The *Aspergillus fumigatus* cell wall is organized in domains that are remodelled during polarity establishment

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Aspergillus fumigatus is a life-threatening and increasingly frequent pathogen of the immunocompromised. Like other filamentous fungi *A. fumigatus* grows in a highly polar manner, adding new cell wall to the apical region of hyphae. mAbs were raised against isolated *A. fumigatus* cell walls. Fifteen antibodies bound reproducibly to isolated cell walls in ELISAs and to the walls of intact cells in immunofluorescence experiments. Surprisingly, individual mAbs showed distinct patterns of localization. Six antibodies labelled exclusively conidial or basal regions, seven labelled apical regions and a single antibody labelled both basal and apical regions of hyphae. Ten antibodies did not label the walls adjacent to septa. In double labelling experiments with representative mAbs there was little or no overlap between epitopes recognized. These labelling patterns suggest that the wall is made up of basal and apical domains that differ in composition or organization and that the wall region flanking septa differs from other regions of the lateral wall. In time-course experiments of early *A. fumigatus* growth, mAb16C4 failed to label isotropically expanding cells and labelled emerging germ tubes and branches. The same mAb failed to label the *Aspergillus nidulans* *swcC* mutant, which is defective in polarity establishment. However, mAb16C4 did label the *A. nidulans* *swcA* mutant, which completes polarity establishment, but is defective in polarity maintenance. Thus, mAb16C4 appears to recognize a cell wall change that occurs during polarity establishment. In immunogold labelling and transmission electron microscopy (TEM) experiments, conidia, basal regions and apical regions of thin-sectioned cells labelled with mAb16C4. That only apical regions labelled in intact cells (immunofluorescence) while conidial, basal and apical regions labelled in thin-sectioned cells (TEM) suggests that the 16C4 epitope is present along the whole hypha, but is masked everywhere except the tip until polarity establishment. That is to say, some remodelling of the wall during polarity establishment exposes the 16C4 epitope. The 16C4 epitope was partially purified from *A. fumigatus* total protein by passage through hydrophobic interaction and anion-exchange columns. The resulting single ELISA-positive fraction showed relatively few bands by SDS-PAGE and silver staining and a strong band by Western blotting with the 16C4 mAb. Sequencing of the fraction yielded a predicted peptide with a 6-amino acid exact match to a region of the Cat1 protein previously identified as an immunodominant *A. fumigatus* catalase that localizes to the cell wall and is secreted to the medium. Experiments are under way to determine if mAb16C4 recognizes Cat1 or another protein that co-purifies with Cat1.

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Abbreviations: DIC, differential interference contrast; TEM, transmission electron microscopy.

INTRODUCTION

Aspergillus fumigatus is a ubiquitous saprobe that breaks down organic material in leaf litter and soil (Debeaupuis *et al.*, 1997). The asexual spores (conidia) of this very common filamentous fungus can reach lung alveoli when inhaled (Pitt, 1994). In healthy individuals, *A. fumigatus* sometimes causes allergic reactions or localized infections; however, in the immunocompromised, it can cause life-threatening invasive aspergillosis. Invasive aspergillosis has a 30–90% mortality rate and is being reported with increasing frequency (Denning, 1998; Ellis, 1999). *In vitro*, *A. fumigatus* conidia break dormancy and expand isotropically for a brief time before primary germ tubes emerge (Momany & Taylor, 2000). These primary germ tubes extend to form hyphae by addition of new material exclusively at their tips – a highly asymmetric, or polar, growth mode employed by all filamentous fungi. In invasive aspergillosis, polar growth of primary hyphae and branches eventually forms the filamentous network typically seen in patient tissues.

Before filamentous fungi can switch from isotropic spore expansion to polar hyphal growth, the spot where the germ tube will emerge must be chosen (Harris, 1997; Harris & Momany, 2004; Momany, 2002). This site specification step, known as polarity establishment, is followed by a redirection of the cellular machinery needed to make new plasma membrane and cell wall at the chosen site, known as polarity maintenance. Work with *Aspergillus nidulans* *swa* mutants has shown that establishment and maintenance are genetically separable steps in polar growth (Momany *et al.*, 1999).

