Biosynthesis and assembly of fungal wall polymers
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SUMMARY

One of the main purposes of studying wall structure and biosynthesis in fungi is to elucidate the role of the wall in hyphal morphogenesis. Growth of fungal hyphae is in general restricted to the curved portion at the tip called the extension zone. So it is obvious that the study of the formation and maturation of the cell wall in this specific region should give us more insight in the shape-determining features of the wall. Therefore it is surprising to see that only few attempts were made by students on hyphal cell walls to investigate the formation of the wall at the apex. Instead, much attention was paid to chemical composition and architecture of the mature wall. In Chapter I models and observations with respect to hyphal tip growth recorded in literature are reviewed.

This thesis was meant to contribute to the understanding of the role of the cell wall in hyphal morphogenesis by studying the biosynthesis and assembly of the wall polymers in Schizophyllum commune. The advantage of using this organism is that the chemical composition and architecture of its mature wall was already available. This wall contains a water-soluble (1-3)-β/(1-6)-β-glucan (mucilage), an alkali-soluble (1-3)-α-D-glucan (S-glucan) and an alkali-insoluble complex of (1-3)-α/(1-6)-β-D-glucan (R-glucan) with chitin ((1-4)-2-acetamino-2-deoxy-β-D-glucan). Attention was especially focussed to the alkali-insoluble complex, because from studies done in this laboratory and from records in literature it appeared that interference with the synthesis of the alkali-insoluble (1-3)-α-D-glucan does not have a major effect on hyphal morphogenesis.

In previous studies on the hyphal wall of S. commune the insolubility of the glucan in the alkali-insoluble R-glucan/chitin complex has been explained by the presence of covalent linkages between this glucan and chitin. The low crystallinity of chitin in the native wall has also been explained by the presence of these linkages. It is assumed that the alkali-insoluble network is the major component that determines the strength and rigidity of the hyphal wall of S. commune. Wall synthesis occurs mainly at the tip and because there are indications that the crosslinking between the components has to occur in the wall it seems probable that the mechanical properties of the wall would change during the construction of this network thus providing a mechanism to regulate the surface expansion of the tip wall needed for the generation of a cylindrical cell.

Because growth in hyphae is restricted to a very narrow region, one of the
main problems in studying wall synthesis is the presence of a large amount of mature wall material. One way to circumvent this problem is to study wall synthesis in regenerating protoplasts.

In Chapter II this system was used to study the biosynthesis of alkali-insoluble cell-wall glucan in *S. commune*. Using double-labelling techniques it was shown that the alkali-insoluble glucan of the cell wall was synthesized from a larger pool of previously synthesized material than the other cell wall fractions. When the synthesis of the different glucan fractions was studied it appeared that a water-soluble glucan was initially synthesized at a higher rate during the first three hours of regeneration than the (1-3)-α-D-glucan and chitin. As known from previous studies, the synthesis of the alkali-insoluble glucan showed a lag period of about three hours. When cycloheximide, which inhibits primarily the formation of the water-soluble and alkali-insoluble glucan, was added after three hours of regeneration, a sharp decrease in the amount of previously synthesized water-soluble glucan was observed during further regeneration. The formation of the alkali-insoluble glucan was unaffected for several hours. From this it appeared that while normally cycloheximide, when added at the start of the regeneration, prevents the formation of the alkali-insoluble glucan the accumulation of this glucan is unaffected for several hours when previously a water-soluble glucan was formed.

In pulse-chase experiments it was confirmed that the alkali-insoluble glucan is formed from a water-soluble fraction. When the synthesis of chitin is inhibited by Polyoxin D, the formation of the alkali-insoluble glucan is also prevented. Under these conditions the water-soluble glucan accumulates indicating that under normal conditions this glucan becomes alkali-insoluble by covalent linkage to chitin. There are indications that the transfer of glucan from water-soluble into alkali-insoluble occurs via an intermediate alkali-soluble state.

From enzymic and chemical analysis it appeared that the portion of the water-soluble fraction involved in the turnover, contains only (1-3)-β-glucan. The alkali-insoluble glucan formed during the first 9 to 12 hours of regeneration contains, apart from contaminating glycogen, also only (1-3)-β-linked glucan.

