A polypeptide of 59 kDa is associated with bundles of cytoplasmic filaments in *Neurospora crassa*

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Complex arrangements of filamentous structures have been isolated from vegetative cells of the fungus *Neurospora crassa*. They were enriched by differential centrifugation and purified by permeation chromatography. The filamentous structures are made up of units of 8–10 nm diameter and were isolated in bundles of up to six to nine units. The main constituent of these structures is a polypeptide with an apparent molecular mass of 59 kDa (P59Nc), which represents 4–5 % of the total *N. crassa* proteins. The filamentous structures are cold-stable and are not affected by high-ionic-strength solutions or by the presence of 10 mM-EDTA or 1 % (w/v) Triton X-100; they were disassembled by raising the pH of the solution or by using Tris-based buffers. The disassembled form assembled into structures sedimentable at 105 000 g after dialysis against the isolation buffer. The sedimentable structures were organized in the form of regular aggregates of 42–45 nm diameter. Immunoblot experiments showed that P59Nc is not immunologically related to fungal tubulin or actin polypeptides and reacted weakly with anti-IFA, a monoclonal antibody which recognizes an epitope common to many of the higher-eukaryote intermediate-filament polypeptides. Immunofluorescence examination of wall-digested hyphae of *N. crassa* using affinity-purified antibodies prepared against P59Nc showed immunostaining of abundant filamentous and dot-shaped structures distributed in the cytoplasm.

INTRODUCTION

Several aspects relative to the cytoskeletal proteins tubulin (Adams & Pringle, 1984; Kilmartin & Adams, 1984; Huffaker *et al.*, 1987), actin (Greer & Schekman, 1982; Adams & Pringle, 1984; Kilmartin & Adams, 1984), myosin (Watts *et al.*, 1985) and microtubule-associated proteins (Pillus & Solomon, 1986) have been studied in non-filamentous fungi such as yeast.

For the case of filamentous fungi, tubulin has also been extensively studied (Davidse & Flach, 1977; Gambino *et al.*, 1984; Morris, 1986), and actin (Allen & Sussman, 1978; Fidel *et al.*, 1988) and microfilaments (Grove & Bracker, 1970; Girbardt, 1979; Howard, 1981; Tucker *et al.*, 1986) have been described. However, there is not yet a good overall picture of their cytoskeleton, and a complex group of cytoplasmic fibrillar structures described at the morphological level remain poorly characterized at a biochemical and functional level. Some of the phenomena postulated to involve cytoskeletal function (other than mitotic spindle) in higher eukaryotic cells (i.e. secretion processes or organellar translocation) also occur in filamentous fungi at an apparently comparable level of complexity (McClure *et al.*, 1968; Howard & Aist, 1980; Oakley & Morris, 1980; Herr & Heath, 1982; Oakley & Rinehart, 1985; Cole, 1986; Wessels, 1986). These processes could also be related to the presence of non-tubulin non-actin filaments (Raudaskoski & Koltin, 1973; Girbardt, 1979; McKerracher & Heath, 1987). The characterization of these filaments in lower eukaryotes amenable for genetic analyses (Tilburn *et al.*, 1983; Case, 1986; Vollmer & Yanofsky, 1986) could be of interest for the study of these processes (see McKerracher & Heath, 1987).

In previous biochemical and electron-microscopic studies of *N. crassa* clathrin-coated vesicles (Rosa & Maccioni, 1987), we observed a co-distribution along several purification steps of a polypeptide of 59 kDa and complex arrangements of 8–10 nm

filaments. We report here ultrastructural studies and biochemical properties of these filaments as well as the association between the 59 kDa polypeptide, which we will hereinafter refer to as 'P59Nc', and the 8–10 nm filamentous structures. Immunocytochemical examination of permeabilized hyphae of *N. crassa* cells using affinity-purified antibodies raised against P59Nc showed P59Nc immunoreactivity in filamentous cytoplasmic structures. The morphological, biochemical and immunocytochemical data indicate that the 8–10 nm *N. crassa* filaments and the constituent P59Nc could be components of an as yet undescribed filamentous network of this lower eukaryotic cell.

