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MITOSIS IN *CLADOSPORIUM HERBARUM*

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Abstract

The paper provides observations on somatic nuclear division in *Cladosporium herbarum* based on light microscopic work outlining the division in fixed and stained hyphae. An intranuclear spindle, developing between a dividing lateral granule or centriole equivalent, is seen in division figures. A double-track anaphase configuration peculiar to hyphomycetous nuclei is also observed.

Introduction

In recent years several well documented publications (Robinow & Caten. 1969; Aist, 1969; Crackower & Bauer, 1970; Day, 1971; Hashmi et al., 1972) have indicated some of the features of the mechanism of somatic nuclear division in fungi. In most of the published work it is observed that the division process does differ from a classical mitosis in that the typical metaplase plate does not exist, and an unusual double-track anaphase configuration is always present. Some reports (Ichida & Fuller, 1968; Wells, 1970; Heath & Greenwood, 1970; McCully & Robinow, 1971; 1973) have also indicated further variations from classical mitotic division in the role of the intranuclear spindle and in the formation of chromatinic elements into chains.

The present work was undertaken to elucidate the process of mitosis in *Cladosporium herbarum* and to determine how it compares with information obtained from similar studies of other fungi.

Materials and Methods

(a) *Preparation of Material*

Pure culture of *Cladosporium herbarum* was maintained on a solid medium containing 0.5% yeast extract, 2% glucose and 1.5% agar (yeast-extract glucose agar).

An inoculum was prepared by dispersing conidia of *Cladosporium herbarum* in 10 ml. of sterile distilled water containing a drop of Tween 80. The suspension was then centrifuged, the supernatant poured off, and the conidial pellet was suspended in 1 ml. of filtered fresh 5% egg white (v/v) in distilled water which also contained 1% glucose (w/v). A drop from this suspension was spread over a 22 x 22 mm. coverslip and allowed to dry at room temperature for 10-15 min. Rectangular sheets of opened out dialysis tubing (cellulose) about 1.5 cm. x 2.0 cm. were boiled for 10 min in distilled water, then lightly blotted between absorbent paper and placed over the dried film of conidia. At the time of fixation the coverslip was detached

and instantly plunged into a Columbia staining dish containing Helly's fixative. This is essentially the method of Clutterbuck & Roper (1966) which was later adapted by Robinow & Caten (1969) for staining fungal nuclei.

(*b*) *Fixation*

Fixation of material to be stained with acid fuchsin and aceto-orcein was in modified Helly solution containing 5% mercuric chloride, 3% potassium dichromate in water with 0.6 ml of formaldehyde added immediately before use (Robinow, 1961). After 12 min in fixative, the coverslips were rinsed in 70% alcohol and either stained immediately or stored in Newcomer's (1953) preservative at 4°C.

Material to be stained in HCl-Giemsa was fixed in a solution suggested by Robinow (personal communication, 1976) which contained absolute alcohol, 50 ml ; formaldehyde, 5 ml ; acetic acid, 2 ml ; mercuric chloride (saturated solution), 1 ml and distilled water, 42 ml,

(c) *Staining*:

(1) **Acid Fuchsin:**

Robinow & Marak (1966) used this stain in low concentration to demonstrate a fiber apparatus in the yeast nucleus. Since then it has been used for the same purpose elsewhere (Heale et al., 1968; Aist, 1969; Crackower & Bauer, 1971; Hashmi et al., 1972).

After rinsing several times in 70% alcohol the fixed material was stained for 2 min in 1:60,000 acid fuchsin in 1% acetic acid and examined in 1% acetic acid with a x40 Zeiss water immersion objective lens. Once sufficiently stained, the preparation was mounted in a drop of 1% acetic acid and sealed with wax from a small candle or Glyceel (G. T. Gurr, London, England).

(2) **HCl-Aceto orcein:**

Fixed specimens were hydrolyzed in NHCl for 10 min at 60°C, rinsed several times with distilled water, left in 60% acetic acid for 5 min and stained with 1% synthetic orcein in 60% acetic acid (aceto-orcein: Elliot, 1960) for 30 min. These specimens were examined in the stain and sealed with wax.

(3) **HCl-Giemsa:**

Fixed specimens were hydrolyzed as above, rinsed with distilled water and stained for 1 hr. or more with Gurr's Improved Giemsa stain R66 diluted 1 in 10 with Gurr's Giemsa (phosphate) buffer at pH 6.8 The quality of the staining was determined with a x 40 Zeiss water immersion lens. The overstained preparations were differentiated in 40 ml of distilled water to which a loopful of glacial acetic acid was added. After achieving the proper differentiation the material was mounted in a drop of Giemsa buffer.

