Nuclear Structure and Division in the Fungi Imperfecti

II. *Scopulariopsis brevicaulis*

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Preface

In a previous report (Bakerspigel 1960) the writer described the structure and behavior of the nuclei in the mycelium of the imperfect fungus, *Phyllosticta*. He showed that their manner of division was similar to that described in *Penicillium* (Pontefract 1956) but that it differed from the behavior of the vegetative nuclei in *Mucor* and *Phycomyces* (Robinow 1957a, b) and several other fungi studied thus far (Bakerspigel 1958, 1959a, b). Since then the writer has studied the nuclei in other imperfect fungi; viz., *Scopulariopsis brevicaulis, Macrophomina phaseoli* and *Trichophyton mentagrophytes*. To extend our knowledge of nuclear behavior in the Fungi Imperfecti the following report will record observations made on *Scopulariopsis brevicaulis*. This fungus is related to the *Penicillia* belonging to the same form order, the *Moniliales*.

Materials and methods

Culture. The strain of *Scopulariopsis brevicaulis* used in this study was isolated by the writer from an infected toe-nail of a patient suffering from Tinea pedis. It was grown and maintained on Sabouraud glucose agar medium at 24°C.

Cytological preparations. Preparations of conidia and mycelium were made and stained using the methods previously described by the writer (Bakerspigel 1958; 1959a, b). These included fixation with acetic acid-alcohol and osmium tetroxide vapors followed by staining with the HCl-Giemsa, Feulgen, Azure A-SO₂ and iron alum hematoxylin techniques. Phase contrast microscopy was also employed to study nuclei in living preparations.

Observations

*Nuclei in conidiophore and in ungerminated conidia* (figs. 1–4, 30, 31). Conidia are borne on the conidiophores either singly or in short chains and contain 1–3 nuclei. A conidium may receive one or more nuclei from the

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conidiophore to which it is attached. These migrating nuclei are either mature or the products of a recent division in the mycelium from which the

Figs. 1-10. Nuclei in ungerminated and germinating conidia of Scopulariopsis brevicaulis. 1-3 and 5-10, HCl-Giemsa. 4, Azure A- SO₄. All figures × ca. 3200. 1a, a binucleate conidium. 1b, a nucleus fixed while entering a conidium. 2, two densely stained nuclei in a conidiophore. Probably the products of a recent division. 3, a trinucleate conidium. 4, a binucleate conidium. 5, three nuclei in a young germ tube. Arrow points to a filamentous (chromosomal) complex in a dividing nucleus. 6, mature, interdivisional nuclei in a young germ tube. 7, arrows point to nuclei at beginning of division. 8, a dividing nucleus, a, chromatin. b, faintly stained nucleolus. 9, arrow points to the bar of chromatin during later stage of division. Nucleolus is not evident. 10, dividing nuclei. a, chromatin shown as a filamentous complex. b, nucleolus. c, bar of dividing chromatin. d, remnant of nucleolus.
conidiophore arose. In stained preparations the nuclei in ungerminated conidia appear to be granular and spherical or oval in shape. Nucleoli were not discernible in these nuclei. Actual division has not been observed in these conidia and it is suggested that they receive their nuclei by migration from the conidiophores to which they are attached.

In contrast to the nuclei in ungerminated conidia interdivisional nuclei in conidiophores appear as loosely arranged masses of chromatin also without any visible nucleolus. In addition divisional sequences have not been observed to occur in conidiophores though what appeared to be post-divisional products (sister nuclei) have been noted in some conidiophores (e.g. fig. 2).

*Nuclei in germinating conidia and mycelium* (figs. 5–24, 32–38). In young germ tubes and in older hyphae mature, interdivisional nuclei are composed of crescentic masses of densely stained chromatin each cupping a single, globular nucleolus. This nucleolus is Feulgen-negative, stains faintly with Giemsa (e.g. figs. 6, 16) and densely with iron alum haematoxylin (figs. 28, 29). As secondary hyphal branches are produced nuclei migrate into them from the primary hyphae. The chromatin of these nuclei enter first trailing their nucleoli behind them (fig. 15).

The first division occurs in the germ tube when it is approximately 2–3 times the length of the conidial diameter. At the beginning of division the chromatin becomes separated from the nucleolus. As division continues the chromatin becomes rearranged, in a manner unknown at present, into tightly bound complexes of chromosomal filaments (e.g. fig. 24). In some of these complexes one or more of the filaments have a knob-like projection at a free end (fig. 24). However, counts of individual chromosomes could not be made from these preparations. As division proceeds these complexes contract into densely stained bars which then constrict at their midregions and pull apart each forming two, densely stained, featureless portions. As the chromatin divides the nucleoli decrease in size and at the end of division their remnants may be found lying between the two separated portions of chromatin. These remnants then completely disappear and new nucleoli reappear in the maturing sister nuclei.