MATERIALS AND METHODS

Materials

Mes, phenylmethanesulphonyl fluoride (PMSF), porous glass beads [300 nm (3000 Å) normal pore size; 120–200 mesh], fluorescent goat anti-rabbit IgG were from Sigma Chemical Co., St. Louis, MO, U.S.A.

Cell culture and harvesting

The wild-type *N. crassa* strain St. Lawrence 74-OR23-1A FGSC9871 was used throughout this work. Vegetative mycelium was grown in Vogel's (1956) N medium supplemented with 2 % (w/v) sucrose, 2.5 µg of D-(+)-biotin/ml, and 0.75 % (w/v) of yeast extract (Difco) and nutrient broth (Difco) as described by Rosa & Maccioni (1987). Vegetative cells were harvested by filtration, washed with 2 vol. of cold distilled water, frozen in liquid N₂ and ground to a fine powder in a cooled mortar.

Subcellular fractionation

The frozen powdered mycelium was thawed, then homogenized in an Ultra-Turrax T25 (Janke und Kunkel; IKA Labortechnik)

Abbreviations used: PMSF, phenylmethanesulphonyl fluoride; P59Nc, a 59 kDa polypeptide from *Neurospora crassa*; PBS, phosphate-buffered saline (0.02 M-sodium/potassium phosphate/0.130 M-NaCl, pH 7.0); FITC, fluorescein isothiocyanate; FGSC, Fungal Genetic Stock Center.

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apparatus, in two times its wet weight of 50 mM-Mes/NaOH (pH 6.5)/0.5 mM-MgCl₂/1 mM-EGTA/1 mM-PMSF/1 % Triton X-100 (MMGT buffer). The homogenate was rapidly filtered through cheesecloth, and unbroken cells and debris were removed by centrifugation at 2500 *g* for 10 min. The supernatant was adjusted to 100 µg of RNAase/ml, incubated for 20 min at 37 °C and centrifuged at 22000 *g* for 20 min. The 22000 *g* supernatant was sedimented by centrifugation at 105000 *g* for 60 min to obtain a 105000 *g* pellet and the corresponding 105000 *g* supernatant.

Glass-permeation chromatography (g.p.c.)

G.p.c. was performed essentially as described by Gravotta & Maccioni (1985) and Rosa & Maccioni (1987). A column (1 cm × 90 cm) of porous glass beads pretreated with 1 % (w/v) poly(ethylene glycol) and equilibrated with MMG buffer (buffer MMGT without Triton X-100) was used. Approx. 15 mg of protein was applied to the column, and elution was performed with MMG buffer at a rate of 3.6 ml/h. Fractions (1.8 ml each) were examined by electron microscopy and SDS/PAGE.

Purification of P59Nc

Fractions from g.p.c. enriched in P59Nc were pooled and centrifuged at 105000 *g*, 60 min. The filaments in the pellet were washed by suspension in MMG buffer supplemented with 0.4 M-NaCl for 60 min and centrifuged again at 105000 *g* for 60 min. The washed pellet was suspended in 50 mM-Tris/HCl (pH 7.5)/0.5 mM-MgCl₂/1 mM-EGTA (TMG buffer), left for 60 min and centrifuged again at 105000 *g* for 60 min. More than 90 % of the P59Nc remained in the supernatant after centrifugation. The supernatant was dialysed twice against 100 vol. of MMG buffer for 60 min and centrifuged at 105000 *g* for 60 min to recover P59Nc in the pellet. A second cycle of disassembly-reassembly produced a highly purified preparation of P59Nc.

Immunological procedures

A 500 µg portion of twice-cycled P59Nc was emulsified in 5 vol. of Freund's complete adjuvant. The emulsion was injected intradermally at several sites on the back of an adult rabbit at day 1. A second dose of 250 µg was given at day 30. Serum collected at day 37 gave a single immunoprecipitate band at different concentrations of the *N. crassa* homogenate in an Ouchterlony immunodiffusion test. Affinity-purified antibodies were obtained by chromatography on a P59Nc-Sepharose-4B column prepared according to conventional procedures (Cuatrecasas & Anfinsen, 1971; Keen, 1983). Immunoblot experiments were performed as described by Towbin *et al.* (1979). Blots with anti-IFA (a monoclonal antibody which recognizes an epitope common to many of the higher-eukaryote intermediate-filament polypeptides) (40 µg/ml) were carried out essentially as previously described (Lawson, 1983; Shapland *et al.*, 1988).