Photomicrographs were made with an Exakta camera attached through a bellows to a Zeiss model GFL microscope equipped with a Ziess planapochromat bright field oil immersion objective lens in conjunction with a x10 compensating flat-field eye piece and an achromatic-aplanatic condenser (N.A. 1.4). A broad

band green interference filter was used for photographing stained preparations. Photographs were taken on 35 mm Kodak Panatomic-X film by exposing 4-6 seconds. Prints were made at a final enlargement of x3000.

Results

Acid Fuchsin Staining.

Acid fuchsin selectively stains nucleoli, spindles and dense lateral granules situated in close association of nucleoli (Figs. 1A-1H). The nucleolus in interphase and dividing nuclei appears as a spherical body or an irregular hump surrounded by an unstained area of chromatin. During early stages of nuclear division, the lateral granule divides into two similar components (Fig. 1B upper nucleus, Fig. 1C) and between these a thin spindle is formed. The spindle increases in length and is seen attached on both ends by the two original granules (Fig. 1D). The spindle, in its early stages of development, is a thick and straight structure often situated inclined to the long axis of the hypha (Figs. 1E-.G). At telophase, however, it expands into a less stainable thin fibre and lies parallel to the hyphal axis (Fig. 1H).

HCl-Acetoorcein and HCl-Giemsa Staining

Both staining procedures reveal dividing chromatinic configurations at all stages of mitotic division. In interphase nuclei (Figs. 1I-1L) the chromatin is situated on one side of the nucleolus and appears finely granular. The lateral granules and spindles are not revealed by these procedures. As has been reported elsewhere (Robinow & Caten, 1969; Hashmi et al, 1972) four stages of nuclear division can be clearly distinguished here. In stage I, the chromatin assumes the form of chromatinic filaments that are clustered together to one side of the nucleolus (Fig. 2A). Although the nucleolus is not stained here, its position is clearly delineated. In stage II, chromosome constellations change from tight clusters to narrow, oblong chromatinic bodies aligned parallel to each other (Fig. 2B, upper nucleus). This track stage has no equivalent in the conventional form of mitosis and occurs only in fungi. In stage III, the track nucleus breaks away in the middle and the chromatin moves to the poles (Fig. 2B lower nucleus, Figs. 2C-2G). Chromatinic elements that may be lagging chromosomes (lc in Figs. 2C and 2G) have sometimes been observed between two separating chromatinic masses. This stage is comparable to the anaphase of standard forms of mitosis. In stage IV which is similar to the conventional telophase, two small daughter nuclei are reconstructed from densely contracted and deeply staining chromatinic masses (Figs. 2H-2K). These nuclei later become granular and less stainable with aceto orcein.

Discussion

The process of mitosis in *Cladosporium herbarum* in characterized by the appearance of spindles in the division figures with a concomitant division of lateral granules. This process is comparable to that observed in several other filamentous fungi and yeasts (Girbardt, 1962; 1968; Robinow & Marak, 1966; Heale et al, 1968; Robinow & Caten, 1969). Robinow & Caten (1969) in *Aspergillus nidulans*, Crackower (1969) in *Penicillium tardum* and Hashmi et al (1972) in *Trichothecium roseum* demonstrated, by a duplicate staining technique, that the formation and elongation of the spindle is correlated with the progress of division of the chromatin. There is no doubt that the intranuclear spindles of *Cladosporium herbarum* also play a similar role. Ultra-structural studies of several fungi have demonstrated some variations in the mode of formation and structure of this spinlde. Chromosomal and pole-to-

