Mycologia, 98(6), 2006, pp. 850–859.

Assembling the Fungal Tree of Life: constructing the Structural and Biochemical Database

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Abstract: A major goal of the Assembling the Fungal Tree of Life project is to create a searchable database <http://aftol.umn.edu> of selected ultrastructural and biochemical characters from published and new data for use in phylogenetic and other analyses. While developing this database such issues as evaluating specimen fixation quality in published micrographs, organizing data to accommodate characters that were dependent on location and developmental stage, and requiring accountability of data contributors were addressed. Character states for three traits, septal pore apparatus, nuclear division and spindle pole body cycle, are illustrated, and character states are resolved with maximum parsimony and plotted on a summary cladogram of known phylogenetic relationships of the Fungi. The analysis illustrates the inherent phylogenetic signal of these characters, the paucity of comparable characters and character states in subcellular studies and the challenges in establishing a comprehensive structural and biochemical database of the Fungi.

Key words: cytology, informatics, morphology, phylogeny, ultrastructure

INTRODUCTION

Structural data have played a major role in reconstructing the evolutionary history of the Fungi. Macromorphological data were supplemented with light microscopic characters more than 100 y ago to provide the outline of 20th century classifications. Microscopic characters were refined further beginning in the 1960s with the greater resolving power of the electron microscope, and new structures and relationships were revealed (Bracker 1967). With the advent of analyses of molecular sequence data in the 1990s many unexpected or unexplained taxonomic relationships were uncovered, e.g. the phylogenetic separation between Blastocladiales and Chytridiales (James et al 2000, this issue), unexpected phylogenetic diversity of Zygomycota (Benny et al 2001, Cavalier-Smith 2001, Lutzoni et al 2004, White et al this issue), the surprising basal position of *Neolecta* within the Ascomycota (Landvik 1996, Kurtzman and Sugiyama 2001) and the separation of the loculoascomycetous taxa within the filamentous ascomycetes (Lutzoni et al 2004), mimicry among smut taxa which resulted in their classification in two subphyla (Bruns et al 1992, Swann and Taylor 1995, Bauer et al 1997, Swann et al 2001, Aime et al this issue, Begerow et al this issue) and the taxonomic placement of gasteromycetous taxa among the reorganized Agaricomycetes (Hibbett et al 1997, Hibbett and Thorn 2001, Binder and Hibbett this issue, Hosaka et al this issue). These newly discovered relationships require the reevaluation of structural characters at all taxon levels to recognize homologies.

Subcellular or ultrastructural characters are unevenly studied. Reports on subcellular characters are widely scattered in the literature and not easy to retrieve and compare. In addition the extent of their prior analysis is difficult to assess. Such characters provide additional challenges in that the quality of the image depends on the method of cell preservation and familiarity of the user with the results of the methods used in evaluation. While molecular sequence databases are available for Fungi (e.g. GenBank, WASABI, UNITE, EMBL and COGEME) structural databases are not available at a time when the volume and complexity of the data exceed the ability of an individual to comprehend it. To assess the new homologies revealed by molecular data, structural databases for the Fungi are a necessity.

This paper provides an introduction to the Structural and Biochemical Database for the Fungi being compiled by the Assembling the Fungal Tree of Life (AFTOL) project and uses the characters associated with the septal pore apparatus, nuclear division and the spindle pole body (SPB) to illustrate how structural characters support current molecular phylogenetic analyses of the Fungi.

Accepted for publication 8 November 2006.
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MATERIALS AND METHODS

Database development.—During preliminary development of the database, data were entered into a Microsoft Excel® spreadsheet (Microsoft Corp., Redmond, Washington). Initially to populate the online database, the Excel® file was converted to an Oracle® database file (Oracle Corp., Redwood Shores, California) via Perl scripts written and maintained by the Java and Web Services group, University of Minnesota. Subsequent data updates were made with a custom Web application constructed with additional Perl scripts.

Mycological publications were reviewed to determine the types of subcellular and biochemical characters to include in the database. Taxa were chosen for initial inclusion in the database based on the completeness of the studies and the quality of cell preservation. Newly defined characters and characters previously shown to be phylogenetically informative are included in the database. The traits chosen for the database are the septum and septal pore organization, nuclear division, spindle pole body form and cycles, meiospore and meiosporangium differentiation, selected cytoplasmic features (e.g. Golgi apparatus, Spitzenkörper, microscala, colacosomes), motile cell structure, specialized cell structures (e.g. cystidia, paraphyses), haustorium-host interface, and selected biochemical characters (e.g. metabolic pathways and cell wall composition). Diagrams used to illustrate these characters and character states were made either by a professional artist or by using Adobe® Illustrator® CS2 (Adobe Systems Inc., San Jose, California).

The fixation quality in electron micrographs from source references was evaluated and recorded in a separate data field. For objective analysis of specimen fixation we noted the protocol employed in each study. We then assessed the quality of the micrographs based on what could be expected in terms of fixation artifacts from each method (Hayat 1970, Hoch 1986). We checked the appearance of certain cell components, continuity and smoothness of cell membranes, cytoplasmic rearrangement compared to living cells and minimal extraction of cellular material (Flegler et al 1993). Mitochondria also were examined for inner and outer membrane integrity and lack of swelling. Qualitative judgment was made on the completeness of each study, taking into consideration specimen fixation and the thoroughness with which the organism was presented in text and figures. Micrographs are included to illustrate character states of a taxon with permission of publisher(s) and/or author(s).

Taxonomic classifications are based on Eriksson (2005) (Ascomycota), Hibbett and Thorn (2001) (Basidiomycota), James et al (2000) (Chytridiomycota) and Benny et al (2001) (Zygomycota), and these have been adapted to reflect the classification being developed by the AFTOL project in collaboration with other mycologists (Blackwell et al this issue, <http://www.clarku.edu/faculty/dhibbett/AFTOL/AFTOL.htm>). Taxa of uncertain placement above the genus level also are included. If a genus and/or specific epithet have changed, both the name from the source reference and the current name are provided.

The database can be searched with three criteria, partial or full genus and/or species names, rank and character state. From the search results, coded character states from selected taxa can be compiled automatically and displayed in NEXUS format (Maddison et al 1997) for easy implementation in commonly used phylogenetic software.