For indirect immunofluorescence of *N. crassa* hyphae, mycelium picked up from liquid cultures was fixed for 30–40 min in 3.7 % paraformaldehyde and, after several washes with phosphate-buffered saline (PBS)/0.2 M-glycine, was treated with the cocktail of wall-lytic enzymes described by Quigley *et al.* (1985). The partially digested hyphae were permeabilized with methanol at –20 °C for 8 min, washed with PBS, and treated for 20 min with a 1:20 dilution of goat serum in PBS to prevent non-specific interactions. After two washes with PBS the mycelium was incubated with the affinity-purified anti-P59Nc (5 µg of IgG/ml) and then with the secondary antibody [fluorescein isothiocyanate (FITC)-labelled goat anti-rabbit IgG]. Preparations were observed in a Zeiss Axiomat epifluorescence

microscope. Photographs were made on 400 ASA Kodak Tri-X Pan film; the exposure time was 30 s.

Other procedures

Twice-cycled microtubules were obtained from rat brain (Shelanski *et al.*, 1973). For electron-microscopic studies, drops of the samples were applied to individual carbon-coated Formvar grids and negatively stained with 1 % uranyl acetate (Rosa & Maccioni, 1987). Specimens were observed in a Hitachi HU-11B electron microscope operating at 50 kV. SDS/PAGE was performed according to Laemmli (1970). Samples were previously boiled in a buffer containing 125 mM-Tris/HCl, pH 6.8, 3 % (w/v) SDS, 5 % (w/v) β-mercaptoethanol and 10 % (v/v) glycerol. The gels were stained with Coomassie Blue or by a silver-staining procedure (Switzer *et al.*, 1979). Protein concentration was determined by the method of Lowry *et al.* (1951), with BSA as standard.

RESULTS

Distribution of P59Nc through subcellular fractions

The pattern of polypeptides present in *N. crassa* subcellular fractions is shown in Fig. 1. P59Nc represents about 5 % of the polypeptides present in the 2500 *g* supernatant (lane 1). No appreciable amount remained in the first pellet, which consisted mainly of cell-wall material (not shown in the Figure). P59Nc was virtually absent in the 22000 *g* pellet (lane 2) and represented the most abundant polypeptide in the 105000 *g* pellet (lane 4). Although P59Nc represents 25–30 % of the total protein of this fraction, a variable amount remained in the final supernatant (lane 5). Immunoblot experiments (not shown) confirmed that this soluble species is P59Nc and not another, co-migrating, polypeptide. This soluble form could represent a fraction of very

Fig. 1. Enrichment of P59Nc by differential centrifugation

N. crassa homogenate was prepared and fractionated by centrifugation as described in the Materials and methods section. The starting 2500 *g* supernatant (lane 1) was centrifuged at 22000 *g* to obtain a pellet (lane 2) and a supernatant (lane 3). The latter was centrifuged at 105000 *g*, rendering a sediment highly enriched in P59Nc (lane 4) and a final 105000 *g* supernatant (lane 5). A portion (40 µg) of total protein from each fraction was loaded into gel slots for SDS/PAGE. The resolving gel contained 6 % (w/v) acrylamide and was stained, after SDS/PAGE, with Coomassie Blue. The arrow indicates the position of P59Nc.

Fig. 2. G.p.c. of the 105000 g pellet

The column was equilibrated and developed as indicated in the Materials and methods section. The elution pattern was obtained by continuous monitoring of eluates at 280 nm. Aliquots of the fractions were analysed by SDS/PAGE. The inset shows the polypeptide patterns of fractions 38, 40, 42, 44, 46, 48 and 50 (lanes 2–8 respectively); the pattern of the 105000 g pellet (lane 1) and supernatant (lane 9) are also shown. The arrow indicates the position of P59Nc. The gel contained 7.5% (w/v) acrylamide and was silver-stained.

small filaments or a non-assembled state of the protein. The 105000 g pellet was the starting material for further purification of P59Nc and characterization of filaments.

