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Glucans, Walls, and Morphogenesis: On the Contributions of J. G. H. Wessels to the Golden Decades of Fungal Physiology and Beyond

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Bartnicki-García, S. 1999. Glucans, Walls, and Morphogenesis: On the contributions of J. G. H. Wessels to the golden decades of fungal physiology and beyond. *Fungal Genetics and Biology* 27, 119–127. This is a collection of impressions on the career of J. G. H. Wessels and his work in the areas of cell wall metabolism and apical morphogenesis. It highlights the finding of massive cell wall glucan metabolism during differentiation, the discovery of covalent linkages between wall polymers, the changes in chemical and physical properties of the wall at the fungal apex, and the steady-state model for tip growth. A tandem VSC-SS model for hyphal morphogenesis is proposed that combines the spatial control of wall synthesis provided by the vesicle supply center model with the temporal regulation intrinsic in Wessels's steady-state model.

Index Descriptors: apical wall structure; chitin; α-glucan; β-glucan; hyphal morphogenesis; metabolism; tip-growth models; Wessels.

The forthcoming 7th Fungal Biology Conference, and this accompanying publication, are fitting vehicles to commemorate the official end of the career of Joseph G. H. Wessels, long-time friend, astute scientist, and consummate fungal biologist. Wessels's work on: the molecular biology of fruit body formation in higher fungi and the discovery and characterization of a novel and remarkable type of biological molecules: the hydrophobins is discussed elsewhere in this issue (Wessels, 1999; Wosten *et al.*, 1999). Here, I plan to dwell mainly on the other half of Jos Wessels's career, his work on cell wall structure and function, and his rapid ascent to prominence in fungal physiology.

This is not a detached account of his scientific progress but a collection of impressions I gathered over the years as his contemporary. From the outset, there was great rapport between us, as we realized that we held similar views and aspired to similar goals in the then almost completely virgin field of fungal morphogenesis. On two widely separate occasions, we visited each other's labs, in the hope of getting some collaboration going. Regrettably, our interaction never materialized into a joint project. Nevertheless, we had numerous opportunities to exchange ideas and comments on each other's work at international scientific meetings.

Although our overall values have been quite similar, our outlook on specifics became substantially different over the years, sometimes so significantly that on more than one occasion we have found ourselves looking at each other from opposite sides of a scientific controversy. Yet, I feel we both have done it with respect for the opposing arguments. Divergency of viewpoints is not to be disdained; if handled correctly, it is a powerful catalyst for progress and valuable insurance against complacency.

As I reminisce about all of this, I take pride in the realization that our friendship of more than 3 decades did not interfere with our quest for scientific truth, and we have never ceased to cast a critical eye on each other's work. A good example is in the record of our correspon-

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dence. Wessels had asked me for a copy of the manuscript describing the new mathematical model for fungal morphogenesis I had just presented at a meeting we both attended in Oxford (1988). I could see, during and after the presentation, that he was troubled by the premises and conclusions of our VSC (vesicle supply center) model. His written critical remarks were very perceptive and extremely useful, and they were prefaced by an eloquent statement that is worth repeating, as it distills the essence of this aspect of our relationship: "There are, however, some major points that worry me and I hope that you (will) excuse me for bringing these to your attention. But then, we have so often been disagreeing on scientific matters that you could hardly expect me to harmonize this time." Indeed, such harmony has eluded us, even to this day. I am afraid life is too short to completely dispel our divergent views on certain topics that we both cherish deeply. I am further afraid that should Heaven and Hell provide us with the means of communication, we will probably continue to argue the merits of our scientific interpretations—eternally.

Scientists who do not know Jos Wessels well, particularly the younger ones, may see only the serious facade of a classic European professor. But the Jos Wessels I have known, to this day, is a warm and friendly human being. He has played two roles and has played them well: the serious leader with an elevated academic position and the good-natured colleague imbued with intense curiosity. In both roles, the mission has been the same: the incessant quest for discovering the secrets of the fungi. Total devotion to science is common among scientists but few succeed as handsomely as Jos Wessels did. What I find most admirable and enviable about Wessels, besides the indispensable scientific insight and instincts of an accomplished scientist, are his personal integrity and organizational skills. The former earned him the esteem and respect of colleagues worldwide; the latter allowed him to build a very efficient laboratory with continuously increasing productivity . . . to the very end.

