Nuclear Division in *Neurospora crassa* During Conidiation and Germination

Vegetative nuclear division in *Neurospora crassa* has recently been studied by a number of investigators[1-4], but their interpretations of the stained mitotic figures still vary greatly. The present study of nuclear division in developing and germinating conidia allows the additional correlation of stages in the division of the nuclei with sequential morphogenetic changes: constriction of the hyphae (conidiation) and lengthening of the germ tube (germination).

Cultures of *N. crassa* (strain Lindegren+) were grown in M medium and conidia germinated in C medium[5]. Conidia for germination experiments were harvested by shaking in sterile distilled water and filtering through 4 layers of cheese-cloth, and used immediately. All tissues were fixed in a solution of glacial acetic acid, chloroform, and 96% ethanol (1:3:6) for 24 h. They were then washed once in 70% ethanol, 3 times in distilled water, and hydrolysed for 8 min in 10% perchloric acid at 60 °C. After cooling and washing 3 times in distilled water and once in 0.01 *M* phosphate buffer, the cells were suspended in Giemsa strain and phosphate buffer (1:5) for 10–30 min. Stained cells were washed with the buffer, wet mounted and studied immediately.

In order to obtain hyphae which would differentiate conidia at approximately the same time, cultures were grown for 3 days in M medium which permits only vegetative growth[5]. On the third day, the growth medium was removed and replaced with sterile 0.1 *M* phosphate buffer. Aerial hyphae were formed and conidia developed from them within 15 h; stages in the formation of the conidia could be obtained by sampling before and after this time.

Nuclei observed in these conidiogenous hyphae, before the beginning of differentiation, show many different morphologies (Figure 1a, b, c) and stages of division. There does not appear to be any synchronization of division. In larger hyphae the nuclei appear to be more numerous (Figure 1d) and in old hyphae we have observed a diminution of nuclei and active division. Hyphae which produce conidia are always relatively thin. Their first detectable sign of differentiation is the constriction of the cell wall, and although the cause of this change is unknown, it may be correlated with the nucleus: in each case that we have studied, the constrictions begin between each nucleus. From this time on, constriction of the outer wall progresses and eventually a septum is formed separating the individual conidia. Nuclear division also

[1] C. E. SOMERS, R. P. WAGNER and T. C. HSU, Genetics *45*, 801 (1960).
[2] E. W. B. WARD and K. W. CIURYSEK, Am. J. Bot. *49*, 393 (1962).
[3] A. BAKERSPIGEL, Am. J. Bot. *46*, 180 (1959).
[4] J. WEIJER, A. KOOPMANS and D. L. WEIJER, Can. J. Genet. Cytol. *7*, 140 (1965).
[5] G. TURIAN, Nature *202*, 1240 (1964).