G.p.c.

Fig. 2 shows the elution pattern (based on A_{280}) of the 105000 g pellet of a *N. crassa* cell homogenate chromatographed in a controlled-pore-glass column. The SDS/PAGE analysis of some of the fractions shows that P59Nc is highly enriched in those fractions eluted at the front of the main u.v.-absorbing peak. An electron-microscopic examination of the fractions indicated that the column produced an effective elimination of major constituents of the fungal microsomal fraction, such as ribosomes and small vesicles, which were eluted in fractions 48–62 (results not shown)

Morphological characterization of 8–10 nm filaments

The microscopical examination of eluates from a g.p.c. experiment carried out under the same conditions as those described above is depicted in Fig. 3. A dense background of short filaments (small arrows in Fig. 3, part b) and many longer filaments that are associated in pairs (large arrows) or bundles of greater number of units (arrowheads) are observed in fractions enriched in P59Nc (Fig. 3, part a). The maximal length observed in different experiments was 0.75 μm . This maximal observed length appears to be a consequence of initial breakage in the

Fig. 3. Characterization of 8–10 nm filaments

A g.p.c. fraction enriched in P59Nc (SDS/PAGE in a) was examined under the electron microscope after negative staining (b–d). The structures obtained after disassembly of the filaments and back-dialysis of the supernatant against MMG buffer (see Fig. 4, lane 5) are shown in e after negative staining. Rat brain microtubules used as reference for the width determination are shown in f. Bars represent 200 (b) or 100 (c–f) nm.

homogenization step rather than reflecting the maximal size of naturally occurring filaments (see below under 'Cellular topography of P59Nc'). Because the correct edge determination in negatively stained structures is difficult to achieve (Steven *et al.*,

Fig. 4. Solubility properties of P59Nc

G.p.c. fractions enriched in 8–10 nm filaments were pooled and subjected to several treatments as described in the text. After each treatment the solution was centrifuged to obtain a pellet and a supernatant. The solubilization of P59Nc was monitored by SDS/PAGE, loading equivalent amounts of paired pellets (lanes 1, 3, 5 and 8) and supernatants (lanes 2, 4 and 6). Lanes 1 and 2, NaCl treatment; lanes 3 and 4, TMG buffer treatment; lanes 5 and 6, back-dialysis of the solubilized P59Nc against MMG buffer; lane 8, P59Nc after a second cycle of disassembly–reassembly; lane 7, molecular-mass standards (205, 116, 97.4, 66, 45 and 20 kDa, respectively, from top to bottom). The gel contained 6% (w/v) acrylamide and was silver-stained. The arrow at the left shows the position of P59Nc. The small arrow indicates *N. crassa* clathrin.

1985), width measurements were standardized with rat brain microtubules (Fig. 3, part f). Under these conditions it was estimated that the *N. crassa* filamentous units have an apparent diameter of 8.82 ± 1.47 nm.

A detailed inspection of Fig. 3, parts c and d, shows a wavy edge in some of the filaments, suggesting a helical conformation of the filamentous backbone. The periodicity of the waves was measured as 26 nm. The filaments appear to be formed by two substructures of 5 nm diameter entangled in a double-helical arrangement (Fig. 3, part c, inset). A complex bundle of filaments is shown in Fig. 3, part d.