MORPHOGENETIC PIONEERS

Wessels and I are bona fide contemporaries. We started our mycological careers facing similar challenges and, since 1965, when we became aware of each other's thoughts and aspirations, a deep sense of mutual empathy developed despite the geographic distance that separated us. We both felt strongly that cell wall metabolism was central to understanding fungal morphogenesis, an appreciation that was still largely foreign to the rest of the discipline of mycology. This new outlook on fungal biology posed a major risk on two fronts. First, the application of morphogenetic concepts to fungi may seem like a natural course of events today, yet, morphogenesis, long the domain of anatomists and embryologists, needed leaders to promote its application to fungi. Two names stand out. Walter J. Nickerson and Edward C. Cantino, who extended to the fungi concepts and principles previously reserved to much higher forms of life and who sensed an urgent need to explain morphology in biochemical terms. Whereas Cantino (1956, 1968) explored a multitude of metabolic and cytological events of the water mold *Blastocladiella emersonii* and made daring, elaborate attempts to relate them to morphogenesis, Nickerson (1954, 1963) focused on the connection between cell wall structure and morphogenesis, with particular emphasis on the dimorphism of a wide variety of pathogenic and saprophytic fungi. The importance of cell walls in morphogenesis was a relatively new concept and a second reason for apprehension in Wessels newly launched career.[2]

THE GOLDEN ERA OF FUNGAL PHYSIOLOGY

Wessels' rise took place at a very exciting time for the entire discipline of fungal physiology. During roughly two splendid decades, the sixties and seventies (give or take an extra decade depending on the specific subject), a good number of laboratories in different corners of the world sprouted and flourished studying fundamental aspects of the physiology, biochemistry, and ultrastructure of fungi, what we would call today generically fungal biology. This explosion of research was catalyzed by the urge to explain cellular behavior in the biochemical terms and concepts amassed in the previous decades. Radioisotope labeling and various kinds of chromatographic procedures made biochemical experimentation readily accessible to biologists. During this period, the spectacular discoveries made by electron microscopists on the structure and organization of the fungal cell (Bracker, 1967; Heath, 1976), on a seemingly daily basis, played a crucial symbiotic role in the explosive growth of physiological and biochemical investigations on fungi.

In those golden decades, all aspects of fungal physiology

[2] Private correspondence (Feburary 5, 1968).

came under scrutiny and major advances were made on many different fronts. A sizeable number of fungal genera became the subjects of considerable experimental scrutiny (*Achlya, Aspergillus, Blastocladiella, Candida, Coprinus, Mucor, Neurospora, Penicillium, Phycomyces, Phytophthora, Saccharomyces, Saprolegnia, Schizophyllum,* etc.). The following is a partial list of topics that had a direct impact on the field of fungal morphogenesis.

The kinetics of mycelial growth and colony development were formulated with great precision (Trinci, 1969), including the processes of septation and branching (Trinci, 1979). The accompanying concepts of peripheral growth zone and duplication cycle were developed. The main features of the chemical and physical structure of fungal cell walls were elucidated (Bartnicki-Garcia, 1968; Burnett, 1979). Basic aspects of the molecular biology of sporulation and spore germination in fungi were determined (Lovett, 1968; Brambl *et al.*, 1978). Dormancy in *Neurospora crassa* ascospores defied full clarification (Hecker and Sussman, 1973) but complete success was attained in the identification of the self-inhibitors of germination of rust uredospores (Macko *et al.*, 1972). The search for physiological understanding of hyphal morphogenesis was initiated in earnest (Robertson, 1965; Bartnicki-Garcia, 1973). The process of cell wall biogenesis began to be explored with different organisms (Gooday and Trinci, 1980). The importance and role of secondary metabolism in fungal ecology and fungal differentiation became clear (Bu'lock 1976; Bennett, 1983). The hormones of lower fungi were chemically identified and found to act in astonishingly low concentrations (Barksdale, 1969; Gooday, 1974). Quick answers to the biochemical basis of morphogenesis by a straightforward genetic approach, akin to the one-gene one-enzyme relationship that had been so successful in elucidating basic metabolism, did not materialize; single gene mutations affecting morphology were shown to produce a morass of pleiotropic uncertainties (Brody, 1973). The contributions from Wessels and his team described below on the physiology and morphogenesis of *Schizophyllum commune* were a salient part of this exciting period.

Rapid progress was also made in other aspects of the physiology of fungi. Yet, despite the success and the abundance of research material, the mine of fungal physiology became nearly abandoned, as the next generation of workers, plus many of their mentors, departed to find fortune harvesting the veneer of molecular genetics.