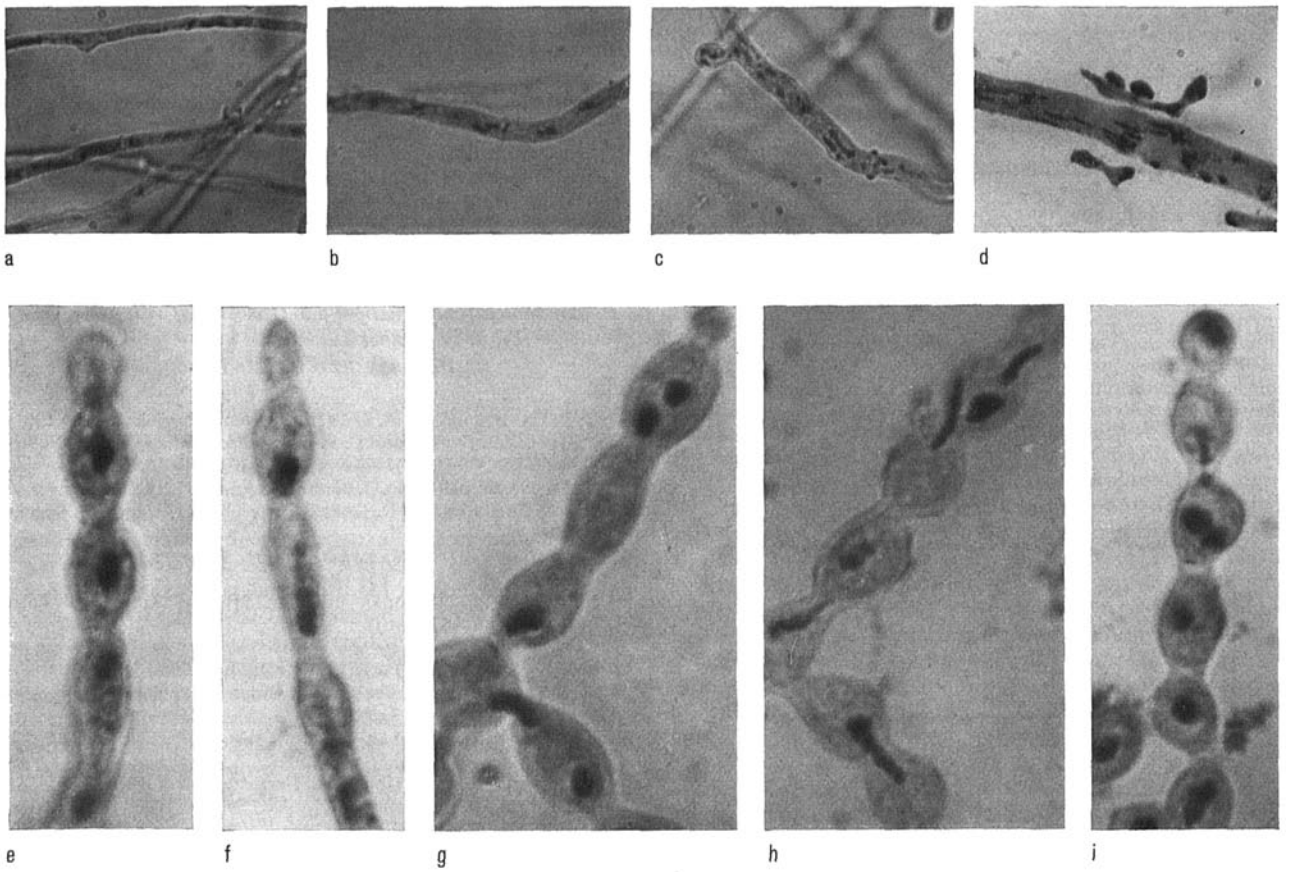

Fig. 1. Mycelia and conidiating hyphae of *N. crassa* fixed with acetic acid-chloroform-alcohol, hydrolysed with 10% perchloric acid, stained with Giemsa. a–c = young hyphae; d = large hyphae; e–i = conidiating hyphae with septa forming in i. Scale: a–d, 2 mm = 8 μ; e–i, 1 cm = 8 μ.

continues (Figure 1 e–i) but no longer influences the cell wall. Movement of the chromosomes during division generally results in multinucleate conidia (Figure 1i) but some conidia may lack nuclei (Figure 1g) and, in some cases, the formation of the septum separates the nucleus into 2 different cells (Figure 1h). These 2 latter events may account for the relatively poor viability of the conidia (67%) of this strain of *N. crassa*. The last nuclear division occurs just as the septum separating the conidia is formed. We have seen no evidence of nuclear division in mature conidia, although statistical counts indicate that division may occur occasionally. The average number of nuclei is 1.79 in the mature compared to only 1.47 in the newly formed conidium.

Rapid germination of conidia was achieved by harvesting them from cultures grown in C medium for 10 days (maximum viability), washing the conidia 3 times in water and suspending them in fresh C medium (10^6 conidia/ml). During germination the conidia were shaken to maintain aerobic conditions at 25 °C. The time for germination was found not to be consistent between conidia, and although heat shocks decreased the average time for germination, they did not significantly synchronize this process. Because of the great difference in time for a conidium to germinate (1–8 h), it is not possible to predict stages of nuclear division with time. However, the number of nuclei do relate clearly to the length of the germ tube (Figure 2). The average number of 1.79 nuclei increases slightly by the time the first bud of a germ tube appears on the swollen conidium, indicating that a division is possible before the germ tube extends. By the time this tube is about 3 times the length of the conidium, the number of nuclei has doubled.

The question of whether nuclear division in *N. crassa* is classical cannot be answered simply. The patterns of division which we have observed in germinating conidia show a relatively classical sequence of mitotic stages: nondividing nuclei in conidia typically spherical and dense (Figure 3a); enlarged and less dense nuclei in the swelling, pregerminating conidia (3b); prophase-like characters (Figure 3c, d) followed by late prophase or metaphase-like characters (Figure 3e) when the germ tube appears; and an anaphase stage (Figure 3f) which shows indications of a spindle. However, these figure stages are not usually localized in one area of the cell during the process of division but become displaced and distorted due to the movement of the cytoplasm during growth, especially in the young rapidly extending germ tubes and thin actively growing hyphae, where the dividing nuclei are often forced out and may display an elongated appearance (Figure 3g). The same can be said of hyphae constricting to form conidia (Figure 1). In older hyphae where movement of the cytoplasm is not as rapid, and in large hyphae, the distortion of the nucleus is not as pronounced and a

