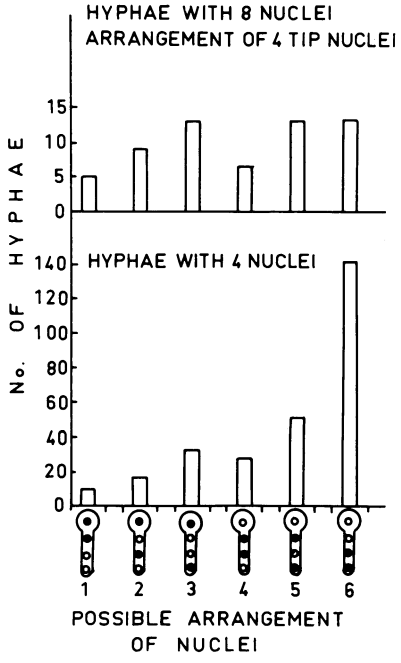

FIG. 6. *Arrangement of the two labeled nuclei in hyphae with four or eight nuclei. The frequency of the possible arrangements in hyphae with four nuclei is shown in the lower part. The upper part shows the arrangement of the last four nuclei in hyphae with a total of eight nuclei; both labeled nuclei are in the last four (64% of total examined). Symbol: ●, labeled nucleus.*

division, and in the case of arrangement 6 of the third nuclear division, moved past the labeled nuclei in the direction of the spore.

DISCUSSION

Hyphae with four or eight nuclei contained two nuclear populations, one pair of labeled nuclei and two or six nuclei with considerably less radioactivity. Although the total number of silver grains over various hyphae originating from the same batch of radioactive conidia showed considerable scatter (Fig. 1), a distribution of this kind would be expected from the relatively low grain counts and the statistics of isotope disintegration. For example, if the true isotope content of hyphae in Fig. 1 was equivalent to 12 grains per hypha, 19 of 20 hyphae would be expected to fall in the range of 5 to 19 grains. Since hyphae with two nuclei showed equal labeling in both of the nuclei, whereas longer hyphae did not, and in view of the number of hyphae scored, we believe that our conclusions are valid, despite the statistical uncertainties associated with low grain counts.

We have demonstrated previously (7, 14) that the nuclei in individual germinating conidiospores replicate and divide in synchrony during growth on glucose media. This finding excludes the possibility that a particular nucleus does not divide or divides less frequently than the others and thus leads to a nonrandom segregation of the original chromosome set.

Conidia of strain 46 NX do not germinate in media lacking adenine and their content of free purines, which in our experiments would be labeled, must therefore be extremely small. Since we used an adenine-requiring mutant and tritiated adenine, the original conidiospores contained labeled RNA as well as DNA. It might be argued that breakdown of RNA could liberate labeled nucleotides which are then incorporated into newly formed DNA. However, most species of cellular RNA are stable (11), and unstable messenger RNA would be made during germination from unlabeled adenine rather than be carried over with the conidiospore (5). If labeled nucleotides are recycled into the pool, they would be greatly diluted by the cold adenine supplied and their contribution to radioactivity in the DNA should be negligible. The observed nonrandomness must then be due to a division mechanism which tends to segregate the 16 chromatids containing the DNA strands of the original conidial nucleus into two nuclei only.

The radioactivity of the original chromosome set was mainly, but not exclusively, located in two of the progeny nuclei. Such minor redistribution of label after successive mitotic divisions was also found by Lark (9, 10) in animal and plant cells. This result would be expected if, together

with a completely nonrandom segregation of sister chromatids, sister-sister chromatid exchanges occurred during nuclear replication and division. In eukaryotic nuclei labeled with tritium, such exchanges invariably occur (17) and could adequately explain our results.

Thus, the segregation of all chromatids containing DNA strands of equal age into one nucleus is a feature of mitosis in such divergent groups as plant, mammalian, and fungal cells. In bacteria containing episomes, evidence has been presented that the old parental strands of both the genome and the episome segregate together (2). Cosegregation of template strands of like age thus seems to be a basic mechanism in the indexing and distribution of genetic material during vegetative growth.