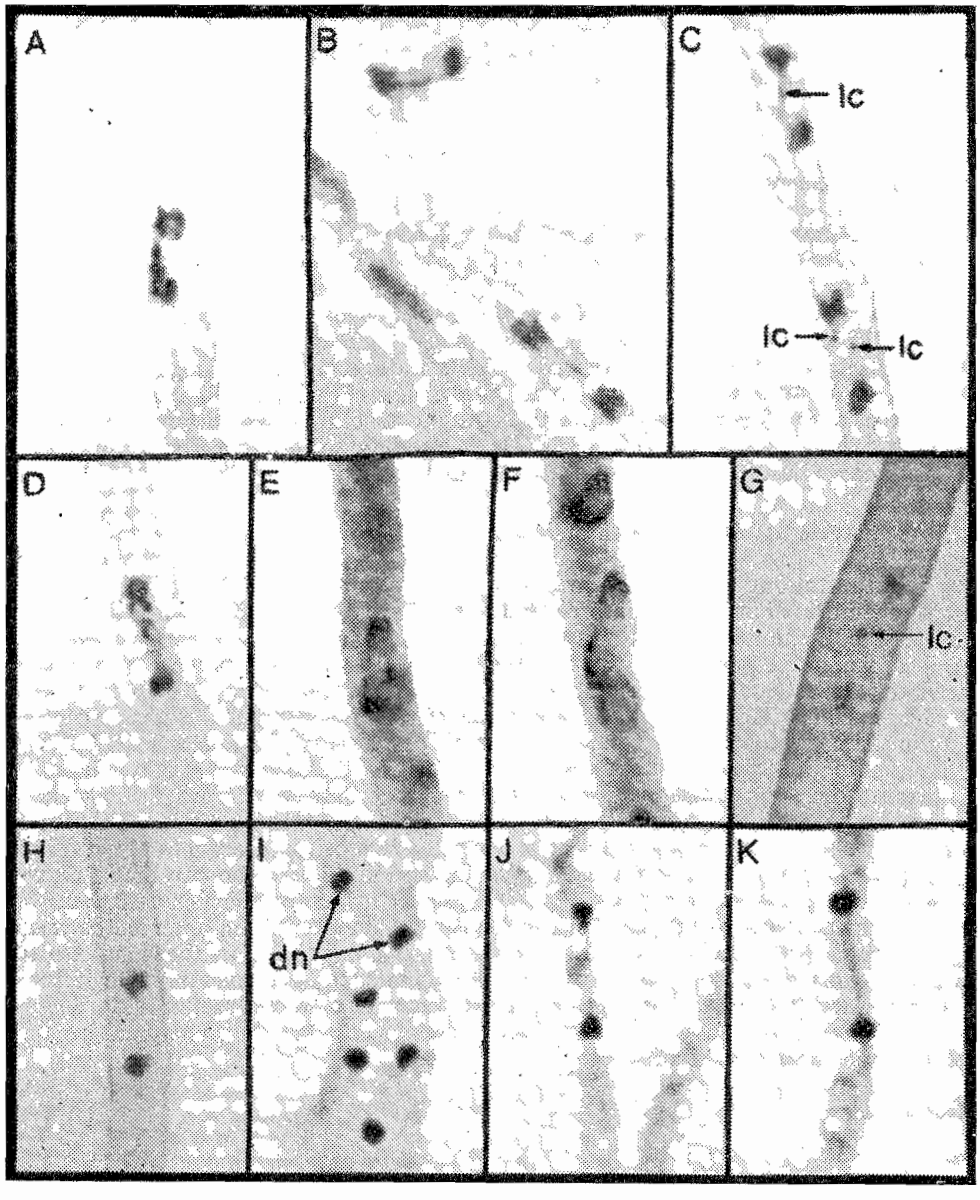

Fig. 1. *Cladosporium herbarum:* Helly, Acid Fuchsin, HCl-Aceto orcein and HCl-Giemsa.
A-D: Hyphae stained with Acid Fuchsin showing dense nucleoli (nu), chromatin (ch) and lateral granules (lg). Figure D shows a short spindle (sp) developing between a dividing lateral granule.
E G: Spindles (sp) alongside nucleoli stained with Acid Fuchsin.
H: A dividing nucleus, at stage IV, showing a long spindle anchored on both ends with daughter nucleoli (dnu). The original nucleolus (nu) does not divide and subsequently disintegrates.
I-J: Hyphae stained with HCl-Aceto orcein showing interphase nuclei. Chromatin (ch) is situated on one side of the nucleolus (nu) and shows a granular structure.
K-L: Hyphae stained with HCl-Giemsa showing interphase nuclei Chromatin (ch) is densely stained and does not show its granular structure.