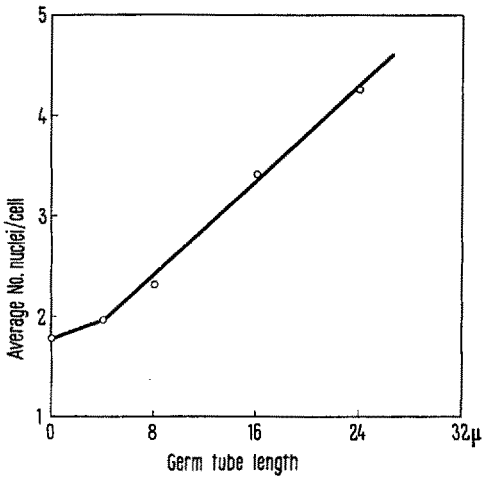

Fig. 2. Average number of nuclei per germinating conidium as a function of length of germ tube at 25 °C in C medium.

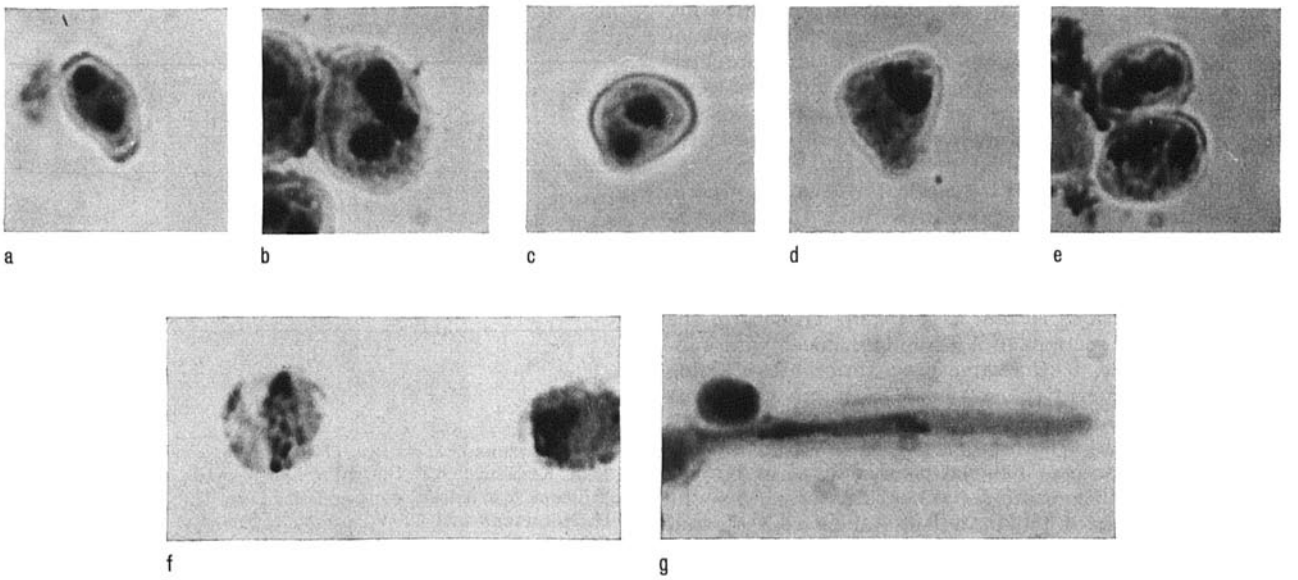

Fig. 3. Conidia and germination stages of *N. crassa* at 25 °C fixed with acetic acid-chloroform-alcohol, hydrolysed with 10% perchloric acid, stained with Giemsa. a = conidium prior to germination with resting nuclei; b = swollen conidium prior to formation of germ tube; c and d = appearance of germ tube at different stages of prophase; e = metaphase (germ tube not in field of view); f = anaphase at left; g = distortion of dividing nuclei carried into germ tube. Scale: 1 cm = 8 μ.

more typical mitosis is observed. For certain developmental stages of *Neurospora*, a facultative mechanism of longitudinal division of filamentous nuclei as advanced be KEEPING[6] and WEIJER et al.[4] must therefore be considered[7].

Résumé. La différenciation conidienne de *Neurospora crassa* s'accompagne de divisions nucléaires dans les hyphes en constriction jusqu'à l'achèvement de la septation inter-conidienne. Dans les hyphes étroits conidiogènes, les figures mitotiques sont souvent très étirées et peuvent correspondre à un autre mécanisme de division nucléaire que celui, plus classique, des conidies en germination.