In some preparations (fig. 11, a) when the sister nuclei have just separated a densely stained granule can be seen trailing behind each nucleus. These granules are probably the division products of a single granule similar to that described in the vegetative nuclei of *Gelasinospora tetrasperma* and *Neurospora crassa* (Bakerspigel 1959 a, b). The function of such granules is still unknown and it can only be suggested that they may play some role in the kinetics of division possibly that of separating the sister nuclei at the end of division. Such a suggestion is not unreasonable in view of the fact that the nucleoli in this fungus do not elongate and divide and therefore could not play a similar role at the end of division as described in *Neurospora* and *Gelasinospora*. 
In a unicellular germ tube or in a single cell of multicellular hyphae the nuclei at any one time are usually in the same state while nuclei in neighbouring cells are in a different state. This situation is similar to that
already noted in *Penicillium* (Pontefract 1956), *Phyllosticta* (Bakerspigel

Figs. 24-29. 24, three dividing nuclei in an older hypha of *Scopulariopsis brevicaulis*. a, filamentous chromosomal complex. b, remnants of nucleolus. Note the knob-like tips in the complexes of these nuclei. *HCl-Giemsa*, ×ca. 3100. 25-27, living nuclei. Phase contrast microscopy, ×ca. 3100. a, Optically clear regions. b, nucleoli. c, a filamentous mitochondrion. 25, an interdivisional nucleus in a conidial remnant. 26, nucleus at beginning of division in a conidial remnant. 27, interdivisional nucleus in an older hyphal cell. 28, 29, *Iron alum haematoxylin* preparations, ×ca. 3100. 28, two, mature, interdivisional nuclei in a hyphal cell. 29, maturing sister nuclei in a hyphal cell.
1960) and *Trichophyton mentographytes* (unpublished data). It is dissimilar to that observed and reported in the vegetative mycelium of *Endogone sphagnophila*, *Neurospora crassa*, *Gelasinospora tetrasperma* (Bakerspigel 1958, 1959 a, b) as well as in *Mucor* and *Phycomyces* (Robinow, 1957 a, b).

Living nuclei studied with the phase contrast microscope (figs. 25-27). It is difficult to study living nuclei in ungerminated and germinating conidia.
because of the density of the cytoplasm. However, when an interdivisional nucleus could be observed for any length of time it appeared to be composed of an optically dense, irregularly oval-shaped nucleolus surrounded by an optically clear ring or cap-shaped region. The nucleolus is not homogeneous containing regions of lesser density. The clear region changes its shape continually so that at times it is flame-shaped.

During the early part of division the clear region becomes elongated and withdrawn from the nucleolus (fig. 26). This configuration is similar to those in the stained preparations illustrated in figs. 10, 19, 20. As division continues the entire nucleus seems to "disappear" into the cytoplasm and then reappear within a few seconds. This process was seen to occur a number of times during this stage of division. Following this the clear portion of the nucleus quickly pinches apart into two small, tear-drop-shaped portions.

Within a few minutes after division the original nucleolus, which had become progressively smaller and less dense could not be found or if still visible had become much smaller finally disappearing completely from view. Maturation of the sister nuclei could not be followed completely because of the density of the cytoplasm. Nevertheless the reappearance of nucleoli in these nuclei was observed to occur within a few minutes post-division. These nucleoli were small, globular, dense bodies that gradually enlarged as the nuclei matured.

Discussion

The description of nuclear behavior given above parallels that previously reported in Penicillium (Pontefract 1956) and Phyllosticta (Bakerspigel 1960). It is worth noting that here again a nuclear membrane could not be discerned either in living or stained preparations. This is not surprising when one reads in the few reports on the electron microscopy of nuclei in Neurospora crassa (Tsuda 1959, Shatkin 1959), Penicillium (Tsuda 1956), Coccidioides immitis (O’Hern and Henry 1956) and Allomyces macrogynus (Turian and Kellenberger 1956) that the thickness of the double nuclear membrane measures 80–950Å. Furthermore there is no evidence as to whether any of these nuclear membranes break down during division and if so when they are reformed. None of these studies have attempted to follow a divisional sequence or to show stained preparations for comparison.

In the present report the Feulgen-negative body within the nucleus is called the nucleolus. This is a change from the descriptive term "central body" which had been used by the writer and Professor Robinow in several previous publications. An explanation for the change has been given in a recent paper on the nuclear cytology of the Saprolegniaceae (Bakerspigel 1960).

Summary

The nuclei in the imperfect fungus Scopulariopsis brevicaulis divide with-
out the aid of a visible spindle or metaphase plate. In this respect they behave in a manner similar to the nuclei in other imperfect fungi such as *Penicillium* and *Phyllosticta*. Complexes of chromosomal filaments are present in the dividing nuclei of *S. brevicaulis*. However, it has not been possible to count these filaments nor to determine how they become segregated during division.

**Literature cited**


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