The cell wall is the main defence of the fungus against a hostile environment. Fungal cell wall components are often the targets of the host immune system in a fungal infection. Several studies have characterized the sugars, proteins and lipids that make up the *A. fumigatus* cell wall (Fontaine *et al.*, 1997; Ghfir *et al.*, 1997; Guest & Momany, 2000; Hearn & Sietsma, 1994). mAbs have been raised against galactomannan from the *A. fumigatus* wall (Ste-Marie *et al.*, 1990). In the current work we describe the generation and characterization of a panel of mAbs raised against isolated *A. fumigatus* cell walls. Immunofluorescence experiments with these antibodies showed that the cell wall appears to be organized in unique basal and apical domains and that the apical domain reorganizes during polarity establishment.

METHODS

Strains and growth conditions. Experiments were carried out using *A. fumigatus* strain 237, a clinical isolate provided by David Holden (Royal Postgraduate Medical School, Hammersmith Hospital, London) or *A. nidulans* strains AJB16 (*swa*A-2) and AGA24 (*swa*C) (Momany *et al.*, 1999). All cultures were incubated in complete medium (1% glucose, 0.2% peptone, 0.1% yeast extract, 0.1% Casamino acids, nitrate salts, trace elements and 0.1% vitamins, pH 6.5). Nitrate salts, trace elements and vitamins are described by Kafer (1977). *A. fumigatus* was incubated at 37 °C and *A. nidulans* was incubated at 30 (permissive) or 42 °C (restrictive).

Cell wall preparation. Cultures of *A. fumigatus* 237 were grown at 37 °C for 48 h, filtered through a #1 Whatman filter and washed with ddH₂O followed by 0.5 M NaCl. Fungal hyphae were broken in Disruption Buffer (DB; 20 mM Tris, 50 mM EDTA, pH 8.0) in a glass homogenizer until microscopic examination revealed hyphal ghosts. Cell walls were separated by centrifugation at 3000 g for 10 min. The pellet containing the cell wall fraction was washed with DB with stirring for 4 h at 4 °C followed by a wash with ddH₂O under the same conditions. The pellet was dried by vacuum filtration and lyophilized.

Preparation of mAbs. Mice were injected with isolated *A. fumigatus* 237 cell walls to generate mAbs following the protocol of Ste-Marie *et al.* (1990) at the University of Georgia Monoclonal Antibodies Facility (<http://www.rserv.uga.edu/ma/>). Antibodies from monoclonal cell lines were screened for binding to *A. fumigatus* cell walls by ELISA. Antibodies produced by 15 cell lines reproducibly bound isolated cell walls. These mAbs were isotyped by the UGA Monoclonal Antibodies Facility and found to be of the pentameric IgM class.

ELISA. Isolated *A. fumigatus* cell walls at 0.5 mg ml⁻¹ in EPBS (8.2 mM Na₂HPO₄, 1.8 mM NaH₂PO₄.H₂O, 140 mM NaCl, pH 7.4) were incubated in 96-well Immulon 2HB plates (Dynex) overnight at 4 °C. All subsequent washes and incubations were at room temperature. Wells were washed three times with ELISA wash buffer (10 mM Tris, 0.05% Tween 20, 3.1 mM sodium azide, pH 8.0) and blocked with ELISA blocking solution (10 mg BSA ml⁻¹, 0.02% sodium azide, in EPBS) for 30 min. Wells were washed three times with ELISA wash buffer followed by incubation with mAb for 1 to 2 h. mAbs were diluted 1:10 with ELISA diluent (0.05% Tween 20, 0.02% sodium azide, 10 mg BSA ml⁻¹ in EPBS). Wells were washed three times with ELISA wash buffer and incubated with alkaline phosphatase-conjugated goat anti-mouse IgM antibody (Sigma) for 1–2 h (diluted 1:500 in ELISA diluent). Wells were washed three times with ELISA wash buffer and incubated with phosphatase substrate tablets (Sigma) dissolved in 8.33 ml alkaline phosphatase substrate buffer (0.5 mM MgCl₂.6H₂O, 9.6% diethanolamine, pH 9.6) for 30 min. Absorbance at 405 nm was recorded. All ELISA experiments were performed at least two times.

Immunofluorescence. Immunofluorescence experiments were carried out following the protocols of Harris *et al.* (1994). Ten millilitres of complete medium was inoculated with 1×10^4 – 5×10^4 conidia ml⁻¹ and incubated in a Petri plate containing a glass coverslip. The coverslips with adherent fungal hyphae were fixed with 3.7% formaldehyde, 50 mM phosphate buffer (pH 7.0) and 0.2% Triton X-100 for 30–60 min. Coverslips were washed once with water and three times in PBS (pH 7.3) and incubated for 1–2 h with the mAb as indicated. Coverslips were washed in PBS and incubated with FITC-conjugated goat anti-mouse IgM antibody (Sigma) for 1–2 h (diluted 1:200 in PBS). Coverslips were washed in PBS, incubated with 10 µg Calcofluor White ml⁻¹ (Bayer) and 100 ng Hoechst 33258 ml⁻¹ (Sigma), washed briefly in water and mounted on a glass slide. For double labelling experiments Alexa Fluor 546 labelling kit (Molecular Probes) was used to label mAb16C4 according to the manufacturer's instructions. Fungal hyphae were viewed using a Zeiss Axioplan microscope and digitally photographed using Optonics software. All immunofluorescence experiments were performed two times.