Structural stability of the 8–10 nm filaments and solubility properties of the 59 kDa polypeptide

Pooled fractions of the g.p.c. enriched in 8–10 nm filaments (see Fig. 2) were adjusted to 0.4 M-NaCl, left for 60 min at 4 °C and centrifuged at 105 000 *g* for 60 min. Equivalent amounts of paired pellets and supernatants were analysed by SDS/PAGE to assess the solubilization of P59Nc. Almost all of the polypeptide remained sedimentable (Fig. 4, lanes 1–2). This material, that consisted of filaments like those shown in Figs. 3(b)–3(d), was also sedimentable after strong homogenization in a glass cup/Teflon pestle homogenizer in 50 mM-Mes/NaOH (pH 6.5)/0.5 mM-MgCl₂/1 mM-EDTA/1 mM-phenylmethanesulphonyl fluoride (MMG buffer) supplemented with either 0.4 M-NaCl or 10 mM-EDTA and left for 60 min at 4 °C or at room temperature (results not shown). On the other hand, when the filaments were homogenized in 50 mM-Tris/HCl (pH 7.5)/0.5 mM-MgCl₂/1 mM-EGTA (TMG buffer), they were readily disassembled, and almost all of the P59Nc was no longer sedimentable, even after centrifugation at 130 000 *g* for 90 min (Fig. 4, lanes 3 and 4). Similar results (not shown) were obtained with Tris buffer, pH 6.5, or MMG buffer, pH 7.5. Thus solubilization appears to depend on the pH shift as much as on the presence of Tris.

Fig. 5. Western blotting with affinity-purified anti-P59Nc anti-(bovine brain tubulin) and anti- γ -actin antibodies

Purified P59Nc (P, 18 μ g), a 2500 *g* supernatant of *N. crassa* homogenate (H, 50 μ g), purified rabbit muscle actin (A, 12 μ g) and purified rat brain tubulin (T, 25 μ g) samples were subjected to SDS/PAGE (8.5% acrylamide) and electroblotted on to nitrocellulose sheets. A blot was stained with Amido Black ('Amido Black'). Similar blots were immunostained with anti-P59Nc affinity-purified antibodies ('Anti-P59Nc') or anti- γ -actin antibodies ('Anti-actin') or anti-(bovine brain tubulin) antibodies ('Anti-tubulin'). ¹²⁵I-labelled protein A from *Staphylococcus aureus* was used as secondary ligand. A very faint staining of P59Nc with anti- γ -actin antibodies was observed in some experiments; this was considered unspecific, since it was also observed in other polypeptide bands of the *N. crassa* homogenate. Specific signals at 52 and 42 kDa were obtained with anti-tubulin and anti- γ -actin antibodies respectively in *N. crassa* homogenate (arrows), which probably represent the fungal α -tubulin and actin. Position of P59Nc (59 kDa), rat brain tubulins (55 and 50 kDa) and rabbit muscle actin (42 kDa) are indicated at the left of the Amido Black panel.

Fig. 6. Western blotting with anti-IFA

Purified P59Nc (*a* and *b*) and a rat-embryo-fibroblast homogenate (*c* and *d*) were subjected to SDS/PAGE and stained with Coomassie Blue (*a* and *c*) or transferred to nitrocellulose and treated with anti-IFA antibody (*b* and *d*).

The highly overloaded gel shown in Fig. 4 permits the observation of other proteins that were also solubilized. *N. crassa* clathrin (176 kDa) (Rosa & Maccioni, 1987), a protein of 120 kDa and P59Nc are the main solubilized polypeptides. No structures were observed under the electron microscope in this supernatant (results not shown); however, after dialysis against MMG buffer, a homogeneous population of 42–45 nm structures was formed (Fig. 3e). This material was readily sedimentable by centrifugation (Fig. 4, lanes 5 and 6). *N. crassa* clathrin 'baskets' were not formed at this low clathrin concentration (Rosa & Maccioni, 1987), and hence almost all of this protein remained soluble (Fig. 4, lanes 5 and 6). A second cycle of disassembly–reassembly renders P59Nc in a highly purified form (Fig. 4, lane 8). The faint bands observed below P59Nc in this gel were not consistently observed in different experiments (see Figs. 3 and 5) and could represent proteolysis of the molecule. Under the electron microscope the reassembled material shows the same appearance as in Fig. 3(e). No filaments reconstituted in these experiments and no conditions were found which would obtain them.