Septum characters are divided into five developmental stages: hypha excluding ascogenous hypha/ascus, multiperforate septum, basidium, immature ascogenous hypha/ascus and mature ascogenous hypha/ascus (SUPPLEMENTARY TABLE I). The type and nuclear condition of the hypha or specialized cell from which septum character data were described are noted for each entry, as is meiosis or mitosis for nuclear division and spindle pole body character data.

Character homology.—The most critical issue in interpreting the evolutionary significance and phylogenetic application of organismal traits is determining homology of characters and their states. We have used comparative methods to determine initial character and character state homologies for structural characters across kingdom Fungi. The presence of the same or similar structural features at a developmental stage in closely related taxa provides support for homology of a character or character state (e.g. the many characters associated with the septal pore at specific stages in development). We also have relied on character assessments in the literature by mycologists familiar with specific taxonomic groups. Most SPB and nuclear division characters are relatively easy to evaluate because these are usually common to all fungi, but specialized structures, such as those in the cytoplasm around the septal pore or their variations at different stages in development, may or may not be homologous, especially in distantly related taxa.

Molecular phylogenetic analyses can be used to refine character assessment; e.g. once it was clear that smut fungi consisted of two independently derived groups, the subtle differences in organization of the SPBs in each group could be recognized as distinct character states (McLaughlin et al 1995). In a number of cases (e.g. multiperforate septum) we have presumed that characters are homologous until further evidence is available. These characters must be used with caution and need to be reassessed when additional data become available.

Character mapping.—A data matrix of 241 taxa and 47 subcellular characters with 190 character states (SUPPLEMENTARY APPENDIX 1) was compiled from the AFTOL Structural and Biochemical Database and loaded into MacClade v4.08 (Maddison and Maddison 2005). The database contains eight taxa of unknown taxonomic placement. These taxa were omitted from the data matrix. A summary tree representing relationships between phyla, classes and subclasses of the Fungi based on recently published and unpublished data (Lutzoni et al 2004, Matheny et al in press, James et al in press, Spatafora et al this issue) was constructed by organizing the taxa in the data matrix according to these classifications. Characters were plotted on the summary cladogram with ''trace all changes'' with the ''approximate maximum number of changes'' option. Polytomies were treated as simultaneous multiple speciation

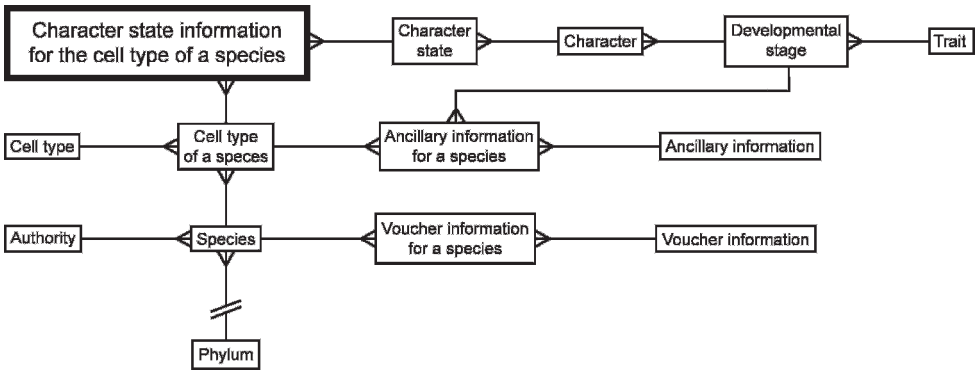

FIG. 1. A simplified Logical Data Structure diagram for the Structural and Biochemical Database. Items in rectangles represent data tables. ''Crowsfoot'' lines connecting rectangles indicate the direction of a one-to-many relationship between tables.

events (''hard''). Polymorphic character state assignments for branches subtending clades were ignored.

RESULTS AND DISCUSSION

Database development.—The database can be accessed at http://aftol.umn.edu. A simplified diagram in Logical Data Structure format (FIG. 1) shows the relationships between data groups. The focus of the database is the table linking species, cell type and character state information. The ancillary information table contains data about bibliographic information and information on fixation method and quality. The voucher information table includes the herbarium or culture collection where the voucher specimen or culture was deposited, and collector data when available. The database contains information from 163 published source references with the journals *Mycologia* and *Canadian Journal of Botany* yielding the highest number of hits at 36 each.

Some source references used different terms for describing similar characters (e.g. parenthesome versus septal pore cap or nucleus associated organelle versus spindle pole body). A single set of terms was employed and a glossary of definitions was constructed, including general diagrams of characters and character states.

There are three levels of database access: general users who may only view data, contributors who may submit new data or changes to the data and administrators who approve content updates and maintain the database. Species entries contain a record of the person who submitted the data and the entry date. Administrators review changes to the database before edits are made.

Many factors affected the quality of the data entered into the database. Source references may have one or two informative figures that provide character state data for only a few characters. Also some studies use figures displaying fair to poor fixation quality and containing features now known to be artifacts. These organisms should be reinvestigated with multiple or current fixation protocols. Another problem is the difficulty in retrieving specimens for additional study. Organisms that were collected and studied but not deposited into a culture collection or herbarium are difficult to obtain for reexamination of cell structure or DNA sequencing or for identity verification.

Micrographs accompany taxon entries whenever possible, which lets a user inspect the data on which entries are based. This also helps the user to understand better a contributor's interpretation of subcellular features. To maximize the availability of micrographs for the database, authors are strongly encouraged to publish in journals that grant permission to display copyrighted figures at no cost to the scientific community. As more journals become available online, entries may include direct links to their source references and figures. Because of the three-dimensional nature of many characters in the database, serial sections are important for understanding the shape and placement of cellular structures and organelles. Although only a limited number of figures may be included in a publication, supplemental micrographs should be included in the Structural and Biochemical Database to provide a complete record of the characters. Images submitted to the Website should be in JPEG format with 72 pixels per inch (29 pixels per cm) and should be no larger than 400 pixels in either height or width. Details about current submission requirements are provided at the site.

The ability to combine character state data from multiple entries and display them in NEXUS format encourages data analysis independent of, or combined with, molecular data. However the NEXUS-formatted text may require editing before analysis.