Immunogold labelling and transmission electron microscopy (TEM). Hyphae of *A. fumigatus* 237 were prepared for study by TEM using freeze-substitution fixation as described previously (Momany *et al.*, 2002) except that LR White resin was used instead of Epon and mAb16C4 was used as primary antibody.

Assay in patient samples. Five paraffin-embedded tissue samples from patients diagnosed with aspergillosis were assayed using the Envision Plus Detection System (DAKO) with mAb16C4. No strong positive signals were seen indicating that the antibody is not suitable for clinical application.

Partial protein purification. *A. fumigatus* conidia were inoculated to complete liquid medium and incubated with shaking at 37 °C for 48 h. Fungal hyphae were filtered through cheesecloth, washed with water, frozen with liquid nitrogen and ground by mortar and pestle while frozen. Powdered hyphae (10 g) were suspended in 40 ml 50 mM sodium citrate, pH 6, 0.02% sodium azide, supplemented with one Complete Protease Inhibitor tablet (Roche), rocked for 30 min at room temperature and then centrifuged at 10 000 g at 4 °C for 10 min. The supernatant was loaded at 5 ml min⁻¹ onto a 25 ml Octyl-Sepharose column (Amersham-Pharmacia, 1.6 × 15 cm) equilibrated with 20 mM Tris, pH 8, then a linear gradient was performed over 20 column vols with 20 mM Tris pH8/50% 2-propanol using an Äkta purifier (Amersham-Pharmacia). Two wavelengths, 280 and 405 nm, were monitored during all chromatography steps. Eluted fractions were assayed by ELISA. Fractions having positive ELISA signals were directly loaded at 5 ml min⁻¹ onto a 5 ml HiTrap Q column (Amersham-Pharmacia) equilibrated in 20 mM Tris, pH 8.0. After washing with the starting buffer, a linear gradient was performed over 20 column vols with 1 M NaCl, 20 mM Tris, pH 8. Eluted fractions were assayed by ELISA and SDS-PAGE. One ELISA-positive fraction was sequenced by the Molecular Genetics Instrumentation Facility at UGA (<http://www.ors.uga.edu/ibf/index.html>).

The following sequence was returned: A/S/Y,G/E/P,A/E,S/F,L/V,L,Y,P,T,G,R,X,X/D,Q,X,X/F. For comparison, the sequence for the *A. fumigatus* Cat1 (GenBank accession U97574.1) from amino acid 650 to 663 is SGASSLYPTGRPLQ.

Electrophoresis and immunoblotting. SDS-PAGE was carried out using standard protocols using the Phast gel system (Amersham Pharmacia). Proteins were visualized by silver staining. Proteins were transferred from the separating gel by electrophoresis to NitroBind nitrocellulose transfer membrane (pore size 0.45 µm; Osmonics Inc.) for 30 min. The membrane was blocked with 5% non-fat dry milk for 1 h then incubated sequentially with undiluted mAb16C4 for 1–2 h, horseradish peroxidase-conjugated sheep anti-mouse Ig (Amersham Pharmacia Biotech) and ECL Western blotting detection reagents (Amersham Pharmacia Biotech). Autoradiography film was exposed to blots.

RESULTS/DISCUSSION

The cell wall is organized in domains that differ between the basal and apical regions of the hypha

Cell walls isolated from *A. fumigatus* clinical isolate strain 237 were used to inoculate mice for the production of mAbs. Antibodies from individual monoclonal lines were screened

Table 1. Binding of mAbs to *A. fumigatus* cell walls by ELISA

Absorbance was measured at 405 nm; ELISAs were performed with unpurified hybridoma culture supernatant against isolated cell walls.