Immunoblotting studies

Anti-P59Nc antibodies purified by affinity chromatography on a P59Nc–Sepharose column were used in Western-blot experiments to examine the possibility of a relationship between P59Nc and other *N. crassa* proteins. Fig. 5 shows that the main immunoreactive polypeptide was the one migrating as purified P59Nc. Already described fungal cytoskeletal proteins such as the two α -tubulins (55 and 52 kDa) and the β -tubulin (50 kDa) of *N. crassa* (Hoang-Van *et al.*, 1989) and actin (42 kDa) (Greer & Schekman, 1983; Fidel *et al.*, 1988) were not detected by anti-P59Nc, neither were detected the overloaded samples of rat brain tubulin or rabbit muscle actin (Fig. 5). In another set of Western-

Fig. 7. Intracellular distribution of P59Nc in *N. crassa*

Hyphae were partially digested, permeabilized and examined by indirect immunofluorescence as indicated in the Materials and methods section. The primary antibody was the affinity-purified anti-P59Nc (5 μ g of IgG/ml) (*c* and *d*) or preimmune rabbit IgG (same animal, 25 μ g/ml) (*a* and *b*). *a* and *c*, phase-contrast optics; *b* and *d*, epifluorescence optics. In *d*, a generalized immunostaining was observed with the rabbit anti-P59Nc IgG. The immunoreactive material in the hyphae at the plane of focus appears as abundant filamentous structures distributed in the cytoplasm and superimposed on a faint background; this is best noted in the inset at higher magnification of a different visual field. No immunostaining was noted when preimmune rabbit IgG was the primary antibody (*b*). Final magnifications: *a*, *b*, *c* and *d*, $\times 98$; inset in *d*, $\times 150$.

blot experiments, anti-(bovine brain tubulin) and anti-(γ -actin) antibodies (Otey *et al.*, 1986; Fidel *et al.*, 1988) did not recognize P59Nc either in the *N. crassa* homogenate or in the purified sample in which 18 μ g of P59Nc was blotted (Fig. 5); the reaction of these antibodies with *N. crassa* 50–55 kDa (anti-tubulin) or 42 kDa (anti-actin) polypeptides was fainter than with the corresponding reference samples of tubulin and actin, probably reflecting the low proportion of tubulin and actin in the *N. crassa* homogenate. The anti-IFA monoclonal antibody, which recognizes an antigenic determinant common to many members of the IF polypeptide family (Pruss *et al.*, 1981; Magin *et al.*, 1987), was also tested. In this case immunoblots of 6 μ g of purified P59Nc gave only a weak signal compared with that of rat-embryo fibroblast total protein (Fig. 6).

Cellular topography of P59Nc

The affinity-purified anti-P59Nc antibodies were used to study the native distribution of P59Nc in the *N. crassa* cells by indirect immunofluorescence. The fixation and immunostaining schedules were developed according to methods described for plant (Wick *et al.*, 1981) and yeast (Kilmartin & Adams, 1984) cells using a cocktail of cell-wall-lytic enzymes (Quigley *et al.*, 1985). Fig. 7 shows the result of a typical immunofluorescence experiment. Defined cytoplasmic structures are the main fluorescent structures observed. These structures were longer than those isolated from homogenates (see above, Fig. 3) and could be traced as the parts in the plane of focus of a continuum of stained filaments; in no case, however, were these structures longer than 15–20 μ m. A background of staining is also observed in the hyphal cytoplasm. It is possible that this non-organized stain represents the non-sedimentable form of P59Nc mentioned above (see Fig. 1, lane 5). The pattern of staining of P59Nc differs from the filamentous and peripheral punctiform pattern of actin (Adams & Pringle, 1984; Kilmartin & Adams, 1984; Tucker *et al.*, 1986) and from the image of fine microtubules oriented in parallel with the longitudinal axis of the hyphae (Hoch & Staples, 1985; Caesar *et al.*, 1988). It should be noted that no immunostaining occurred if the overall procedure was carried out on mycelia not previously permeabilized by treatment with the lytic enzymes and cold methanol; in this case the picture obtained was identical with that of Fig. 7, part b (results not shown). The results of the immunofluorescence experiments are consistent with the structural studies of isolated filaments shown in Fig. 3 and support the contention that the P59Nc 8–10 nm filaments are cytoplasmic structures.