The nuclei containing the original radioactive DNA strands were not distributed at random along the hypha but showed a marked tendency to accumulate near the growing tip. This could only occur if there was little or no circulation of nuclei in hyphae of *A. nidulans*, a finding in agreement with the direct phase-contrast microscope observations of Clutterbuck (1) and of Kessel (*unpublished data*). Although the absence of nuclear circulation explains how a nonrandom distribution is maintained, it does not explain how it arises. Nuclei tended to be evenly spaced along the hypha and did not accumulate as clusters containing the progeny of successive nuclear divisions (Fig. 7). Therefore, there must be some mechanism which separates the products of mitosis during growth and hyphae extension. This mechanism operates with some precision, since diploid nuclei tend to be spread so that the volume of cytoplasm per nucleus is twice that found for haploid strains (1). Our results indicate that, whatever the nature of the mechanism, it can distinguish between daughter nuclei containing DNA strands of different ages. Eberle and Lark (4) have claimed that single cells of *Bacillus subtilis*, prelabeled with thymidine, form chains in which the original chromosome strands are not distributed at random along the chain. The significance of these results is difficult to interpret, however, since in similar experiments Ryter (15) found that the original chromosome strands are distributed at random along the chain.

Little is known about the movement of nuclei in coenocytic hyphae (13, 16) and any interpretation of the results would necessarily be speculative. However, the nuclear membrane of eukaryotic cells, including fungi, is considered to be continuous with the endoplasmic reticulum (12), and nuclei may well be distributed throughout the

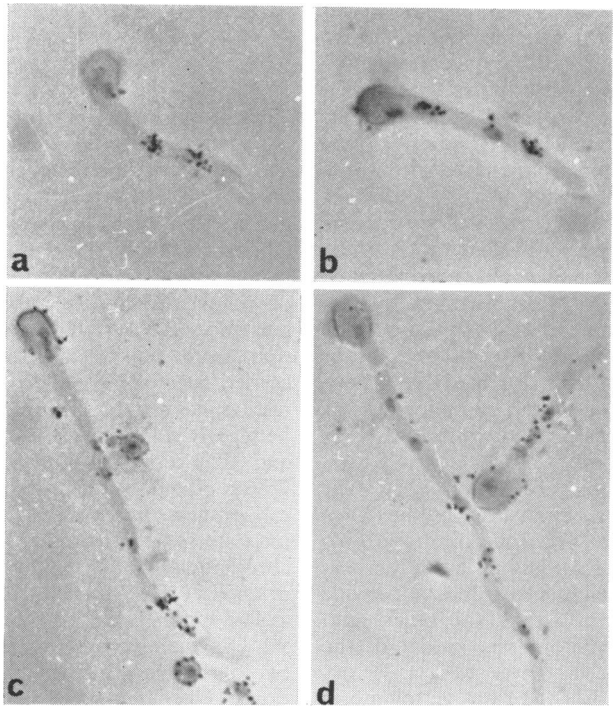

FIG. 7. *Radioautograms of hyphae containing four or eight nuclei. (a) Nuclei no. 3 and 4 labeled. (b) Nuclei no. 2 and 4 labeled. (c) Nuclei no. 7 and 8 labeled (spore end nucleus = 1). (d) Nuclei no. 5 and 6 labeled.* × *1,100.*

growing hypha by the continued synthesis and extension of the endoplasmic reticulum. If, as discussed above, DNA strands of identical age are attached to a structural segregating unit, the following model may be proposed.

To start DNA replication, a nucleus must carry out at least two operations: (i) the permanent attachment of the segregation unit carrying the DNA strands to cell membranes and (ii) the acquisition of a second segregation unit. Subsequent replication and mitosis would then yield one nucleus attached to cell membranes and a free nucleus. In general, nuclei whose older DNA strands have served as templates twice or more would be attached while others would be free. The attached nuclei would move with the extending membrane system while the other nuclei would move with cytoplasmic streaming, etc., until they find a vacant attachment site. The location of new membrane attachment sites would thus determine the position of the nuclei containing the youngest DNA strands. If at least part of the membrane system maintains its location in relation to the growing and elongating tip, the type of distribution observed experimentally would result. There is no information in the literature concerning the sites of new membrane synthesis in filamentous fungi and we are currently investigating this point. Microscopic observations of *Gelasinospora* have suggested that nuclei may be anchored to cellular structures or may occur free in the cytoplasm (3). The type of nuclear migration envisaged here would not, of course, have to be related to the oriented and rapid nuclear migration occurring in certain higher fungi (16). It may be pertinent that, at slow growth rates of *A. nidulans*, some nuclei replicate and divide, whereas others in the same hypha do not (7, 14). The above model would readily fit these findings if the rate at which membrane attachment sites are formed is related to the overall growth rate. At slow growth rates and with no sites available, only those nuclei which are already attached could initiate replication.

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