For example multiple entries for a character can occur in a species, depending upon whether data were acquired from more than one structure, cell type or developmental stage as noted above. The character states may be consolidated to produce a single species entry in the data file. In rare cases species may exhibit multiple character states for the same cell type.

Although the Structural and Biochemical Database was designed for public use, to become a valuable resource it requires mycological community involvement in its development. Undertaking new ultrastructural studies is time-consuming, and more contributors are needed to increase the rate of data entry and acquisition. We anticipate that periodic meetings of the mycological community will be required to refine character definitions and character state descriptions. While data that do not conform to existing characters and states still may be included in the ''Notes'' field for each taxon, such refinement allows for more accurate representation of the organisms.

With the involvement of a large number of contributors, the quality of the data must be ensured to the best of the administrators' abilities. Specific mycological expertise is required to interpret image data accurately and translate them into information that can be analyzed. Criteria for contributors may include scientific community membership, relevant publications in peer-reviewed journals and/or recommendations from colleagues. In addition the identities of contributors who submit new data or edit existing data are recorded with the entries. Contributors also must complete justification statements for changes to the data. These features emphasize the responsibility of contributors and provide accountability to users.

Character homology.—Comparison of characters across phyla presents challenges in homology assessment. Some characters may have a basic structure (e.g. SPB form), which unites a broad group of taxa, and a more detailed form limited to more closely related taxa. Both general and detailed character states are needed to encompass the structural detail for phylogenetic analyses, and these states are treated in separate characters.

Ontogenetic similarity also was used in the determination of character homologies. When there was a clear difference between character states present at two or more developmental stages, we defined subgroups of similar characters according to the developmental stages in which these states are present. For example the septal pore occlusion in the ascus of *Sordaria humana* (FIG. 2G–H) is an elaborate membrane system when immature but develops into a simple membranous cap at maturity (Beckett 1981). Thus we partitioned septal pore occlusion characters in ascogenous hypha or ascus into two subgroups, immature and mature. This was critical to avoid treating potentially nonhomologous but similar looking character states at different developmental stages as homologous.

Some characters present problems in homology assessment. It is unclear whether Woronin bodies (Ascomycota) and microbodies (Basidiomycota) at the septal pore (character 4) are homologous; cytochemical evidence is needed to prove homology (Jedd and Chua 2000, Lutzoni et al 2004). Many types of septal pore occlusions occur in Fungi (character 7) and they have been treated as homologous. Most of these character states are limited to specific taxonomic groups but some states occur in more than one phylum. Multiperforate septa (character 17) may be independently derived in most or all cases but are treated as homologous for now. It is uncertain if the septal pore occlusions in ascogenous hypha and ascus (characters 19, 21, 24, 26) in different classes are homologous; however they are treated as such for the present. The intranuclear element (character 47) in the Basidiomycota may not be homologous with that in the Mucoromycotina (Mucorales). In the former it contains actin (Hoch and Staples 1983) while in the latter it acts as a microtubule-organizing center.

Character mapping.—The data matrix for character mapping (SUPPLEMENTARY TABLE I) contains 42 parsimony-informative, three parsimony-uninformative and two invariant characters. Character states for septa and for SPB and nuclear division characters are illustrated (FIGS. 2–3). The data matrix contains all of the nuclear division studies with usable information and septal data for all of these taxa if it was available. Only 27 taxa have data for both septal and SPB/nuclear division characters states: 20 Basidiomycota, 4 Ascomycota, 1 Blastocladiomycotina (James et al this issue), 1 Chytridiomycota, and *Basidiobolus*. Of those taxa with vegetative septal data 97% have data for 50% or more of the characters, while 55% of taxa with data for SPB and nuclear division characters have 50% or more of the characters.

Relatively few septal, SPB, or nuclear division characters support the clades that link the phyla and subphyla of Fungi (FIG. 4). This partly reflects the incompleteness of the studies available and the plesiomorphic nature of some characters. The basal clade (A) linking the Blastocladiomycotina with higher taxa is supported by a change to an intact metaphase nuclear envelope from one with polar fenestrae in the Chytridiomycotina and the loss of perinuclear endoplasmic reticulum. The timing of spindle pole body migration before nuclear division

