Genet. Res., Camb. (1977), **29**, *pp.* 1–8
With 3 *text-figures*
Printed in Great Britain

DNA syntheses in course of meiotic development in *Neurospora crassa**†

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(*Received* 13 *April* 1976)

SUMMARY

Nuclear DNA syntheses have been measured microspectrophotometrically in course of meiotic nuclear development in *Neurospora crassa*. The purpose of the study has been to establish the timing of these DNA syntheses in relation to meiosis and in the formation of its final products in an ideal genetic material, where the products of the meiotic division can be recovered and identified. Spectrophotometric analysis has indicated that the last premeiotic DNA replication is completed before caryogamy; a postmeiotic DNA replication takes place at the tetrad of the nuclei level; and finally a second postmeiotic DNA replication occurs in the ascospores to result in eight binucleated ascospores. The timing of postmeiotic replication is compatible with the models explaining gene conversion through the postulated mechanisms involving heteroduplex formation.

1. INTRODUCTION

Organisms in which all four products of the meiotic division can be recovered and identified in having a common meiotic origin offer opportunities for obtaining information about meiotic events related to genetic recombination. Organisms like *Neurospora crassa* and *Sordaria fimicola* where linearly ordered eight-spored asci are formed, yield direct information about a larger number of meiotic events than any other organism. '*Neurospora*' type of meiosis characterized by the presence of a single diploid nucleus which immediately undergoes meiosis has long appeared to offer unique possibilities for determining the time of DNA synthesis relative to genetic recombination. It has not been till now possible to follow the timing of DNA synthesis relative to meiosis in an ascomycete adaptable to genetic studies. Biochemical idiosyncrasies in *Neurospora* have made labelling of chromosomes with ^{3}H-thymidine impossible. Likewise, nuclei of nearly all ascomycetes have not been proved to be adaptable to spectrophotometric methods as it has been possible in *Neottiella rutilans* characterized by unusually large nuclei. *N. rutilans*, on the other hand, has not shown any indication that it can replace *Neurospora*/*Sordaria* as a

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† We thank Dr D. R. Stadler for his comments during the preparation of this communication. This work has been supported by Research grants from University Grants Commission, New Delhi and Fellowship grants from the Council of Scientific and Industrial Research (G.A.S.I.) and Indian Council of Agricultural Research (P.C.D.), New Delhi.

parallel experimental system for genetical studies pertaining to recombination. All attempts to culture *N. rutilans* in the past have failed. Hence, a gap has remained all the while. The present text deals with the account of an attempt to fill this gap by adopting similar spectrophotometric methods as earlier used by Rossen & Westergaard (1966) with *Neottiella rutilans*, to measure the time of DNA synthesis in course of developmental phases of meiosis in *Neurospora crassa*. This has no doubt relevance primarily to the mechanism of meiotic recombination.

2. MATERIALS AND METHODS

Wild type cultures of *Neurospora crassa* CBS285–62 obtained from the Central Bureau Voor-Schimmelucultures, Baarn, Netherland, were grown on Potato Dextrose Agar medium with 2 % glucose and 2 % agar. Crosses were made following the technique adopted by Barry (1966). Standardization of the staining method for *Neurospora* nuclei, has been attained after an extensive trial with various fixing fluids, viz. 1:3 acetic acid–ethanol, 1:6:3 acetic acid–ethanol–chloroform, 1:3 propionic acid–ethanol, 1:6:1 acetic acid–ethanol–lactic acid, Newcomer's fixative and Lu's fixative (1962) either singly or in combinations. It has been observed that the procedure for fixation contributes in a large way towards the applicability of the Feulgen method of nuclear staining. It has been found that the staining was most effective when young perithecia at various growth stages were fixed at least for 24 h in Lu's fixative (Lu, 1962) before transferring to Newcomer's fluid. The fixed perithecia were hydrolysed for 8 min at 60 °C in 1 N-HCl, washed in distilled water, treated with methanol for 2–3 min and finally stained in Schiff's reagent. Mycelial clumps fixed in 1:3 acetic acid–ethanol mixture were stained in Schiff's reagent after hydrolysis for 12 min in HCl at 60 °C.

