Electron Microscopy of Nuclear Membranes during Somatic Nuclear Separation in *Neurospora crassa*

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(Accepted for publication 17 January 1972)

Moore (1963, 1964) described karyochorisis in *Cordyceps* in which the invagination of the inner nuclear membrane divided the nucleoplasm into two or more subunits and subsequent invagination of outer nuclear membrane separated the nucleus into daughter nuclei. In *Catenaria* the telophase stage of mitosis is marked by the rounding up of the daughter nuclei with the exclusion from the nucleus of a central, spindle-shaped structure which is then broken down (Ichida, 1968). A similar configuration has been shown in meiosis of *Physarum* (Aldrich, 1967).

This paper deals with nuclear division in germinating conidia of *Neurospora crassa*.

METHODS

Conidia formed by cultures of *Neurospora crassa* (FGSC 821) grown on minimal agar medium (Beadle & Tatum, 1945) for 6 days at 25 °C, were harvested with distilled water and separated from hyphae by filtration through glass wool.

Samples were incubated at 30 °C in minimal liquid medium and centrifuged. Germinating conidia were fixed in 2% (w/v) aqueous KMnO₄ or in 6% (v/v) aqueous glutaraldehyde followed by postfixation in 2% (w/v) aqueous KMnO₄.

The samples were dehydrated in an ethanol series, rinsed in propylene oxide, and then embedded in a mixture of Epon 812 and Epok 533. Sections were cut with glass knives on an LKB ultramicrotome, stained with uranyl acetate and lead citrate (Reynolds, 1963), and examined with a JEM-7 electron microscope.

RESULTS

Fig. 1(a) shows a nucleus with an invagination of the inner nuclear membrane. The outer membrane was clearly continuous across the point of invagination. No nucleus showed simultaneous membranous invaginations on both sides of the dividing nucleus, indicating that the invagination proceeded from one side of the nuclear surface to the other and not centripetally.

Ultimately, an invagination of the inner nuclear membrane seemed to fuse with the nuclear membrane at the opposite side of the nuclear surface, resulting in a partitioned nucleus as seen in Fig. 1(b).

At the next stage of division, the nucleus showed a partition wall consisting of four parallel membranes. Fig. 1(c) probably shows two daughter nuclei immediately after nuclear division, each nucleus being completely enclosed by a double-membraned envelope.

Fig. 1(d) shows a nucleus that had apparently divided into four parts by the invagination

of the inner nuclear membrane at three points. Each partition probably consisted of two membranes. This would eliminate the possibility that some of the nuclei associated together to produce the present nuclear configuration.

DISCUSSION

The process of nuclear separation in *Neurospora crassa* which closely resembles that in *Cordyceps* (Moore, 1964), including the occurrence of the multipartitioned nucleus as shown in Fig. 1(d). The additional membranes may have been formed by further invagination of the outer nuclear membrane, as described in *Cordyceps* (Moore, 1964), or by accumulation of membranous component substances on to either the outside or the inside of the invaginated inner membrane.

Tandler, Erlandson, Smith & Wynder (1969) have reported that dividing mitochondria in the liver of mice given an injection of riboflavin are characterized by a membranous partition separating the inner compartment into two parts. This is accomplished either by fusion of existing cristae, or by elongation of a single crista, followed by the extension of the outer membrane of the mitochondrial envelope between the component membranes of the septum, resulting in a separation of the two mitochondrial moieties, perhaps due to a repulsive force between the outer membranes. The same may be a possible mechanism of nuclear separation in *Neurospora*.

The type of somatic nuclear separation in *Neurospora* is certainly different from yeast cells (Hawker, 1965) and *Catenaria* (Ichida, 1968). Occurrence of multiseparated nucleus may indicate that the daughter nuclei initiated the next division before their separation was completed.

The only report on nuclear division of *Neurospora* studied by electron microscopy has been published by Namboodiri & Lowry (1967). They showed two slender nuclei lying parallel to each other in hyphae of mutant 'clock', which they interpreted as two daughter nuclei immediately after telophase. Although they showed disintegration of nuclear envelope during the early stage of division, this was not observed in the present study. No configuration indicating the invagination of nuclear membrane was shown by Namboodiri & Lowry (1967).

We express our gratitude to Drs E. B. Wagenaar & K. Nakamura, Department of Biological Sciences, University of Lethbridge, for their comments and advice.

Fig. 1(a) Invagination of the inner nuclear membrane. The outer membrane is clearly continuous across the point of invagination.

Fig. 1(b). A nucleus partitioned by the invagination of the inner membrane.

Fig. 1(c). Accomplishment of nuclear separation. Note the daughter nuclei surrounded by intact double membranes.

Fig. 1(d). Multipartitioned nucleus resulting from several simultaneous invaginations of the inner nuclear membrane.

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