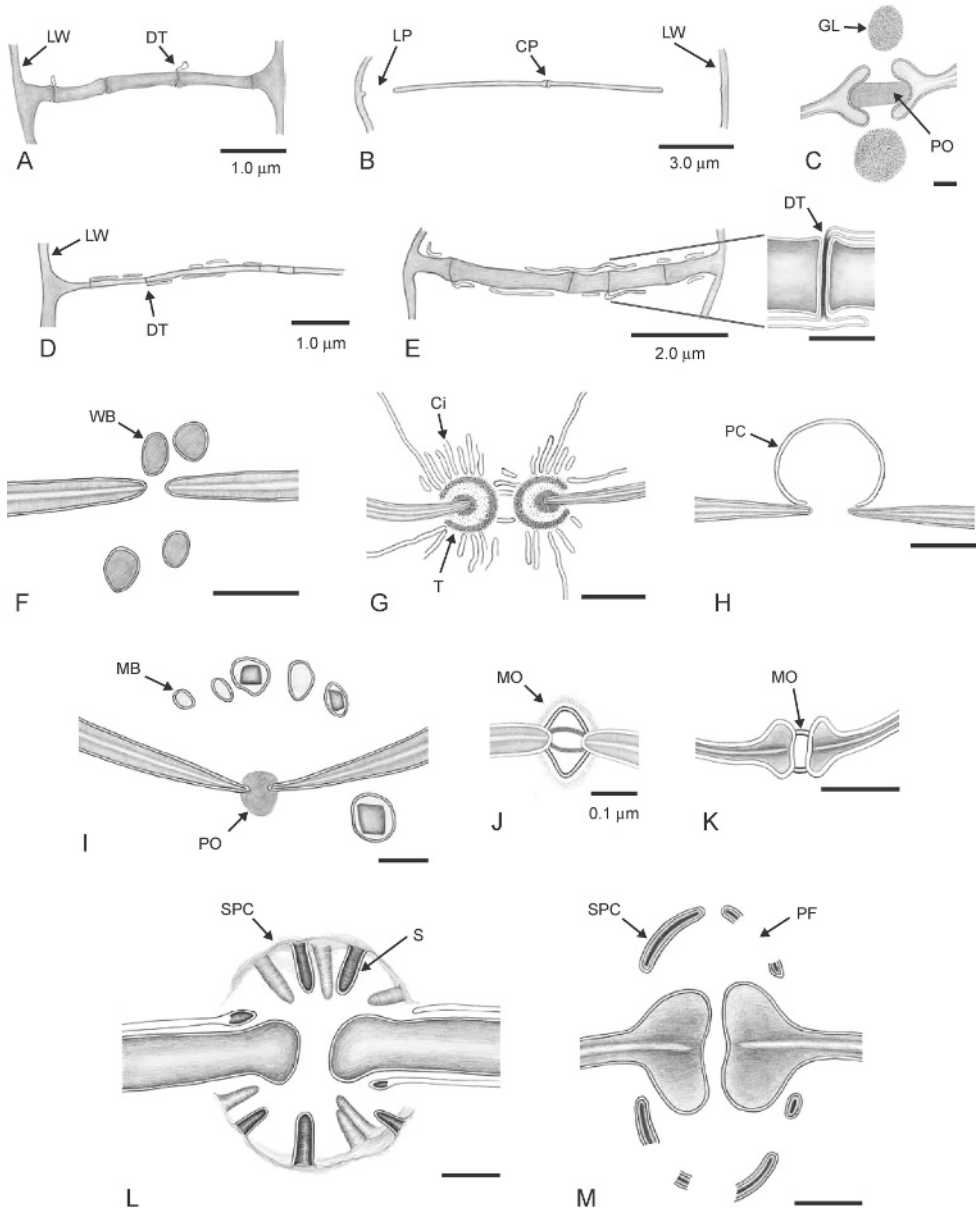

FIG. 2. Variations in septa and septal pore organization in vegetative hyphae (A–C, E–F, I–M), immature and mature ascus (G–H) and a gametangial septum (D). Uniperforate septa except A–B, D–E, multiperforate septa without (B) and with plasmodesmata and desmotubules (DT). A. Chytridiomycotina (Powell 1974). B. Lateral (LP) and central (CP) pore; Blastocladiomycotina (Meyer and Fuller 1985). C. Uniperforate septum with lenticular cavity, nonmembrane-bound pore occlusion (PO), and associating nonmembrane-bound globules (GL); Harpellomycotina (Jeffries and Young 1979). D. Mucoromycotina (Hawker et al 1966). E. Saccharomycotina (Kreger-van Rij and Veenhuis 1972). F. Uniperforate septum with Woronin bodies (WB); Pezizomycotina (Momany et al 2002). G–H. Uniperforate septum with torus (T) and radiating tubular cisternae (Ci) (G) or membranous subspherical pore cap (PC) (H); Pezizomycotina (Beckett 1981). I. Simple septum with nonmembranous pore occlusion with associated microbodies (MB); Pucciniomycotina (*Helicobasidium compactum*, D.J. McLaughlin unpublished). J–K. Simple septum with membranous pore occlusions (MO); Ustilaginomycotina (Bauer et al 1995, 1997). L. Septal pore swelling with elaborated septal pore cap with saccules (S); Tremellomycetes (Berbee and Wells 1988). M. Septal pore swelling with simple septal pore cap (SPC) with perforations (PF); Agaricomycetes (Müller et al 1998). LW, lateral wall of hypha. Bars = 0.25 µm except where indicated.

(character 37) is too seldom studied to support this clade conclusively. Clade B linking the Entomophthoromycotina, *Basidiobolus* and Harpellomycotina with higher taxa is supported by the loss of multiperforate septa and centrioles. Clade C linking the Mucoromycotina and Dikarya is supported by SPB migration during spindle formation, presence of a central spindle and, possibly, the absence of