The DNA content of *Neurospora crassa* at various stages was estimated through Leitz MPV microspectrophotometer at 548 mμ wavelength keeping the diameter of the central plug at 2·0 μ. The photometer was fitted with the light-measuring device Type MFLK (Knott Elektronik) which incorporates the EMI photomultiplier Type 6094A in the photomultiplier housing. The supply unit of this instrument provides a highly stabilized supply potential with a particularly sensitive fine adjustment. This attachment makes the instrument highly sensitive even for very low light intensities and very small measuring fields. It has been possible following this procedure to estimate the amount of DNA per nucleus in arbitrary units (A-units). As DNA measurements through microspectrophotometry has to be based on quite a number of assumptions which can only partially be fulfilled in most biological materials, the method remains always open to criticism (Rossen & Westergaard, 1966). Albeit, it can quite effectively be used to distinguish between cells having 1, 2 or 4 multiples of nuclear DNA content as has been shown by many investigators in the past (cf. Leuchterberger, 1958; Rossen & Westergaard, 1966). The prescribed precautions for employment of microspectrophotometry have been adopted while carrying out the study as far as possible. All measurements have been carried out by one person and the slides have been identically processed during preparation.

During our microspectrophotometric measurements of DNA content of the nucleus, centrally located nucleoli have often hampered our working to a large extent. As far as has been practicable, we have avoided inclusion of such nucleoli during microspectrophotometric analysis in our present study.

3. RESULTS

The study relating to the nuclear developmental phases at meiosis till the ascospores are formed is based partly on the figures as represented in the text (Fig. 1) and also on a sufficient number of observations made under phase-contrast micro-

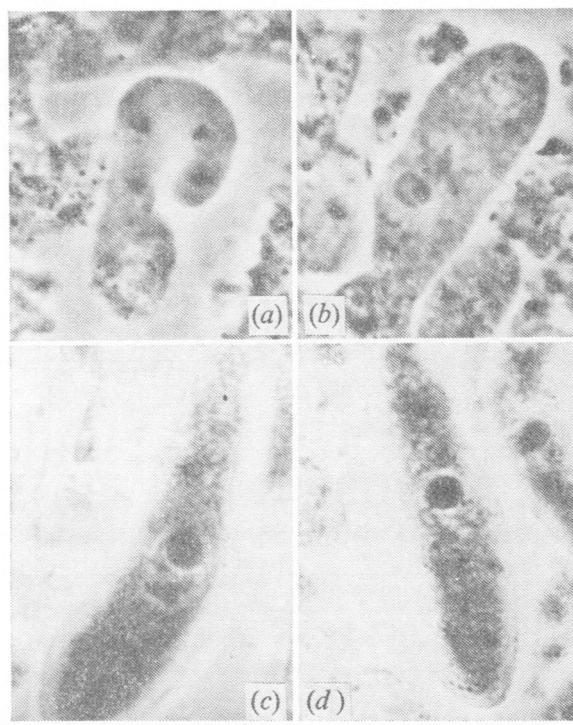

Fig. 1. (*a*) Crozier, with the nuclei at telophase of conjugate division. × 1800. (*b*) Primary ascus nucleus established after caryogamy. × 1800. (*c*), (*d*) Young asci showing initiation of meiosis. × 2100.

scopy. At the initial stage, the terminal cell of an ascogenous hypha bends to follow up with a synchronous mitotic nuclear division of each of the two gametic nuclei to yield a three celled crozier (Fig. 1*a*). A crozier consists of a binucleated subterminal cell and uninucleated stalk and terminal cell. The subterminal cell develops into an ascus upon simultaneous proliferation and nuclear fusion. The ascus proliferation begins during and/or after the fusion of the subterminal cell nuclei. Fusion of such nuclei results in the establishment of the primary ascus nucleus (Fig. 1*b*). The nucleus develops clarity with the onset of meiosis (Fig. 1*c*, *d*). In fact the cytological details of meiosis in *Neurospora crassa* have already been enriched from the past

https://doi.org/10.1017/S0016672300017067 Published online by Cambridge University Press