THE BEGINNING

Ph.D. graduates from the Netherlands have an effectively simple way to introduce themselves to their future scientific peers. They distribute, unsolicited, bound copies of their doctoral theses to researchers in the field around the world. And, so it was that sometime during late 1965, or early 1966, I received a copy of Volume 13 of *Wentia,* a supplement to the Dutch journal *Acta Botanica Neerlandica,* with a story that grabbed my immediate attention (Wessels, 1965). The 113 pages of this volume contained Wessels's doctoral thesis. This was a masterful piece of innovative research with the prophetic title "Morphogenesis and Biochemical Processes in *Schizophyllum commune.*" It set the tone for what would become and remain the major themes in Wessels's entire scientific career.

THE GLUCANS OF *SCHIZOPHYLLUM COMMUNE*

The beautiful mushroom *S. commune* has been the centerpiece of Wessels career. In his doctoral thesis, Wessels laid out a solid framework for understanding development in this fungus, from the deceptively simple mycelium to primordia, and the elaborate pilei (Wessels, 1965). In this initial effort, the methodology for the cultivation and handling of different developmental stages of the fungus was worked out. For biochemical studies, it was essential to achieve a fair degree of synchronization in development, a trick accomplished by controlling the supply of thiamin in the medium. The biochemical studies revealed changes in protein, RNA, DNA, and polysaccharides. A key discovery was the finding that pileus formation did not involve uptake of nutrients but the recycling of components present in the stroma.

Major findings were: (1) The identification of two different types of glucans in the cell walls of *S. commune* which were named S-glucan and R-glucan on the basis of their solubility in alkali. (2) The realization that R-glucan was degraded during the formation of the fruiting bodies to provide C and energy resources for development. This discovery of massive cell wall glucan metabolism during differentiation was the initial impetus for a host of future biochemical and morphogenetic studies. The next few years were spent identifying and characterizing the enzymes involved in the degradation of R-glucan, a β-1,3,

β-1,6-glucan (Wessels, 1966, 1969a; Wessels and Niederpruem, 1967). Such work was greatly aided by the use of mutants defective in fruiting body formation (Wessels, 1969b).

The association with Don Niederpruem, during Wessels's sabbatical leave in Indianapolis, produced a greater emphasis on the physiological and cytological details that accompany development. Much of this impetus became the substance of a joint review (Niederpruem and Wessels, 1969).

Degradation of the complex dolipore septa during nuclear migration was the subject of much attention. Extensive heterocaryotization in *S. commune* and other Basidiomycetes requires septum dissolution to permit nuclear migration. The septum wall was found to be made of R-glucan and chitin, but not S-glucan. Wessels and Marchant followed the process under the electron microscope and found that ER vesicles provided the enzymes for septum dissolution. A β-1,3-glucanase (R-glucanase) and a chitinase operated synergistically in septum wall degradation (Wessels and Marchant, 1974; Marchant and Wessels, 1974). A subsequent study revealed the relative disposition of the chitin and glucan components in the septum wall (Valk *et al.*, 1977).

CELL WALL GLUCANS

Convinced of the importance of wall metabolism in morphogenesis, Wessels made repeated efforts to refine his knowledge of the structure of the walls of *S. commune.* In 1972, a more detailed characterization of the surface of *S. commune* was published (Wessels *et al.*, 1972). In this early work, Wessels displayed his ability as a group leader, orchestrating the expertise of diverse players: the crystallographic skills of his compatriot Dik Kreger, Roger Marchant's familiarity with EM, and the contribution of Onno De Vries, the first of a series of excellent co-workers who joined Wessels's quest in Leiden and later in Groningen. This study corrected the original impression that the alkali-soluble S-glucan was a β-glucan and defined it instead as an α-1,3-linked polymer. It also revealed the β-1,3,β-1,6-glucan nature of the mucilage as an outermost layer on the cell wall. Years later, with the arrival of Hans Sietsma, a more thorough characterization of the wall of *S. commune* was undertaken (Sietsma and Wessels, 1977). By a combination of enzymatic digestion and methylation analysis, this study confirmed the identification of the R-glucan and the S-glucan.

LESSONS FROM WALL-LESS FUNGI

The preparation and properties of living protoplasts (artificially isolated from walled cells) have been a subject of great interest among bacteriologists. In the sixties, mycologists began to take advantage of the many useful experiments that can be done with protoplasts. The Czechoslovakian school of Necas was most influential in this area (Necas, 1971). Their findings provided ample material for pondering the exact role of the cell wall in the physiology and morphogenesis of fungi. Wessels and Vries began to exploit this fruitful avenue of research and developed conditions for making protoplasts of *S. commune* (Vries and Wessels, 1972, 1973). These studies were timely and earned Wessels an invitation to the Third International Conference on Fungal Protoplasts (Wessels and Vries, 1973).