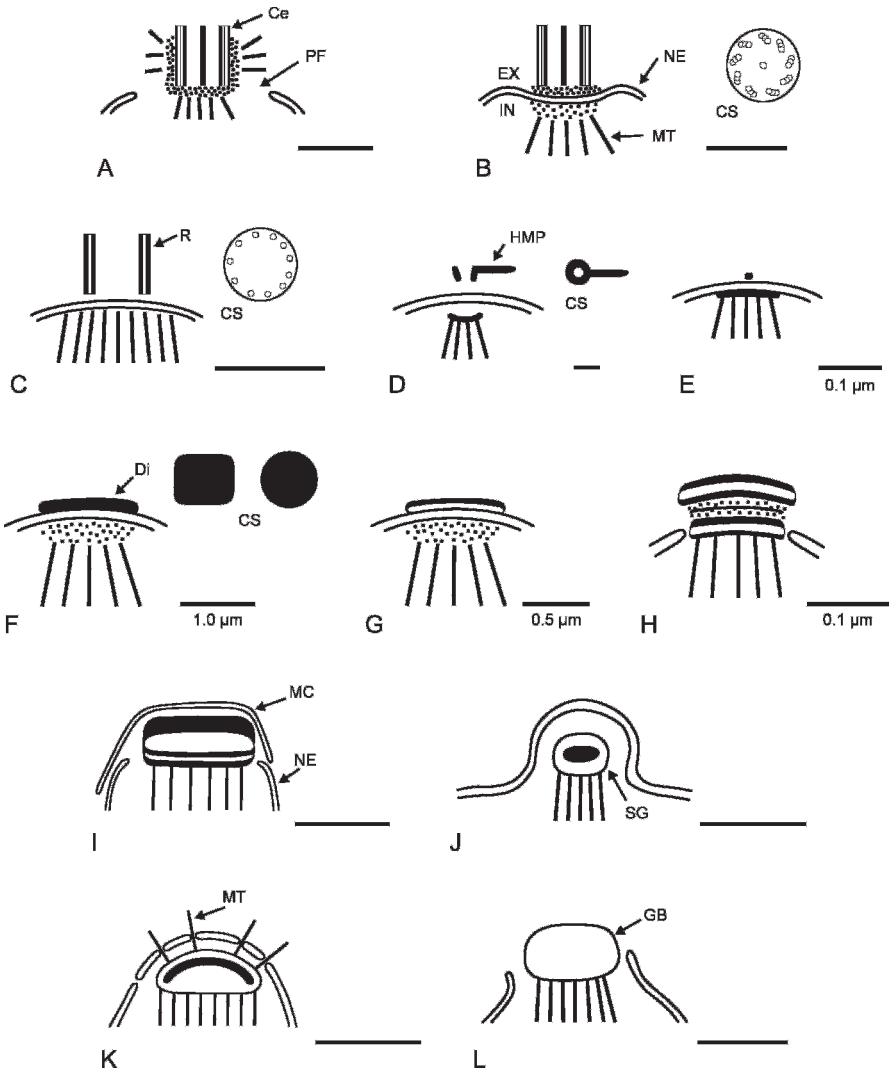

FIG. 3. Overview of spindle pole body forms at metaphase-anaphase and their relationship to the nuclear envelope. A. Centriole-associated material in loose polar fenestra (PF); Chytridiomycota (Powell 1980). B. Centriole-associated extra- and intranuclear components with intact nuclear envelope (NE); Blastocladiomycotina (Ichida and Fuller 1968). C. Ring (R) containing microtubules but lacking centriolar ninefold symmetry; *Basidiobolus* (McKerracher and Heath 1985). D. ''Bottomless saucepan'' with persistent half middle piece (HMP) plus an intranuclear component; Entomophthoromycotina (Butt and Beckett 1984). E. Small amounts of extra- and intranuclear material with intact nuclear envelope; Mucoromycotina (McCully and Robinow 1973). F–H, Ascomycota. F. Quadrilateral plaque or unlayered disk (Di) with intact nuclear envelope; Pezizomycotina (Zickler 1970). G. Two-layered disk with intact nuclear envelope; Pezizomycotina (Schrantz 1970). H. Layered disk in small polar fenestra in nuclear envelope; Saccharomycotina (Moens and Rapport 1971). I–L, Basidiomycota. I. Layered disk in small polar fenestra with membranous cap (MC); Pucciniomycotina (Bourett and McLaughlin 1986). J. Subgloboid (SG) with flat internalized layer and intact nuclear envelope; Pucciniomycotina (McCully and Robinow 1972). K. Subgloboid with concave internalized layer and fragmented nuclear envelope; Ustilaginomycotina (O'Donnell and McLaughlin 1984). L. Globoid (GB) in polar fenestra in nuclear envelope; Agaricomycotina (*Auriscalpium vulgare*; D.J. McLaughlin unpublished). Ce, centriole; CS, cross-section; EX, extranuclear area; IN, intranuclear area; MT, microtubules. Bars = 0.25 μm except where indicated.

a metaphase plate, but the latter character needs confirmation from additional taxa in the Mucoromycotina. Dikarya is supported by the uniperforate septal pore, a plaque or disk-shaped SPB and possession of an intact metaphase nuclear envelope with SPBs plugging the polar fenestrae. The intranuclear element (character 47) is a differentiated region that forms within the nucleus at late interphase opposite the external SPB in many taxa of Basidiomycota and Ascomycota. Whether the internal component of the SPB of the Mucoromycotina is homologous is unclear. The Basidiomycota may be

supported by two characters, septal pore with unelaborated margin and the presence of spindle vesicles; the spindle vesicle character is insufficiently studied to provide support for this phylum. The Ascomycota is supported only by a disk-shaped SPB.

Support from septal, SPB, or nuclear division characters at the level of subphyla is stronger for some than for others (FIG. 4). The Chytridiomycotina is not resolved with molecular data, but the Chytridiomycetes and Monoblepharomycetes share similar SPB-nuclear envelope organization (FIG. 3A). The Blastocladiomycotina is distinguished by its distinctive type of multiperforate septum with lateral pores, and by the intact nuclear envelope and distinctive SPB during nuclear division (FIGS. 2B, 3B). The distinctive uniperforate septum with lenticular cavity containing a thickened plate characterizes the Harpellomycotina (FIG. 2C). The Entomophthoromycotina has no defining characters in the summary cladogram but this reflects the scarcity of nuclear division-SPB studies (FIG. 3D). The septal structure for a species of Entomophthoromycotina indicates a close affinity to the Harpellomycotina but the absence of data for multiple taxa results in this synapomorphy not being supported by the approximate methods used to trace characters on the cladogram. *Basidiobolus* possesses a unique SPB strongly suggestive of a reduced centriole (FIG. 3C). Several synapomorphic characters support the Mucoromycotina, especially the multiperforate septum with plasmodesmata and the distinctive SPB with small amounts of extranuclear and intranuclear material (FIGS. 2D, 3E).

Among the Basidiomycota the Pucciniomycotina is supported by the uniperforate septal pore, which may have a distinctive cytoplasmic organization and may be associated with microbodies (FIGS. 2I, 4). If the microbodies can be demonstrated to be homologous with Woronin bodies, the organization at the pore would be plesiomorphic for this subphylum and for the Ascomycota. The disk or subgloboid SPB (FIG. 3I–