In Chapter III the flow of carbon into the different glucan fractions was studied during hyphal growth by subjecting actively growing germlings to a pulse-chase treatment with \( ^{14}C \) glucose. As in the pulse-chase experiments with regenerating protoplasts it was found that during the chase
period the label in the alkali-insoluble fraction increased at the expense of the water-soluble glucan fraction. Enzymic and chemical analysis of the water-soluble fraction indicated that the portion of the water-soluble glucan involved in the turnover is also a (1-3)-β-glucan. While in protoplasts the turnover of radioactivity from the water-soluble into the alkali-insoluble fraction during the chase period continued for several hours, here the turnover occurred within minutes and the pool of polymeric precursor was much smaller. It was also shown that during labelling the specific radioactivity in the water-soluble glucan fraction rose at least twice as fast as that of the alkali-insoluble glucan fraction while the specific radioactivity in the total glucan fraction and the alkali-soluble glucan fraction rose at a intermediate rate. This also indicates that during the labelling there is a continuous loss of (non-radioactive) glucan from the water-soluble fraction and a continuous addition of previously (non-radioactive) synthesized glucan to the alkali-insoluble glucan.

In pulse-chase experiments combined with autoradiography it was shown that at the tip, apart from alkali-soluble glucan, most of the labelled glucan was water-soluble and only a small amount alkali-insoluble. During the chase, when in growing hyphae the label is transferred to subapical parts, the amount of alkali-insoluble glucan increases at the complete expense of water-soluble glucan. About half of the hyphae that had incorporated radioactivity in the tip during the pulse, failed to continue growth after the chase. Despite the fact that in these hyphae the radioactivity remained in the tip, the water-soluble glucan synthesized initially was converted into alkali-insoluble glucan. This indicates that the assumed crosslinking between glucan and chitin was not dependent on growth per se and was only time dependent.

By applying high shearing forces on hyphae, as generated during the passage of a culture through an X-press, it was shown that growing hyphal tips are mechanically fragile. This treatment removes wall polymers only from growing hyphal tips and not from non growing apices and subapical regions indicating the correlation between the mechanical properties of the wall and the presence or absence of a cross-linked glucan/chitin complex.

It is known that the alkali-insoluble glucan (R-glucan) in the hyphal wall contains both (1-3)-β and (1-6)-β-linkages. In Chapter IV experiments are described done to clarify the apparent discrepancy between the chemical structure of the assumed precursor glucan ((1-3)-β-glucan) and the alkali-insoluble glucan. First the chemical structure of the recently synthesized
R-glucan was analysed. By degrading the chitin in the alkali-insoluble wall fraction of a pulse-labelled culture two labelled glucan fractions were obtained, a water-soluble and an alkali-soluble glucan. Enzymic and chemical analysis of these fractions revealed that the water-soluble glucan consists of a branched (1-3)-β/(1-6)-β-glucan while the alkali-soluble glucan contains only (1-3)-β-linkages. In a pulse-chase experiment it was shown that the initially water-soluble fraction was a precursor for the (1-3)-β-glucan portion of the alkali-insoluble glucan only.

By passing a pulse-labelled culture through an X-press, radioactive labelled tips of growing hyphae could be isolated (see also Chapter III). It appeared that the alkali-insoluble glucan in these growing tips consists of a (1-3)-β-glucan. Autoradiographic experiments combined with periodate oxidations revealed that the (1-6)-linkages in the alkali-insoluble glucan are primarily formed in subapical parts of the wall. Using the same technique it was shown that when tip growth was inhibited by cycloheximide or a low concentration of glucose on the medium glucan synthesis is shifted to subapical regions and under these conditions predominantly (1-6)-linkages are formed in the alkali-insoluble glucan.

Chapter V summarises the previous and recent findings on the molecular structure, synthesis and assembly of the wall in S. commune and presents a new model for apical growth. Chitin and (1-3)-β-glucan are supplied to the tip wall as separate components. A wall consisting of this mixture of polymers is still deformable and can expand under the influence of the internal turgor pressure. Then, however, these polymers become gradually cross-linked. At the same time both components tend to form hydrogen bonds among themselves. The chitin-bonded glucan chains can thus cross-link the partially crystalline chitin molecules forming a rigid network. The process of rigidification of the wall is supposed to be sufficiently slow to allow for the generation of a gradient in rigidity over the apical dome. At the base of the apex the wall is then supposed to be reinforced to such an extent that no further expansion occurs and the hypha has reached its maximal diameter.

An important characteristic of the model is that rigidification of the wall is regarded as a time-dependent and not a growth dependent process. On this base a number of observations on hyphal growth recorded in literature can be explained.