mAb (dilution)	PBS	A.f. 18 h*	A.f. 48 h*	A.f. 72 h*	A.n. 48 h†	Immunofluorescence localization‡
5H9 (1:10)	0	1.0	0.3	0	0.4	Conidium, basal, no septa
8A1 (1:10)	0	1.3	0.9	0.1	0.3	Conidium, basal, no septa
14B5	1.5§	1.5	1.5	1.5	1.5	Conidium, basal, septa
14B5 (1:2)	0	0.25	0.1	0.1	0.5	
1D2	0	0.6	0.1	0	0.1	Basal
4G5	1.4§	1.4	1.1	1.3	1.4	Narrow basal zone
8C12	0.8§	0.9	0.8	0.9	1.2	Narrow basal zone
19H1	1.5§	1.5	1.5	1.5	1.5	Narrow basal zone, septa
19H1 (1:2)	0.5	0.6	0.4	0.5	0.7	
5D4 (1:10)	0	1.3	1.5	1.5	1.1	Apical, no septa
1E2 (1:10)	0	1.4	1.5	1.5	0.9	Apical, no septa
16C4 (1:10)	0	1.3	1.5	1.5	1.2	Apical, no septa
8C8 (1:10)	0	1.3	1.5	1.3	0.9	Apical, no septa
1C4 (1:10)	0	1.2	1.5	1.3	0.8	Apical, no septa
9F1 (1:10)	0	1.4	1.5	1.5	0.9	Apical, no septa
12A6 (1:100)	0	1.0	1.4	1.0	0.5	Apical, no septa
18A3 (1:10)	0	1.3	1.1	0.8	0.3	Apical, basal, no septa

*Cell walls isolated from *A. fumigatus* after 18, 48 or 72 h growth.

†Cell walls isolated from *A. nidulans* after 48 h growth.

‡*A. fumigatus* conidia were inoculated on coverslips in complete medium. After 12–14 h growth, coverslips were fixed and stained with mAb supernatant followed by FITC-conjugated anti-mouse secondary antibody to visualize the epitope location. Basal denotes the germ tube region closest to the conidium; narrow basal zone, the region where the germ tube emerges from the conidium; septa, the outer wall in region of the septa; apical, tips of primary hypha, and branches and regions away from the conidium.

§Despite a high background in ELISA, the mAb showed specific binding in immunofluorescence. The epitope may be shared by the plastic in the assay plate.

by ELISA for binding to isolated *A. fumigatus* cell walls. Fifteen monoclonal lines making antibodies that reproducibly bound to walls were identified from the first fusion (Table 1).

To verify that the mAbs recognized cell wall epitopes, they were used to label whole, fixed *A. fumigatus* germlings in indirect immunofluorescence experiments. Surprisingly, individual antibodies showed distinct patterns of localization (Table 1, Fig. 1). Six antibodies labelled exclusively conidial or basal regions, seven labelled apical regions and a single antibody labelled both basal and apical regions. Ten antibodies did not label the walls adjacent to septa. To investigate whether basal and apical wall domains overlap, double labelling experiments were performed with representative antibodies (Fig. 2). mAb8A1 labelled the conidium and basal region, while mAb16C4 labelled the apical region with little or no overlap. These labelling patterns suggest that the cell wall is made up of basal and apical domains that

differ in composition or organization and that the wall region flanking septa differs from other parts of the lateral wall.

Though our isolated cell walls were washed several times before use as immunogen or as substrate in ELISAs, it is possible that contaminating cytoplasmic proteins were also present. However, it seems likely that our mAbs are recognizing true cell surface epitopes. The immunofluorescence label is clearly cortical (Fig. 2). All of our antibodies are of the pentameric IgM class (data not shown) and it is very unlikely that these bulky antibodies would be able to penetrate the cell wall to label cytosolic proteins. Indeed standard protocols for immunofluorescent labelling of fungal cytosolic proteins require enzymic removal of the cell wall (Pringle *et al.*, 1989).

To further investigate the localization of epitopes recognized by mAbs 8A1 and 16C4, *A. fumigatus* cells were plasmolysed in 2.67 M sorbitol, causing the cytoplasm to shrink away from the wall. When plasmolysed cells were labelled with mAb16C4 or mAb8A1, the antibody clearly followed the smooth contour of the cell wall rather than the wrinkled plasma membrane consistent with recognition of cell wall epitopes (Fig. 3).