Here is a good place to reflect on the importance of these congresses in the career development of young scientists. The international fabric of science is largely woven at these events. For young and largely unknown scientists, as we were in those days, invitations to speak at these events could not have come at a more propitious time. We were not just secondary participants but full partners in the scientific enterprise. I am afraid our science has become much too crowed to provide as many opportunities to young independent scientists to highlight their progress. There were three successive international gatherings that are worth mentioning: The Xth International Congress for Microbiology in Mexico City (1970), the 1st International Mycological Congress in Exeter, England (1971), and the 3rd International Symposium on Yeast Protoplasts in Salamanca, Spain (1972). Each one provided a learning experience that went far beyond the confines of mycology. In Mexico and Spain, we witnessed the enormous efforts of small cadres of dedicated microbiologists placing their countries on the scientific pedestal. Exeter marked a turning point for Mycology; it liberated it from traditional but confining ties that had kept Mycology as a subdiscipline of Botany. Scientifically, all three congresses were extremely successful and gave us a precious opportunity to establish links with many other colleagues from around the world.

The study of protoplasts continued in Wessels' lab and yielded remarkable new insights into the structure of the wall, namely the finding that polyoxin, a specific inhibitor of chitin synthetase, but not glucan synthetase, inhibited the synthesis of *both* R-glucan and chitin by regenerating protoplasts of *S. commune.* It also prevented reversion of

the protoplasts to normal hyphae (Vries and Wessels, 1975). In addition to showing the necessity of R-glucan synthesis for hyphal morphogenesis, these intriguing results became the basis of a major future discovery, tying together the molecules of glucan and chitin in a more enduring fashion than anyone suspected.

COVALENT CROSS-LINKS BETWEEN WALL POLYMERS

The intriguing possibility that cell wall polymers were bonded to one another not solely by physical interaction, but also by chemical links had long been discussed in the field of cell walls. Following the initial lead from the protoplasts investigations, Sietsma and Wessels (1979) found the much needed piece of critical chemical evidence linking chitin and glucan: the isolation of a fragment with both chitin and glucan residues. This finding was later extended to *Saccharomyces cerevisiae* (Mol and Wessels 1987) and *Schizosaccharomyces pombe* (Sietsma and Wessels, 1990). Cross-linkages explained the insolubility of R-glucan and became part of the argument in support of a change in the properties of the wall during hyphal tip growth (see below). The discovery of *in situ* cross-linking of wall polymers is a major discovery whose full significance remains to be explored.

BIOSYNTHETIC STUDIES ON CELL WALLS

In the mid seventies, studies on chitin biosynthesis became increasingly common. At that time a controversy started brewing about the localization of chitin synthetase in the fungal cell. The exact location of an enzyme responsible for cell wall synthesis is not a trivial matter. The precise topology of wall synthesis requires mechanisms for distribution of the enzyme to the right sites. We had collected a veritable mountain of evidence, in the form of sucrose-gradient fractionations and electron micrographs, to back up the claim that a major portion of the chitin synthetase of *Mucor rouxii*, and other fungi, resided in small vesicles, called chitosomes (Bracker *et al.*, 1976; Bartnicki-Garcia *et al.*, 1978). But Cabib and co-workers (Duran *et al.*, 1979) held onto the belief that the enzyme was bound to the plasma membrane and questioned the legitimacy of chitosomes.

Wessels' group undertook the characterization of chitin synthetase in *S. commune.* There were no surprises (Vermeulen *et al.*, 1979). The chitin synthetase from *S. comune* had properties similar to those of the enzyme for most other fungi. They supported a plasma membrane location for part of the enzyme but took a cautious look at the possibility that another portion of the enzyme could be in chitosomes. Much later work done in Wessels' lab by Sietsma, in collaboration with Yarden's lab (Sietsma *et al.*, 1996), provided independent confirmation for the existence of chitosomes in fungi.