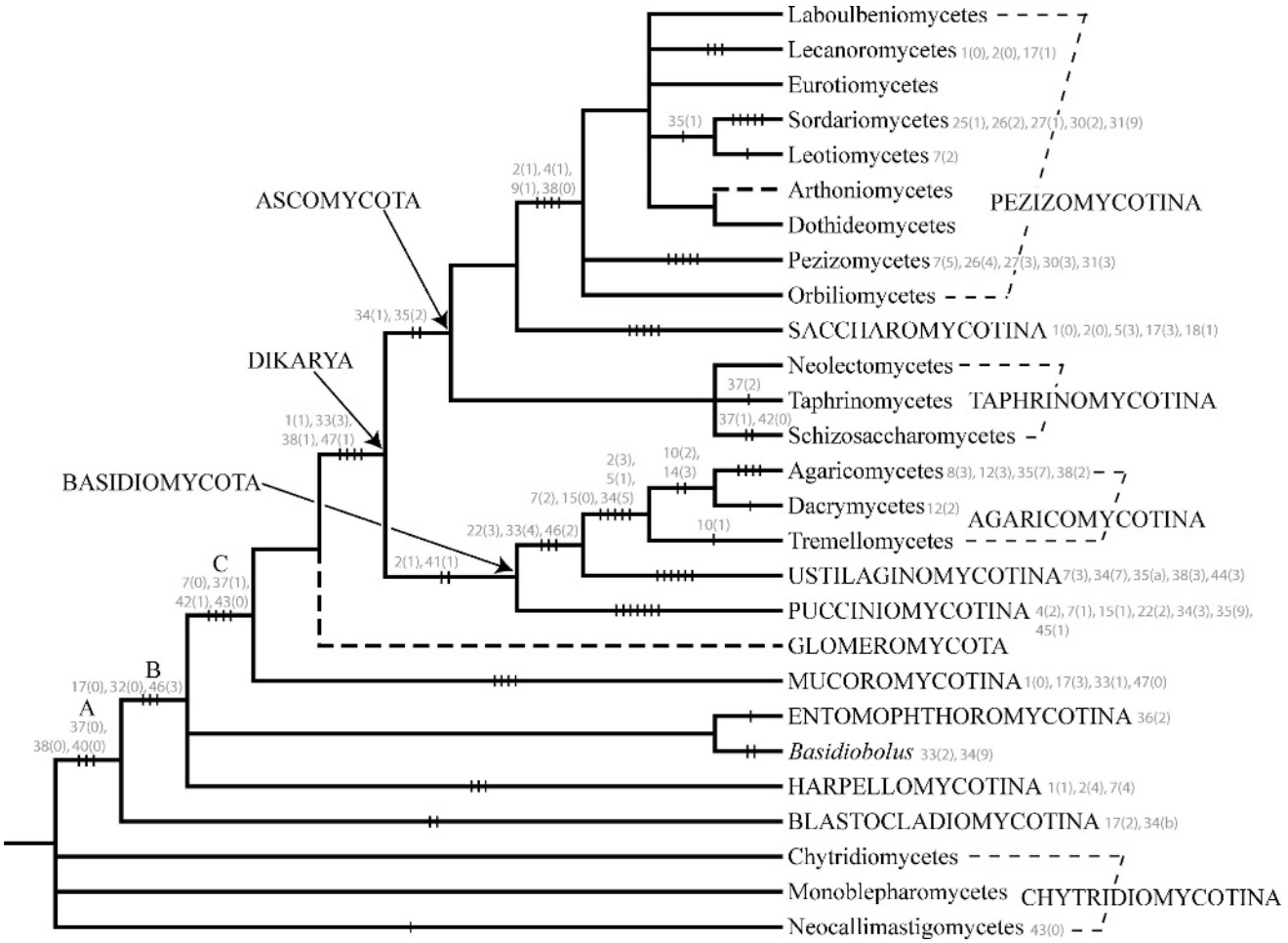

FIG. 4. Summary cladogam of known relationships of the Fungi with changes in character state of selected characters from the Structural and Biochemical Database. Character, number not delimited by ( ); character state, number in ( ). (For character and state descriptions, see SUPPLEMENTARY TABLE I. Dashed branches were omitted from the analysis.

J) and condensed interphase chromatin support the Pucciniomycotina. The Ustilaginomycotina and Agaricomycotina share a globular SPB but the fate of the nucleolus during nuclear division (character 46) is too little studied to draw conclusions. The Ustilaginomycotina is supported by a septal pore closed by distinctive membranous plates and by a subgloboid SPB with a curved internal layer in the limited number of taxa studied (FIGS. 2J–K, 3K). The Agaricomycotina is strongly supported by septal pore characters and by the globoid SPB (FIGS. 2L–M, 3L).

The Taphrinomycotina lacks supporting characters, while the other subphyla of the Ascomycota seem to have more support (FIG. 4). The Saccharomycotina appears to be characterized by the absence of septal pores or the presence of a multiperforate septum with desmotubules (FIG. 2E), but the uniperforate septum also has been reported in *Neolecta vitellina* (Landvik et al 2003). SPBs of the Saccharomycotina are a unifying character at the phylum level (FIG. 3H). The Pezizomycotina is well supported by a uniperforate or multiperforate septum associated with Woronin bodies and by the intact metaphase nuclear envelope (FIGS. 2F, 3F–G).

At the class level in the Basidiomycota the Tremellomycetes is supported by the elaborated septal pore cap (FIG. 2L). The phylogenetic signal can be confused by the possession of two conflicting character states in *Trichosporon sporotrichoides*, which usually lacks septal pore caps but does form them at times (Müller et al 1998). The clade joining the Dacrymycetes and the Agaricomycetes is strongly supported by the substructure of the septal pore cap (FIG. 2M), and pore cap structure also supports each of these classes. The Agaricomycetes is strongly supported by the considerable expansion of the globular SPB during nuclear division and in many cases by the loose polar fenestrae surrounding the SPB (FIG. 3L).

Among the classes of the Ascomycota many lack support because of the paucity of subcellular studies. The Pezizomycetes is supported by the distinctive pore occlusions in vegetative hyphae and in the immature and mature ascogenous hypha/ascus. The clade linking the Leotiomycetes and the Sordariomycetes appears to be supported by SPB form and nuclear envelope organization during nuclear division. The Leotiomycetes receives some support from the vegetative pore occlusion, while the Sordariomycetes are strongly supported by the ascogenous hypha/ascus pore occlusions in immature and mature hyphae (FIG. 2G–H). The Lecanoromycetes receives strong support from the multiperforate septum.

Structural data are important for understanding the evolutionary history of the Fungi. The general shortage of structural studies and their uneven distribution among taxonomic groups make it difficult to draw firm conclusions for many characters on the summary cladogram. The current database, nevertheless, is useful in that it provides guidance on character assessment and on obtaining complete datasets for future studies. It also provides a central comprehensive repository for structural and biochemical data. Constructing the database and using the data revealed problems with the assessment of homology, such as the relationship between vegetative and reproductive septa. Determining the evolutionary significance of structural and biochemical characters depends entirely on the assessment of homology between two or more developmental or organismal traits. These assessments are the most critical and fundamental questions in the evolutionary history of the Fungi, yet remain hampered by lack of sampling. In the future molecular phylogenetic studies will be able to identify and analyze the genes that code for these structures and provide a more in-depth understanding of character evolution. We hope that the challenges that we have highlighted will aid discussion of character coding and homology assessment.