The cell wall apical domain reorganizes with polarity establishment

After breaking dormancy, the spores of filamentous fungi undergo a brief period of isotropic growth before beginning highly polar tip growth (Harris, 1997). To determine whether the apparent domain structure of the wall changes during polarity establishment and early growth, a time-course experiment was performed. *A. fumigatus* conidia were inoculated to coverslips in rich medium, incubated for

Fig. 1. mAbs show distinct localization patterns. *A. fumigatus* 237 was grown for 12–14 h, fixed, incubated with primary mAb, followed by FITC-conjugated anti-mouse secondary antibody, Calcofluor White to label chitin and Hoechst 33258 to label nuclei. Primary antibodies: mAb1E2 (a), mAb8A1 (b) and mAb19H1 (c). Left column, FITC; right column, chitinous cell wall and nuclei. Arrows mark conidia; arrowheads mark septa; the double arrowhead marks a newly formed branch from main hypha.

Fig. 2. Basal and apical wall domains do not overlap. (a, b) *A. fumigatus* 237 was grown for 6 h, fixed and labelled with mAb8A1 (green) and mAb16C4 (red). Left column, differential interference contrast (DIC); middle column, mAb8A1 followed by FITC-conjugated secondary antibody; right column, mAb16C4 directly coupled to Alexa Fluor 546.

Fig. 3. mAbs 8A1 and 16C4 recognize wall epitopes. *A. fumigatus* germlings were plasmolysed in 2.67 M sorbitol before labelling with mAbs and visualization with FITC-conjugated anti-mouse secondary antibody. DIC and fluorescence images of germling labelled by mAb8A1 (a, b) or mAb16C4 (c, d). Bar, 5 μ m.

varying periods of time, fixed and labelled with mAb16C4 (Fig. 4). The antibody labelled emerging germ tubes and branches and failed to label isotropically expanding cells. This pattern suggested that mAb16C4 might recognize a change in the cell wall associated with polarity establishment.

In separate work, the *A. nidulans* temperature-sensitive *swo* mutants were identified. At restrictive temperature these polarity mutants grow isotropically, continue nuclear division and do not send out germ tubes (Momany *et al.*, 1999). The *swoC* mutant is defective in choosing the spot for germ tube emergence (polarity establishment) and the *swoA* mutant is defective in extending the germ tube (polarity maintenance). When cells are shifted from restrictive to permissive temperature, *swoC* does not send out germ tubes within a cell cycle, while *swoA* rapidly sends out multiple germ tubes from one side of the enlarged cell. We reasoned that if mAb16C4 recognized a wall change that occurred

during polarity establishment, it should not label the establishment-defective mutant *swoC*, but should label the maintenance-defective *swoA*. After verifying that mAb16C4 showed the same labelling pattern in wild-type *A. nidulans* as in *A. fumigatus*, we performed immunofluorescence experiments with the *swoC* and *swoA* mutants. At restrictive temperature mAb16C4 did not label *swoC* and did label one pole of *swoA* (Fig. 5a, b). When *swoA* cells were shifted from restrictive to permissive temperature, new germ tubes emerged from the pole of the cell that was labelled with mAb16C4 and each new germ tube showed 16C4 labelling at its tip (Fig. 5c). Thus, mAb16C4 appears to recognize a cell wall change that occurs during polarity establishment.

Because our immunofluorescence experiments were performed on intact germlings, the restriction of mAb16C4 label to the hyphal tip could mean that the epitope is present only at the tip or that the epitope is present in

Fig. 4. mAb16C4 labels areas of polar growth. *A. fumigatus* 237 was incubated for (a) 5, (b) 6, (c) 7, (d) 8 or (e) 9 h, fixed and labelled with mAb16C4 followed by FITC-conjugated anti-mouse secondary antibody. Left column, DIC image; right column, FITC image. Bars: 5 μ m (a–c); 15 μ m (d, e).

Fig. 5. mAb16C4 does not label the polarity establishment mutant *swoC*, but does label the polarity maintenance mutant *swoA*. *A. nidulans* polarity mutants were incubated at restrictive temperature for 12 h (a, b) or at restrictive temperature for 12 h followed by permissive temperature for 4 h (c). After incubation, cells were fixed and labelled to visualize chitin, nuclei and the 16C4 epitope. (a) *swoC*, (b) *swoA*, (c) *swoA* with shift from restrictive to permissive temperature. First column, DIC; second column, Hoechst 33258 and Calcofluor White to label nuclei and cell walls, respectively; third column, mAb16C4 label with FITC-conjugated secondary antibody. Bars, 10 μ m.