Although the glucosyl transferases (β-1,3-glucan synthase or α-1,3-glucan synthases) responsible for wall glucan polymerization in *S. comune* were never characterized, a major effort was, however, devoted to studying the overall wall biosynthetic processes. One of the most significant discoveries was the finding that a water-soluble β1,3-glucan was a precursor to the alkali-insoluble glucan. The finding that polyoxin blocked the conversion made it most remarkable. This became strong supportive evidence for both the existence of an insoluble glucan–chitin complex and the view that the properties of the wall change by modification of the initially deposited polymers (Sonnenberg *et al.*, 1982; Sietsma *et al.*, 1985). Additional work by Sonneberg *et al.* (1985) proved autoradiographically that there is a differential location for the synthesis of β-1,3- and β-1,6-linked glucose units. Whereas the deposition of β-1,3-glucan takes place at the apex, insertion of β-1,6-linked residues occurs preferentially in the subapex.

APICAL ATTRACTION

Sooner or later fungal physiologists are drawn to study, or just marvel at, the mysteries of the growing hyphal apex. This is not surprising since most growth of a fungus takes place in the deceiving simplicity of the hyphal apex. Here, I must take credit for unknowingly accelerating Wessels' entrance into this arena, which he did as a formidable opponent.

By 1973, it was clear to me that the highly localized growth of the hyphal apex required three major components: (1) synthesis of new wall polymers, (2) enzymatic loosening of the existing wall, and (3) turgor, the force that physically expands the wall. Evidence for 1 and 3 was direct and strong but 2 could only be supported by a series of seemingly compelling but nevertheless indirect observations. A crucial one was the tendency of hyphal tips to burst when subjected to even minor changes in the environment

(Bartnicki-Garcia and Lippman, 1972). The bursting was not just a physical explosion; it was the consequence of a rapid chemical reaction with a characteristic temperature coefficient (Q_{10}). From these and other observations, I concluded that a "delicate balance between synthesis and lysis" existed in the growing hyphal apex (Bartnicki-Garcia, 1973). As Wessels confessed to me years later, he simply got tired of people quoting the "delicate balance" as the truth and decided to reexamine the topic and put forth his own and quite different interpretation. There was no quarrel with the fact that wall synthesis was a dominant process in the apex (Bartnicki-Garcia and Lippman, 1969), but wall lysis was a concept that defied unequivocal experimental proof, and it still does, but I had to invoke it, "as plant and fungal physiologists have been doing and still do (Cosgrove, 1987; Money and Hill, 1997)."

In 1981, before the Cell Wall meeting in Göttingen, Wessels and Sietsma (1981) concluded "In the absence of direct evidence for a model in which lysins are required to maintain plasticity at the hyphal apex, we would like to suggest an entirely different growth model in which the synthesized wall is plastic until rigidified at the base of the extension zone. Rigidification would come about by a gradual process of cross-linking of chitin to β-glucan chains by enzymes operating in the wall." This paper was an initial salvo of a number of original research papers, and review articles, on apical wall biogenesis. In subsequent years, Wessels (1986, 1988, 1990) improved the model and renamed it the "steady-state model."

APICAL PLASTICITY AND THE STEADY-STATE MODEL

The steady-state (SS) model of tip growth (Sietsma and Wessels, 1994) is a refinement of previous ideas invoking changes in the physical properties of the wall to explain tip growth (Robertson, 1965; Park and Robinson, 1966; Saunders and Trinci (1979). Accordingly, the hyphal tube is manufactured by a process that transforms and expands the newly deposited plastic wall of the apical dome into a rigid cylindrical wall at the base of the dome. The SS model was so named "because the plastic wall material is continuously synthesized and then converted into a rigid lateral wall with the apex containing a steady state amount of plastic wall."

Wessels and his team gathered considerable experimental evidence to support the notion that the cell wall is more plastic at the extreme apex and becomes more rigid at the base of the apical dome. In a beautiful paper that combined autoradiography, enzymology, and electron microscopy, Vermeulen and Wessels (1984) showed that the newly synthesized chitin at the hyphal tips was not microfibrillar and was highly susceptible to chitinase; this susceptibility diminished as the wall progressed from apex to subapex, and the chitin chains crystallized into microfibrils. Subsequent *in vitro* studies confirmed the susceptibility of newly synthesized chitin to chitinase and the lack of crystallinity of nascent chitin (Vermeulen and Wessels, 1986). These results and the work described above, on the progressive cross-linking of wall polymers by covalent bonds between β-1,3-glucan and chitin, reinforced the view that the physical properties of the wall change with time as the apical wall becomes subapical.