ACKNOWLEDGMENTS

This research was financed by the Assembling the Fungal Tree of Life project, NSF grant EF-0228671 to D.J. McLaughlin and the NSF Research Coordination Network: a phylogeny for kingdom Fungi (NSF 0090301) to M. Blackwell, J.W. Spatafora and J.W. Taylor. The authors thank J. Coyne and D. Johnson for their programming expertise, K.A.T. Silverstein for his assistance with the database model, and K. Kirkeby and A. Gale for their drawings. We also want to thank the many participants in the AFTOL project and the Deep Hypha Research Coordination Network who contributed their time and ideas to developing the Structural and Biochemical Database.

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SUPPLEMENTARY TABLE I. Characters and states from the Structural and Biochemical Database

Septum/Pore Cap – Hypha excluding ascogenous hypha/ascus

1. Uniperforate septum: 0 = absent, walled off pore, or apparently walled off pore; 1 = a single central pore.
2. Uniperforate septal pore margin: 0 = uniperforate septal pore absent; 1 = unelaborated margin; 2 = uniperforate septal pore absent but with some type of discontinuity within the septum, e.g., disruption of central layer of cross wall, wall swelling, and/or deposits within cross wall, suggestive of a blocked or disrupted pore; 3 = septal pore swelling; 4 = with lenticular cavity (bifurcate).
3. Protruding non-membrane-bound bodies associated with septal pore(s): 0 = absent; 1 = present.
4. Membrane-bound bodies associated with septal pore(s): 0 = absent; 1 = Woronin bodies; 2 = microbodies.
5. Membranous structures associated with septal pore(s): 0 = absent; 1 = septal pore cap; 2 = endoplasmic reticulum not associated with plasmodesmata; 3 = endoplasmic reticulum associated with plasmodesmata.
6. Non-membrane-bound, electron-dense bodies associated with septal pore(s): 0 = absent; 1 = present.
7. Septal pore occlusion: 0 = absent; 1 = pulley wheel-shaped occlusion; 2 = non-membranous [thin non-membranous plate(s) and/or occluding material]; 3 = membranous plates continuous with plasma membrane; 4 = thickened non-membranous plate (flattened disc, biumbonate, or irregular); 5 = granular lamellate structure; 6 = subspherical occlusion, non-membrane-bound, with translucent finger-like extensions; 7 = subspherical occlusion, non-membrane-bound, lacking translucent finger-like extensions; 8 = non-membranous occluding material.
8. Organelle trafficking: 0 = organelle trafficking absent; 1 = mitochondria; 2 = nuclei; 3 = ribosomes/small particles or organelles.
9. Woronin body type: 0 = Woronin bodies absent; 1 = globose; 2 = hexagonal; 3 = rectangular/cylindrical.
10. Septal pore cap basic structure: 0 = pore cap absent; 1 = elaborated cap with abseptal or adseptal extensions (cupulate, reticulate, or tubular extensions); 2 = simple cap (cap with unelaborated abseptal surface).
11. Detailed structure of elaborated septal pore cap: 0 = elaborated cap absent; 1 = abseptal extensions (smooth vesicular-tubular membranous structures); 2 = multiple saccules; 3 = cap reticulate.
12. Detailed structure of simple septal pore cap: 0 = simple cap absent; 1 = cap imperforate, flat; 2 = cap imperforate or uniperforate, curved; 3 = cap multiperforate, small pores; 4 = cap multiperforate, large pores.
13. Saccules of elaborated pore cap lined with external electron-dense layer: 0 = absent; 1 = present.
14. Substructure of pore cap: 0 = absent; 1 = cap with uniform electron-transparent contents; 2 = cap with uniform electron-dense contents; 3 = cap with three internal layers (central electron-dense layer between two less electron-dense layers.
15. Zone of organelle exclusion at pore: 0 = absent; 1 = present.
16. Pore cap enclosed by endoplasmic reticulum: 0 = absent; 1 = present.

Multiperforate septum

17. Multiperforate septum: 0 = absent; 1 = uniformly distributed simple pores; 2 = simple pores with variable-sized, large pores adjacent to hyphal wall; 3 = plasmodesmata; 4 = thickened septum with central pore closed by plasmodesmata.
18. Desmotubules in plasmodesmata: 0 = absent; 1 = present.

Basidium/Basidiomycete sporocarp

19. Zone of exclusion outside septal pore cap (abseptal) in basidiomycete sporocarp: 0 = absent; 1 = present.
20. Septal pore cap in basidiomycete sporocarp enclosed by endoplasmic reticulum: 0 = absent; 1= present.
21. Zone of exclusion in simple septum bordered by microbodies in basidiomycete sporocarp or equivalent tissue: 0 = absent; 1 = present.
22. Primary septum within basidium: 0 = absent; 1 = without pore; 2 = walled off pore, or apparently walled off pore; 3 = septal pore swelling; 4 = incomplete septum/septa in basidial apex.
23. Septal pore cap at primary septum within basidium: 0 = absent; 1 = present.

Ascogenous hypha/ascus – immature

24. Immature septal pore in ascogenous hypha/ascus: 0 = absent; 1 = simple with single central pore.
25. Immature septal pore associated structures in ascogenous hypha/ascus: 0 = absent; 1 = endoplasmic reticulum associated with toroid occlusion.
26. Immature septal pore occlusion in ascogenous hypha/ascus: 0 = absent; 1 = non-membranous [thin non-membranous plate(s) and/or occluding material]; 2 = toroid occlusion, i.e., donut-like with central pore; 3 = toroid occlusion containing pulley-wheel-shaped occlusion; 4 = occlusion a convex or biconvex band or more complex hemisphere.
27. Immature pore occlusion detailed structure in ascogenous hypha/ascus: 0 = absent; 1 = torus with radiating tubular cisternae; 2 = torus lacking tubular cisternae; 3 = translucent lamellate torus and granular matrix; 4 = hemispherical usually with narrow, electron-opaque inner and broad, electron-translucent outer bands; 5 = hemispherical with radiating tubular elements; 6 = cone to dumbbell-shaped with V-shaped striations and usually an electron-translucent torus; 7 = double-translucently banded torus in granular matrix becoming hemispherical with dense inner zone and less opaque outer zone; 8 = electron opaque, convex or biconvex bands; 9 = electron-opaque, hemispherical with short radiating tubular elements.