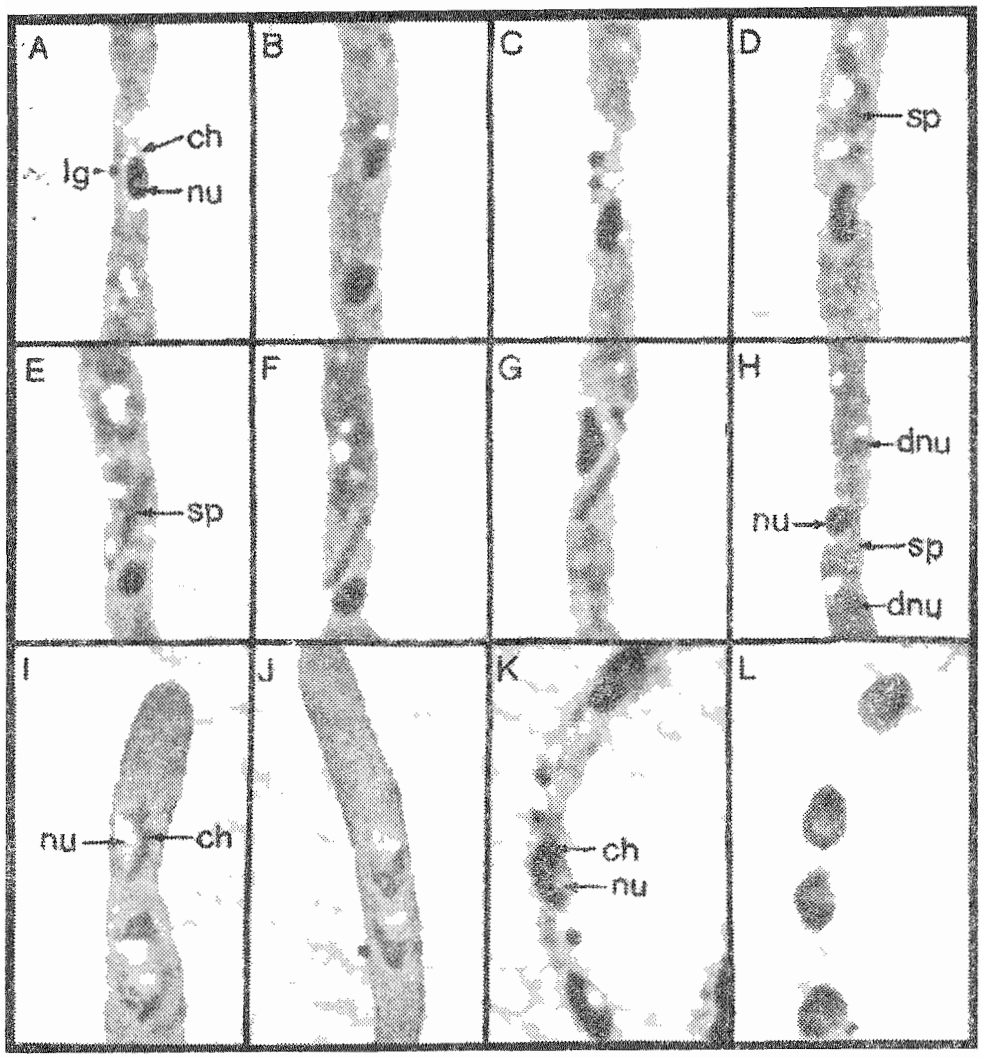

Fig. 2. *Cladosporium herbarum:* Helly, HCl-Aceto orcein and HCl-Giemsa.

A: Nucleus at Stage I. Unravelled cords beginning to line up in parallel fashion.

B: Upper nucleus at Stage II. Rectangular double-track configuration in which chromatin is arranged in two rows. Lower nucleus at late Stage II.

C-G: Nuclei at Stage III. Random transverse breaks in the rectangular configuration as chromatin begins to move apart towards opposite poles. Sometimes lagging chromosomes (lc) are seen between the two dividing chromatin masses.

H-K. Nuclei at Stage IV. Daughter nuclei (dn) are reconstructed from densely contracted chromatinic masses.

pole intranuclear fibres have been reported in *Catenaria anguillulae* (Ichida & Fuller, 1968) and *Ascobolus stercoriarus* (Wells, 1970) while observations on *Saprolegnia* (Heath & Greenwood, 1970), *Schizosaccharomyces pombe* (McCully & Robinow, 1971) and *Mucor hiemalis* (McCully & Robinow, 1973) suggest the presence of only chromosomal fibres.

The chromatin configurations of dividing nuclei of *Cladosporium herbarum* reported here extend and confirm the observations on mitotic division in *Fusarium oxysporum* (Aist, 1969), *Aspergillus nidulans* (Robinow & Caten, 1969), *Penicillium nidulans* (Robinow & Caten, 1969), *Penicillium chrysogenum* and *P. notatum* (Crackower & Bauer, 1971), *Trichothecium roseum* (Hashmi et al., 1972) and several other fungi. The peculiar anaphase configuration, where nuclear material is arrangd in a track-like structure without the intervention of a metaphase plate, is again seen here. What has remained unknown, however, is the method of transition of fungal chromosomes from interphase stage to the unique rectangular arrangement. A plausible theory put forward by Robinow & Caten (1969) is that there is a condensation of chromatin into distinct bodies which line up into two parallel rows of chromatinic masses in which individual chromosomes could no longer be distinguished and an intranuclear spindle presumably pulls the chromosomes to opposite poles.

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