Table 1. *Spectrophotometric estimation of DNA content at developmental stages of meiosis in* Neurospora crassa

Category of developmental stage	Vegetative hyphae nuclei	Prefusion nuclei	Primary ascus nuclei	Diad nuclei
	a	*d*	*e*	*f*
No. of observations	25	20	22	20
Average $\bar{X}$ (A-units)	2·495	5·183	10·361	5·376
Standard error	0·145	0·382	0·225	0·186
DNA content per nucleus (pg)	0·017*	0·044	0·071	0·037

* From Minagawa, Wagner & Strauss (1959)

Note: The differences between the categories *a*-*d*-*e* are statistically significant and between the categories *d* and *f* are statistically insignificant.

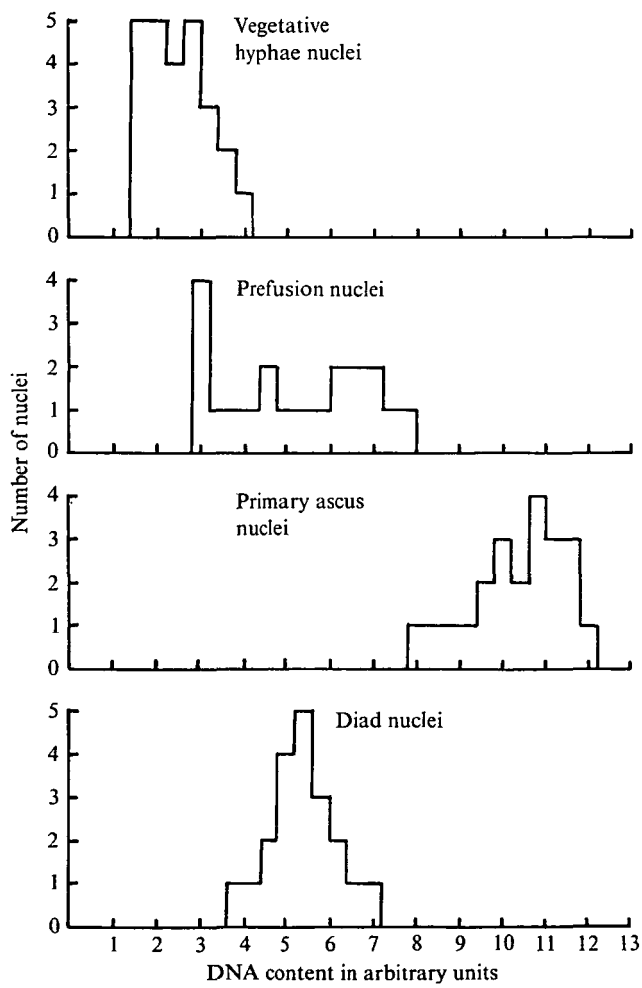

Fig. 2. Histograms showing DNA content (in arbitrary units) of four categories of nuclei in *Neurospora crassa*. Each column on the ordinate represents four units. The abscissa shows the number of nuclei in each group.

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studies (McClintock, 1945; Singleton, 1953; Barry, 1967; Gillies, 1972). Our observation of the nuclear events prior, during and after meiosis are in full agreement with the findings of previous authors.

The spectrophotometric estimation of nuclear DNA content at different development phases of meiosis and ascospore formation collected from at least 20–25 nuclei

