

porate cholesterol to fulfill an essential membrane function. L-form membranes retain DD-carboxypeptidase, an enzyme involved in cell wall peptidoglycan synthesis in a tightly membrane-bound form requiring detergent action for solubilization. From this behaviour the enzyme would be expected to belong to the 'intrinsic' membrane proteins whose function normally depends on their association with membrane phospholipids. However, treatment with phospholipase C and/or extraction with acetone/NH<sub>4</sub>OH removed up to 90% of the phospholipid from L-form membranes but left DD-carboxypeptidase activity largely intact.

### Site of Mannan Synthesis in Yeast

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Thin sections of *Saccharomyces cerevisiae* and *Candida utilis* were floated on colloidal gold (5 nm  $\varnothing$ ) labelled with their homologous antimannan antibodies. Mannan was found concentrated in the developing septum, at the periphery of the cell wall and near the plasmalemma. In bud scars, mannan overlaid an area attributed to chitin. Mannan was also located in vesicles near the plasmalemma especially in the bud and in myelinic structures within the cytoplasm which was otherwise almost free of marker. Similar results were obtained with colloidal gold labelled with Concanavalin A. These results indicated that branched mannan is synthesized within the cytoplasm with the same immunochemical specificity as that of cell wall mannan. The experiments confirmed that fully synthesized mannan is present in the plasmalemma, that mannan overlays chitin in bud scars (HORISBERGER and ROSSET, *Experientia* 32, 798, 1976, and HORISBERGER et al., *Arch. Microbiol.*, 109, 1976) and that multimembrane bodies could be the sites where polymerization of the mannosyl units takes place (CORTAT et al., *Biochem. biophys. Res. Commun.* 53, 482, 1973; KOSINOVA et al., *Arch. Microbiol.* 99, 255, 1974).

### Papulacandin, a New Antibiotic, Active Especially Against Yeasts

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Papulacandin is produced by a strain of *Papularia sphaerosperma* (Pers.), Höhnel, which belongs to the Fungi Imperfecti. This lipophilic antibiotic was isolated by extracting the culture broth with ethyl acetate and purifying the crude extract using silicagel chromatography. It consists of a mixture of several components. The majority of the activity is present as component B, which contains two unsaturated fatty acids. Papulacandin is highly active against *Candida albicans* and several other yeasts. It shows very slight activity against a number of fungi and is inactive against bacteria. The mode of action is fungicidal but only on budding cells; resting cells are unaffected. The mycelial form of *Candida*, owing to its slower growth rate, seems to be less sensitive to the antibiotic. Papulacandin shows no cross resistance with polyene antibiotics. No reduction of its antibiotic activity was observed in the presence of various sterols. On comparing Papulacandin with two other yeast active antibiotics found in our screening, namely Echinocandin and Conocandin, cross resistance with the former was demonstrated but no cross resistance with the latter.

Echinocandin (KELLER et al., *Helv. chim. Acta* 57, 2459, 1974) is a polypeptide with a fatty acid residue, whereas Conocandin (pers. communication J. MÜLLER, Ciba-Geigy) is itself a fatty acid of unusual structure. Papulacandin does not cause leakiness in the cell membrane of *Candida albicans*. This could be shown by assaying for nucleic acid release through the cell envelope. There was also no effect on nucleic acid synthesis. A slight inhibition of protein synthesis was found. The new antibiotic inhibits the synthesis of the structural glucan of the cell wall. The mannan component does not seem to be affected. Thus Papulacandin apparently shows a mode of action analogous to that of penicillin on bacteria.

### Regulation of Cell Size at Division in the Fission Yeast *Schizosaccharomyces pombe*

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Cell division is regulated by a control which maintains a constant size at division and therefore coordinates division to cellular growth. Mutants altered in that regulation have a normal growth rate but divide at half the size of the wild type. They define two unlinked genes *wee1* (10 mutants) and *wee2* (1 mutant). *Wee1* mutants are semi-dominant, suggesting a gene-dose effect, whereas the *wee2* mutant is almost dominant over wild type. *Wee2* is very close and possibly allelic to *cdc2*<sup>-</sup> mutants which are defective in nuclear division. A tentative model for size control over cell division will be presented.

### Determination of Enzyme Activities in Permeabilized Cells of Various Microorganisms

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For investigating metabolic pathways in microorganisms, simple methods for determining enzyme activities are required. We have developed a procedure for permeabilizing cells which allows in situ measurement of enzyme activities using minimal cell mass. The method was originally devised for assaying amino acid biosynthetic enzymes in *Saccharomyces cerevisiae* but has also proved to be successful for assaying the analogous enzymes in *Schizosaccharomyces pombe* and *Escherichia coli*. Cells, preferably grown on minimal medium, are harvested during any growth phase by centrifugation. After washing the cells with distilled water and potassium phosphate or Tris-HCl buffer (both 0.1 M, pH 7.6), they are resuspended in the respective buffer containing 0.05% Triton X-100 to provide a final cell concentration of 100 mg cells (wet weight) per ml buffer. After thorough mixing, the suspension is frozen at -20°C. The cells could be stored in this state for several weeks without loss of activity for most of the enzymes tested. Prior to the enzyme assays, the cell suspension is thawed carefully in a water bath at 30°C and then placed in an ice bath. Using this method we tested biosynthetic enzymes of the arginine, histidine, and tryptophan pathways. Enzyme activities in the permeabilized cells proved to be generally higher and significantly more stable than those in crude extracts.

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