Ascogenous hypha/ascus – mature

28. Mature septal pore in ascogenous hypha/ascus: 0 = absent; 1 = simple with single central pore.
29. Mature septal pore associated structures in ascogenous hypha/ascus: 0 = absent; 1 = endoplasmic reticulum associated with toroid occlusion; 2 = endoplasmic reticulum associated with pore cap membrane.
30. Mature septal pore occlusion in ascogenous hypha/ascus: 0 = absent; 1 = toroid occlusion, i.e., donut-like with central pore; 2 = subspherical pore cap membrane; 3 = occlusion a convex or biconvex band or more complex hemisphere.
31. Mature pore occlusion in ascogenous hypha/ascus, detailed structure: 0 = absent; 1 = torus lacking tubular cisternae; 2 = translucent lamellate torus and granular matrix; 3 = hemispherical usually with narrow, electron-opaque inner and broad, electron-translucent outer bands; 4 = hemispherical with radiating tubular elements; 5 = cone to dumbbell-shaped with V-shaped striations and usually an electron-translucent torus; 6 = double translucently banded torus in granular matrix becoming hemispherical with dense inner zone and less opaque outer zone; 7 = electron opaque, convex or biconvex bands; 8 = electron-opaque, hemispherical with short radiating tubular elements; 9 = simple membrane enclosing cytoplasm; a = torus with radiating tubular cisternae.

Nuclear Division/Spindle Pole Body

32. Centriole: 0 = absent; 1 = present.
33. Basic organization of the spindle pole: 0 = centriolar associated material; 1 = spindle pole body consists of small amounts of extranuclear material; 2 = ring-like spindle pole body; 3 = spindle pole body a plaque or disc; 4 = spindle pole body globular.
34. Spindle pole body form, interphase-prophase: 0 = quadrilateral plaque; 1 = unlayered disc; 2 = a 2-layered disc; 3 = a 3-layered disc; 4 = a 7- to 9-layered disc (inner and outer plaques plus intermediate zone); 5 = a globoid; 6 = a subgloboid with flat internalized layer; 7 = a subgloboid with internalized layer convex with respect to the spindle; 8 = notched ring with middle piece and intranuclear component; 9 = ring containing microtubules but lacking centriolar 9-fold symmetry; a = slight amount of extranuclear and intranuclear material on either side of nuclear envelope; b = centriolar-associated extranuclear and intranuclear components with intact nuclear envelope; c = centriole-associated material.
35. Spindle pole body form, metaphase-anaphase: 0 = quadrilateral plaque with intact nuclear envelope and internal microtubule organizing center; 1 = unlayered disc with intact nuclear envelope and internal microtubule organizing center; 2 = a 2-layered disc with intact nuclear envelope and internal microtubule organizing center; 3 = a 4-layered disc directly connected to spindle; 4 = a 5-layered disc directly connected to spindle; 5 = a 7- to 9-layered disc directly connected to spindle; 6 = an ellipsoid that expands by more than 100% of its interphase-prophase size directly connected to spindle; 7 = a globoid that expands by more than 100% of its interphase-prophase size directly connected to spindle; 8 = a globoid with limited enlargement directly connected to spindle; 9 = a subgloboid with flat internalized layer directly connected to spindle; a = a subgloboid with internalized layer convex with respect to the spindle and directly connected to spindle; b = notched ring with persistent half middle piece and clear zone between intranuclear component and nuclear envelope; c = ring containing microtubules but lacking centriolar 9-fold symmetry; d = slight amount of extranuclear and intranuclear material on either side of nuclear envelope; e = centriolar-associated extranuclear and intranuclear components with intact nuclear envelope; f = centriole associated material.
36. Spindle development site: 0 = gap in nuclear envelope; 1 = cytoplasm; 2 = intranuclear (nuclear envelope intact).
37. Spindle pole body migration: 0 = migration before spindle formation; 1 = migration during spindle formation; 2 = formation of adjacent fan-shaped arrays of microtubules that reorient during spindle formation; 3 = integration of spindle pole body into invaginated nuclear envelope before spindle formation.
38. Metaphase nuclear envelope: 0 = intact; 1 = intact with small polar fenestrae plugged by the spindle pole bodies; 2 = loose polar fenestrae, including extensions of nuclear envelope into the cytoplasm at the spindle pole, but mainly intact; 3 = partially dispersed; 4 = nearly or entirely dispersed.
39. Telophase nuclear envelope: 0 = retention of complete nuclear envelope around the chromatin with median constriction/fragmentation; 1 = retention of parts of a disrupted nuclear envelope around the chromatin; 2 = new envelope forms within old envelope; 3 = dispersed in interzone; 4 = reappearance of nuclear envelope after metaphase; 5 = retention of the nuclear envelope around the chromatin with constriction/fragmentation near the poles, and interzone cut off from the daughter nuclei.
40. Perinuclear endoplasmic reticulum: 0 = absent; 1 = present.
41. Spindle vesicles: 0 = absent; 1 = present.
42. Central spindle: 0 = absent; 1 = present.
43. Metaphase plate: 0 = absent; 1 = present.
44. Spindle pole body cap: 0 = absent; 1 = continuous with the nuclear envelope; 2 = distinct from nuclear envelope; 3 = fragments of a discontinuous membrane that do not form a true cap.
45. Interphase chromatin condensation: 0 = absent; 1 = present.

SUPPLEMENTARY TABLE I. Continued

46. Nucleolus behavior: 0 = nucleolus dispersed and no longer recognizable during prophase; 1 = nucleolus discarded between prophase and metaphase; 2 = nucleolus or part of it is more persistent and is discarded after metaphase; 3 = nucleolus persistent throughout division.
47. Transient intranuclear element at late interphase-prophase: 0 = absent; 1 = present within nucleus opposite the extranuclear spindle pole body.