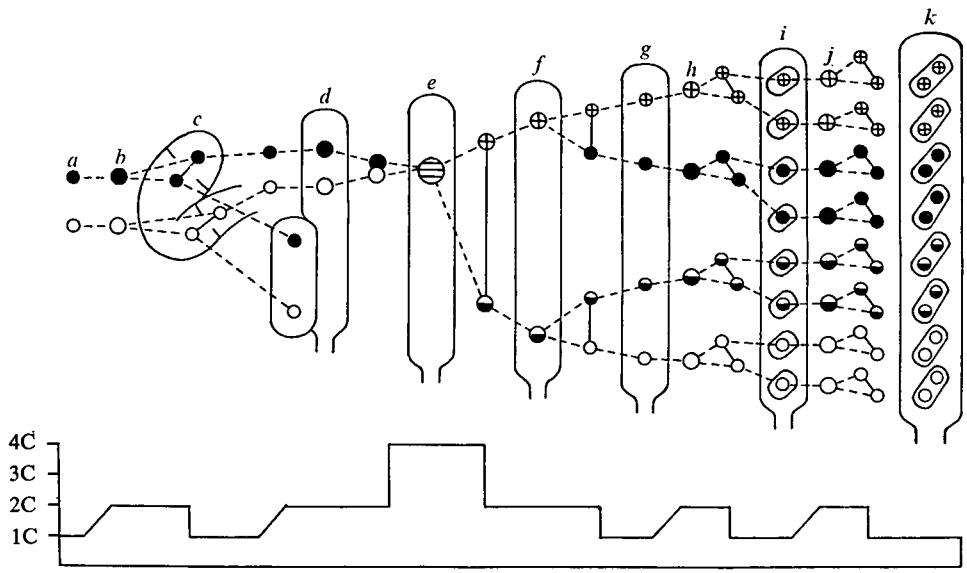

Fig. 3. Nuclear history in a linearly ordered eight-spored ascomycete, *Neurospora crassa*. (*a*) Haploid nuclei of opposite mating type in the terminal cell of the crozier; (*b*) the same nuclei after DNA replication; (*c*) last premeiotic mitosis in the crozier; (*d*) the two gamete nuclei of opposite mating type, prior to fusion, but after completion of the last premeiotic DNA replication, in the initial ascus; (*e*) fusion (Zygote) nucleus in diploid condition prior to meiosis; (*f*) the two nuclei resulting from the first meiotic division; (*g*) tetrad nuclei after second meiotic division; (*h*) the same nuclei after the first postmeiotic DNA replication; (*i*) eight haploid nuclei resulting from the first postmeiotic mitosis; (*j*) octad nuclei after second postmeiotic DNA replication; (*k*) eight binucleate ascospores resulting from second postmeiotic mitosis. The dotted lines in the diagram show the line of descent of the nuclei in each ascospore. Histogram at the bottom represents the amount of DNA per nucleus in terms of C-value at the stages pictured directly above (cf. Table 1). The DNA values shown at tetrad stage and onwards are based on the fact that two rounds of DNA replication takes place, one at tetrad and the other at octad stage of development. (Figure modified from Emerson, 1969.)

Table 2. *Nuclear DNA content in A-units at tetrad and octad stages of development*

(The DNA values shown are the averages of four nuclei out of eleven tetrad stage asci and eight nuclei out of thirteen octad stage asci studied respectively).

Developmental stage	DNA content per nucleus (A-units)
Tetrad	2.30, 2.41, 2.61, 2.83, 3.18, 3.41, 3.82, 4.18, 4.54, 5.14, 5.45
Octad	2.25, 2.41, 2.56, 3.11, 3.40, 3.61, 3.95, 4.32, 4.57, 4.77, 4.92, 5.21, 5.36

in each case is indicated in Table 1 and Fig. 2. The actual DNA content in pg has been calculated on the basis of already known (Minagawa, Wagner & Strauss, 1959) haploid nuclear DNA content of *Neurospora crassa*. The DNA content at different stages of development follow a pattern outlined in Fig. 3. Table 1 and Figs. 2 and 3 indicate the occurrence of a two-fold increase in DNA content of the prefusion nucleus in relation to that of vegatative hyphae nucleus at G_1. Likewise and interestingly enough the invariable four-fold increase of DNA in the primary ascus nucleus points to the fact that the premeiotic DNA replication is completed before the sexually competent nuclei are fused to undergo the course of meiosis. The range of variation of DNA content at tetrad and octad levels fall between 1C and 2C (Table 2) level thereby indicating the occurrence of a cycle of DNA replication at tetrad of the nuclei level and another round of DNA replication in the young ascospores, ultimately resulting in the formation of eight binucleated ascospores.