DISCUSSION

We have characterized an as yet undescribed type of cellular filament in the lower eukaryote *N. crassa*. The isolated filaments have a diameter of 8–10 nm and present complex morphological arrays. They appear to be homopolymers of a polypeptide of 59 kDa (P59Nc) and are readily disassembled in common buffers (i.e. Tris buffer). P59Nc was one of the major polypeptides in *N. crassa* homogenates, and it was the main polypeptide in the 105 000 g pellet; the abundance of such a protein suggests that it could be present in all the hyphae.

The ultrastructural organization of the isolated filaments revealed by the electron-microscopic studies and the intracellular organization of P59Nc revealed by the immunofluorescence studies suggests a relationship of these filaments and their constituent protein with cytoplasmic structures distinguishable from that of fungal microtubules and actin filaments (Kilmartin & Adams, 1984; Hoch & Staples, 1985; Tucker *et al.*, 1986; Caesar *et al.*, 1988). We were unable to find immunological relationships between P59Nc and tubulin or actin polypeptides,

since in Western-blot experiments neither did anti-P59Nc antibodies recognize these proteins nor did antibodies against these proteins recognize P59Nc. In addition, anti-IFA, which recognizes a common epitope present at the C-terminal domain of the α -helix (Magin *et al.*, 1987), reacts only weakly with P59Nc. Even so, the mechanochemical properties of P59Nc, its supra-molecular array in 8–10 nm filaments, and the cellular distribution observed by immunofluorescence studies, support the possibility that a new component of the lower-eukaryotic cellular matrix has been isolated.

Filaments of intermediate size of the type of those present in higher-eukaryotic cells have been recently found in several non-vertebrate cells (Walter & Biesman, 1984; Barnick *et al.*, 1986; Chang & Piperno, 1987). In relation to filamentous fungi, no detailed description of intermediate filaments is available at present. The filaments of 8–10 nm described here could be related to the cytoplasmic bundles of 'microfilaments' observed several years ago in sections of filamentous fungi (see McKerracher & Heath, 1987) and in the cytoplasm of the neck of the bud in yeast (Byers & Goetsch, 1976; Soll & Mitchell, 1983).

In spite of the failure to find convincing immunological relationships between P59Nc and well-established components of the cytoskeleton, such as tubulin and actin, the weak reaction with the anti-IFA antibody would suggest a distant relationship with intermediate-filament polypeptides from higher eukaryotes. It is possible that filamental structures of intermediate size belonging to evolutionarily distant species such as fungi and mammals could play equivalent functional roles, even when the compositions of their constituent polypeptides have diverged substantially. In relation to the biochemical properties, it is noticeable that the *N. crassa* filaments readily disassemble when the nature or the pH of the buffer solution is changed and do not reassemble into filaments after reversion to isolation buffer. The disassembling properties are reminiscent of those of nuclear lamins from higher cells, which disassemble in Tris buffer above pH 7.5 (Aebi *et al.*, 1986).

Filamentous fungi carry out many cellular processes that could involve the 8–10 nm filaments as a particular component of cytoskeletal function; they possess complex and diverse programs of development, with highly specialized forms of polarized growth [see Cole (1986) and Wessels (1986) for reviews]. In general, growth occurs by elongation of the hyphal tip cell (McClure *et al.*, 1968; Grove & Bracker, 1970; Howard, 1981; Howard & Aist, 1980; Herr & Heath, 1982; Cole, 1986; Wessels, 1986), whose intracellular architecture appears specialized for the process of membrane expansion (see McKerracher & Heath, 1987). Other pivotal events in the development of filamentous fungi that are postulated to involve cytoskeletal function are the process of nuclear movement (Oakley & Morris, 1980) and the phenomenon of 'septation' (see Girbardt, 1979) also occurring in yeast (Cabib *et al.*, 1982). However, only microtubules and microfilaments were previously considered as fungal cytoskeleton. The finding of P59Nc and the 8–10 nm filaments in *N. crassa* open the possibility of applying modern techniques of genetic analysis to study the functional role of these structures in the processes mentioned above.

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