other regions, but accessible to the antibody only at the tip. To eliminate the question of epitope accessibility, we performed immunogold labelling and TEM experiments. In contrast to the intact cells used for immunofluorescence experiments, the hyphae used for TEM were sectioned before incubation with the antibody. mAb16C4 recognized an epitope that was present in the walls of conidia (Fig. 6a, b) and in the walls of hyphae (Fig. 6d). The wall is much thinner at the hyphal tip and so harder to visualize; however, mAb16C4 clearly labelled the cell surface and tip vesicles, consistent with a protein transiting the secretory system en route to the cell wall (Fig. 6c). The fact that only apical regions labelled in intact cells (immunofluorescence) while conidial, basal and apical regions labelled in thin-sectioned cells (TEM) suggests that the 16C4 epitope is present along the whole hypha, but is masked everywhere except the tip until polarity establishment. That is to say, some remodelling of the wall during polarity establishment exposes the 16C4 epitope. Previous work by others shows that hyphal cell wall polymers are modified during maturation in the subapical regions (reviewed by Sietsma

& Wessels, 1994). mAb16C4 might be recognizing such a change.

Because *A. fumigatus* is medically important and there is a need for better diagnostic tools, we tested our 16C4 mAb for detection of hyphae in clinical samples. Paraffin-embedded thick sections from confirmed aspergillosis patients were incubated with the antibody. Unfortunately mAb16C4 did not label these sections. This might indicate that the epitope is sensitive to the procedures used. Alternatively, it might indicate that the 16C4 epitope is lost as hyphae age or that it is not made in human hosts.

mAb16C4 recognizes a protein that co-purifies with a catalase

Western blotting experiments using mAb16C4 showed a single band of approximately 70 kDa, so we attempted to isolate the protein using standard biochemical techniques. Total *A. fumigatus* protein was loaded onto an octyl-Sepharose column, a hydrophobic interaction medium, and the eluted fractions were assayed by ELISA. Positive fractions were then loaded onto a Q anion-exchange column for further purification. The single ELISA-positive fraction from the Q column was examined by SDS-PAGE and silver staining, and by Western blotting (Fig. 7). The positive fraction showed relatively few bands and a strong signal in Western blotting with mAb16C4. Mass spectroscopy of the ELISA-positive fraction yielded four bands of 70, 38, 35 and 4 kDa. Sequencing of the fraction yielded a predicted peptide of 14 residues with 6 amino acids unambiguously identified. These 6 amino acids were an exact match to a region of the Cat1 protein previously identified in *A. fumigatus*. Cat1 is an immunodominant catalase that localizes to the cell wall and is secreted into the medium (Calera *et al.*, 1997). Consistent with purification of a catalase, the ELISA-positive chromatography fractions showed significant absorbance at 405 nm, suggesting the presence of a chromophore such as the haem iron found in catalases.

It is possible that mAb16C4 recognizes an epitope of the Cat1 protein. However, it is also possible that mAb16C4 recognizes another protein that co-purifies with Cat1. The *A. fumigatus* Cat1 subunits are reported to have an apparent 90 kDa mass in SDS-PAGE, rather than the 70 kDa mass we observed for the 16C4 epitope, though this discrepancy could also be explained by degradation during protein isolation. Three catalases have been characterized from *A. fumigatus*: CatA is produced in conidia, and Cat1 and Cat2 are produced in mycelia (Paris *et al.*, 2003). In addition, four other catalase homologues have been identified within the *A. fumigatus* genome (Paris *et al.*, 2003). mAb16C4 might recognize one of these other catalases. Further experiments are in progress to positively identify the protein recognized by mAb16C4. Regardless of the identity of the 16C4 epitope, our results support the idea that the fungal cell wall is arranged in basal and apical domains and that the apical domain reorganizes with polarity establishment.

Fig. 6. mAb16C4 labels cell walls in basal and apical regions in immunoelectron microscopy. Transmission electron micrographs of *A. fumigatus* 237 thin sections labelled with mAb16C4 followed by goat anti-mouse antibody coupled to 10 nm gold particles. (a) Conidium and basal region of hypha, including first septum. Inset, region in box enlarged threefold. (b) Conidium; a single nucleus is visible in the middle of the cell. (c) Hyphal tip; the Spitzenkörper is visible as a collection of vesicles. Note that some vesicles are labelled. (d) Wall from central portion of the hypha.

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Fig. 7. mAb16C4 recognizes a protein of ~70 kDa. Total protein (lanes 2, 4) and the positive fraction from the Q column (lanes 1, 3) were analysed by PAGE, silver-stained (lanes 1, 2), Western blotted and hybridized with mAb16C4 (lanes 3, 4). See text for details. The positions of molecular mass markers are shown on the left.

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