The major findings of the present study which have not been earlier recorded in *N. crassa* can be indicated as follows: (1) the premeiotic DNA replication is completed in the gametic nuclei before the caryogamy and no replication is observed after caryogamy till the tetrad of nuclei are resulted; (2) a second round of DNA replication (postmeiotic) takes place at tetrad stage of development which gives rise to eight haploid spores after a nuclear division; (3) finally, a third round of DNA replication (postmeiotic) occurs in the young ascospores which results in eight binucleated ascospores after a nuclear division within the ascospores.

4. DISCUSSION

The DNA measurements from individual nuclei have been quite variable as could be seen from the standard error values in Table 1 and histograms in Fig. 2. However, the 1C, 2C, and 4C levels of absorption values have been found to be quite distinct from one another (except the nuclei in S-phase). Although it is difficult to stain the chromosomes of *Neurospora* with Schiff's reagent so as to make them ideally amenable to microspectrophotometric measurements, our preparations have sufficiently been distinct enough to carry out such measurements, following the method described in this text.

The present evidences indicate that in *Neurospora* meiotic chromosomes, replication takes place earlier than caryogamy. It takes place during the interphase after a premeiotic mitosis of the two gametic nuclei. The variation in the nuclear DNA content (A-units) was maximum in 'prefusion' nuclei as could be seen from the standard error values in Table 1. This could be due to the fact that some of the nuclei selected for microspectrophotometric measurements were still in the S-phase. Moreover, in no case did the primary ascus nucleus provide evidences for occurrence of DNA replication until the formation of the tetrad nuclei. This indicates that the caryogamy follows premeiotic DNA replication. Evidences for the time of premeiotic DNA synthesis in *Chlaydomonas reinhardii* (Sueoka, Chiang & Kates, 1967) and this study supplement the earlier contention of Rossen & Westergaard (1966) that the two events are separable. The discrepancy (Grell, 1969) in the cytological

description of prefusion nuclei between *Neottiella rutilans* (Rossen & Westergaard, 1966) and *Sepultaria sepulta* (Wilson, 1937) can possibly be removed by the present findings. Our evidence indicates that meiosis follows caryogamy as in *Podospora anserina* (Backett & Wilson, 1968) and *Neottiella rutilans* (Rossen & Westergaard, 1966). The ascus cytology evidenced through this study follows the general pattern of meiosis in fungi as observed in *Aspergillus* (Elliot, 1956, 1960; Olive, 1965), *Hypomyces solani* f. *cucurbitae* (El-Ani, 1956), *Cyatus stercoreus* (Lu, 1964), *Coprinus* (Raju & Lu, 1970) and *Neurospora crassa* (McClintock, 1945; Singleton, 1953; Barry, 1967).

We infer from our observations that the tetrads of nuclei resulting from meiosis have to begin with the DNA content characteristic of unreplicated haploid nuclei and then undergo a replication prior to first postmeiotic prophase. Actual measurements of DNA have shown a complete range between unreplicated and replicated haploid amounts (Table 2). The nuclei of young ascospores have been found to have the amount of DNA per nucleus characteristic of unreplicated haploid nuclei. A similar indication of postmeiotic replication is indicated in the work of Rossen & Westergaard (1966). According to models of recombination proposed by Holliday (1964) and Whitehouse & Hasting (1965), crossing over is associated with hybrid DNA formation. If a mutant site lies within the hybrid DNA, then heterozygosity in the molecules may be corrected or repaired to give gene conversion. The mechanism of reciprocal and non-reciprocal recombination at chromosomal level of an eight-spored ascomycete is conveniently explained on this basis by supposing that the chromatid consists of a single DNA duplex, for which overwhelming evidences exist. Hence, the present evidence for replication at four nuclear stages is in line with the models which explain recombination through hybrid DNA formation.

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