Nuclear Division in \textit{Neurospora crassa} During Conidiation and Germination

Vegetative nuclear division in \textit{Neurospora crassa} has recently been studied by a number of investigators\textsuperscript{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 \textit{N. crassa} (strain Lindegren\textsuperscript{+}) were grown in M medium and conidia germinated in C medium\textsuperscript{4}. 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 95\% 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 stain 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\textsuperscript{4}. 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

\textsuperscript{1} C. E. Somers, R. P. Wagner and T. C. Hsu, Genetics 45, 801 (1960).
\textsuperscript{3} A. Bakerspiigel, Am. J. Bot. 46, 180 (1959).
\textsuperscript{5} G. Turian, Nature 202, 1240 (1964).
continues (Figure 1 e–i) but no longer influences the cell wall. Movement of the chromosomes during division generally results in multinucleate conidia (Figure 1j) 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 (57%) 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 (10⁶ 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: non-dividing 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