D. E. BIANCHI[8] and G. TURIAN

Laboratoire de Microbiologie, Institut de Botanique générale, Université de Genève (Suisse), 16th September 1966.

[6] E. S. KEEPING, Neurospora Newsletter *8*, 27 (1965).

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[8] U.S. National Science Fellow, 1965–66. Present address: Biology Department, San Fernando Valley State College, Northridge (Calif., USA).

High Frequency of Mast Cells in Spleens of A-Strain Mice

Mast cells are most frequent in the connective tissues of animals of various species, while in lymphoid tissues their incidence is much more limited[1,2]. Comparison of the relative frequency of mast cells in different animals' spleens[3] showed that they are abundant both in the capsule and parenchyme of cows, calves, sheep, dogs and horses, while in pigs and rabbits they are much less frequent and in rats practically absent. Negative findings of mast cells in the spleen were reported in rats and rabbits[4] and in the hedgehog[5]. A small amount of mast cells was observed in the red pulp of mouse spleen[6].

In the present paper, the finding of an exceptional abundance of mast cells in the spleen of inbred mice of a few genetically related strains, in contrast to their extremely low frequency in several other mouse strains, is described. The strongly positive strains are the A-strain (which has been maintained by strict brother-sister mating in Prague from 1956, when a few breeding pairs were kindly provided by Dr. N. A. MITCHISON, Edinburgh, and which is now denoted A/Ph) and its presumably congenic line A.CA. Comparison was made with the incidence of mast cells in the spleen and thymus of mice of several other strains; furthermore, spleens of rabbits, rats, chickens and ducks were investigated with a negative result.

After killing the animal by cervical dislocation, the respective organs were fixed overnight with 4% formol in McIlvan buffer solution at pH 3.8; the tissues were then cut into 10 μ thick sections on a freezing microtome. Selective staining of mast cells was performed by toluidine blue (0.5% solution, 10 min at pH 2.0). To control the technique, some sections of A/Ph mouse spleen were submitted, as a rule, to the same procedure while staining tissues of the 'negative' strains. The number of mast cells/mm^3 of tissues was calculated according to the following formula by FLODERUS[7]: $x = n(1000/a + d - 2h)$, where n = the number of mast cells counted in 1 mm^3, a = thickness of the section (10 μ in this case), d = the average diameter of the mast cell (taken as 5 μ), and h = the diameter of the smallest nucleated segment just resolvable under given conditions of microscopic observation (0.3 μ).

Table I gives the average values of this parameter (each based on 8–10 animals) for 2- to 4-month-old mice of several strains. In Table II, 5 additional mouse strains

Table I. Comparison of mast cell frequency in spleen of mice of various strains (average values from 8–10 mice)

Strain	No. of mast cells/mm^3 of spleen	Strain	No. of mast cells/mm^3 of spleen
A/Ph	26,042 ± 667	C57BL/10	264 ± 41
A.CA	23,125 ± 227	C57BL/6	90 ± 21
A.SW	2,430 ± 204	B10 BY	521 ± 233
CBA/J	111 ± 14	B10 D2	354 ± 37
CBA/T6T6	111 ± 29	B10 Y	264 ± 43
C3H	194 ± 35	B10 A	174 ± 26
PCTL	28 ± 20	B10 AR v	139 ± 27
LPR III	56 ± 23	B10 LP	90 ± 22
NZB	90 ± 44	B10 AR II	28 ± 21
H	347 ± 62		

Table II. Individual values of the frequency of mast cells in mice of various strains

Strain	No. of mast cells/mm^3 of spleen		
	1	2	3
C3H/NB	0	0	56
C3H.K	264	90	285
R III	90	139	56
B10 BR	56	397	397
B10 M	111	347	370

[1] A. A. KATZBERG, Anat. Rec. *118*, 393 (1954).

[2] M. A. KELSALL and E. D. CRABB, *Lymphocytes and Mast Cells* (The Williams & Wilkins Company, Baltimore 1959), p. 99.

[3] H. HOLMGREN and O. WILANDER, Z. mikrosk.-anat. Forsch. *42*, 242 (1937).

[4] P. CONSTANTINIDES, Science *117*, 505 (1953).

[5] R. HÄRMÄ and P. SUOMALAINEN, Acta physiol. scand. *24*, 90 (1951).

[6] D. METCALF, Aust. J. exp. Biol. med. Sci. *43*, 533 (1965).

[7] S. FLODERUS, Acta path. microbiol. scand. Suppl. *53*, 21 (1944); cited by M. SUNDBERG, Acta path. microbiol. scand. Suppl. *107*, 1 (1955).