With regard to hyphal morphogenesis, the main question is whether the change in wall properties from plastic to rigid would explain the origin of shape in a hypha. I find this explanation difficult to accept. The wall of the apical dome, even at its potentially most plastic point, must be strong enough to resist deformation by the high turgor pressure of the cytoplasm, otherwise the cell would bulge and explode under normal growth conditions. Therefore, it is difficult to see how any additional rigidification of the apical wall would limit wall expansion to create a cylindrical wall.

It is important to keep in mind that the plasticity invoked to allow wall extension must be intrinsically short-lived and that the plastic/rigid state of the wall may depend not only on the age of the wall but also on the proportion of new wall/old wall at any given point. The latter would be determined by the frequency of vesicle discharge at any given point in the growing region. The very tip is more plastic because it continuously receives the highest density of vesicle discharge (Bartnicki-Garcia, 1973, 1990). Accordingly, the overall extensibility of the wall would not depend primarily on the position in the hyphal tube (apical dome vs cylindrical subapex) but on the density of vesicle discharge at any given point.

Although, the change in physical properties of the cell wall advocated in the SS model is not likely to explain the origin of shape, it does explain a fundamental change in the physical properties of the growing cell wall, and this must be taken into account in explanations of fungal cell wall biogenesis.

Theoretically, the polarized growth of a hypha may be explained by the vesicle supply center (VSC) model. Briefly, the model predicts that the Spitzenkörper, or its equivalent, functions as a VSC, distributing vesicles in all directions. As the hypha elongates, the linear advance of

the VSC would automatically set up a gradient of exocytosis that determines the shape of the apical dome as well as the subapical tube (for details see Bartnicki-Garcia *et al.*, 1989, 1995). The VSC model deals exclusively with the pattern of distribution of wall-building vesicles; it does not explain any details about the wall-building process. The VSC model ends when the vesicle merges with the cell surface. Here is where the SS model could take over continuing the explanation of the process of wall biogenesis.

MODEL RECONCILIATION

The SS and the VSC models are not necessarily incompatible; they account for different features of the wall-building process during tip growth. To fully harmonize both models, I feel it is necessary to introduce lysins (i.e., wall-softening or wall-plasticizing enzymes) to the SS model. Wessels (1984) agreed that lysins are needed for wall growth but he limited the need to the initiation of growth from a rigidified wall, such as in branching or spore germination. I maintain that there is no basic difference between these processes: both initiation and continuation of apical growth require a steady supply of plasticizing agents. This contention is perhaps most vividly proven by the findings of Bracker *et al.* (1997) during manipulation of the Spitzenkörper with a laser beam (optical tweezers). When a Spitzenkörper that was actively engaged in the elongation of a growing tip was forced away from its usual position in the apex, it would start deforming any wall adjacent to it: it could make a bump on the subapical wall, change the direction of growth of the hyphal tube, or even start a new branch. Clearly, the vesicles emanating from the Spitzenkörper have the power to render any wall region plastic.

The inclusion of lysins in the SS model complements the key premise of the model, namely, the progressive change in physical properties of the expanding wall. The modified SS model, would allow the growing wall, which is always a mixture of preexisting wall plus new wall, to be fully plasticized. The newly deposited wall would be plastic because of the nascent nature of the polymer chains and the lack of cross-links, and the existing wall would be rendered plastic by the action of lytic agents. Once plasticized, the wall would expand under the force of turgor. But such expansion needs to be discrete or the wall would bulge out and eventually break. The expansion-limiting factors would be the rigidifying processes invoked in the SS model (microfibril formation and cross-linking between polymer chains), to which I must add inactivation of both lytic and synthesizing enzymes. The latter is supported by autoradiographic evidence showing that the apical accumulation of chitin synthetase disappears in the subapical region (McMurrough *et al.*, 1971). Likewise the lysins must have an intrinsic short half-life to limit their plasticizing effect.

Fungal morphogenesis requires an orderly process of cell wall growth, regulated in both space and time. A tandem VSC–SS model would embody the spatial and temporal controls needed for wall biogenesis. The VSC model would explain the *spatial* control of wall synthesis, i.e., the origin of the gradient of exocytosis, while the SS model would account for the necessary *temporal* control of wall extensibility.

A tandem model may be the answer to the harmony that eluded us before.

EPILOGUE

With great skill and perseverance, Joseph G. H. Wessels built and directed a formidable research team that enabled him to conquer new microbiological frontiers. Thanks to his efforts, and those of his outstanding co-workers, the University of Groningen became a preeminent center for the study of fungal biology during the past 3 decades. In so doing, the long tradition of Dutch excellence in Microbiology that began in Delft in the 18th century continued to the very end of the 20th century.

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