YEAST

Control of Budding

from our Microbiology Correspondent

WITH so much being written these days on the subject of morphogenesis and its biochemical foundations it is refreshing to come across a piece of work in which cellular differentiation has been resolved in some detail at the molecular level. Dr Enrico Cabib and his colleagues at the National Institutes of Health in Bethesda have been following a very simple morphogenetic event, that of budding in yeasts. Their latest findings (Cabib and Farkas, *Proc. US Nat. Acad. Sci.*, **68**, 2052; 1971) bring together the details of a chitin synthesizing system which is responsible for initiating septum formation between the mother and daughter cells. The specificity both of timing and localizing this event seems to be produced by conversion of a chitin synthetase zymogen to the active enzyme by an activating factor (AF).

Earlier this year, Cabib and Bowers (*J. Biol. Chem.*, **246**, 157; 1971) had confirmed previous reports that chitin is concentrated in a disk-like plug from which the bud has detached. They have now shown that chitin synthesis occurs in a stepwise fashion in synchronized populations of *Saccharomyces carlsbergensis*. Similarly the chitin synthetase of *Saccharomyces* species which was described as a particulate enzyme (Keller and Cabib, *J. Biol. Chem.*, **246**, 160; 1971) is revealed to be largely in an inactive or zymogen form which can be activated about 15-fold by treatment with trypsin. The endogenous AF can be recovered in high-speed supernatants of the sonicated enzyme preparations. Although the details of the AF have not been elucidated, it probably catalyses a specific proteolysis of the zymogen. Cabib believes that the enzyme is firmly bound to a particulate cell fraction whereas AF is enclosed in more fragile vesicles.

The nature of the synthesizing system is still more complex. A heat stable protein was discovered in the cytoplasm which was thought to be an allosteric inhibitor of the enzyme (Cabib and Keller, *J. Biol. Chem.*, **246**, 167; 1971). But further work has confirmed that the inhibitor interacts with AF and not with the enzyme *per se*.

In order to explain the triggering of chitin synthesis at a precise time and site, Cabib and Farkas offer the following hypothesis. Because chitin synthetase is largely in a zymogen state and the amount of it in the cell is surprisingly constant, it is presumed to be uniformly distributed in some structural component of the cell, most probably the plasma membrane. Following bud formation, AF-containing vesicles might fuse with the plasma membrane at the site of septation resulting in enzyme activation of chitin synthesis. Indeed, the presence of vesicular material at bud sites has been observed several times in yeasts. The role of the inhibitor is more enigmatic. One possibility is that it may serve as a safety device to immobilize AF that might be released into the cytoplasm and thereby prevent any indiscriminate enzyme activation and subsequent chitin formation. Experimental testing of this elegant model doubtless will be made in the near future and news of its veracity and generality will be eagerly awaited.

MICROSCOPY

Cryoultramicrotomy

from a Correspondent

A MEETING and workshop on cryoultramicrotomy was held in Paris on October 18 and 19 and was the second meeting on specialized techniques in microscopy organized jointly by the Royal Microscopical Society and the Société Française de Microscope Electronique.

Dr W. Bernhard (Institut de Recherches Scientifique sur le Cancer, Villejuif) opened the session by reviewing the problems posed by cryoultramicrotomy, and showed that by carefully applying the technique to material which had been lightly fixed it was now possible to obtain morphological information from specimens equal to that obtained by more conventional techniques of embedding and thin sectioning. This excellent introduction to the meeting was followed by a series of contributions by commercial representatives on the physical principles of cryoultramicrotomes. No major advances in instrumentation were described, but all the speakers in this session stressed the importance of accurate temperature control of both the knife and specimen, and the necessity of a dry atmosphere in the cutting area.

The remainder of the first day was taken up by descriptions of some biological applications of the cryoultramicrotome. Dr L. Seveus (LKB, Stockholm) and Dr G. Werner (Saarlandes University) showed that fixed material, frozen, thin sectioned and then dried at low temperature, can give the same type of image obtained by more conventional means, although membranes can only be seen in negative contrast. Organelles such as mitochondria, although recognizable in unfixed material, rarely show internal membranes. Dr T. Appleton (University of Cambridge) gave several useful hints for the handling of frozen sections, and for drying the sections at low temperatures. He also showed that ultracellular membranes in unfixed material seem to appear as an array of linear dots, and said that the interpretation of morphological data obtained by cryoultramicrotomy should not be based solely on the usual appearance of these structures in the electron microscope. Dr L. Paavola and Professor A. K. Christenson (Temple University) showed how necessary it is to work with unfixed material in studies of steroid synthesis in mammalian tissue.

On the second day, Dr S. Hodson and Dr J. Marshall (Institute of Ophthalmology, London) showed that even if sectioning is carried out at −180° C there is still likely to be some danger of surface melting of the specimen. Their studies showed that although this melting is considerably diminished in very thin section, it might seriously jeopardize high resolution localization of diffusable substances. The session continued with a paper on sectioning single cells and a discussion on whether it is really necessary to encapsulate the specimens before freezing. Dr A. Viron (Laboratoire de Biologie de Végétale, Orsay) and Dr S. Halpern (Institut de Recherches Scientifique sur le Cancer, Villejuif) described some results following cryoultramicrotomy of plant material, and it became clear that the watery vacuole of mature plant cells still presents a considerable barrier to successful cryosectioning of plant tissues. There then followed an exhaustive round table discussion which included contributions by Professor H. Moor (Technische Hochschule, Zurich), Dr P. Echlin (University of Cambridge) and Dr M. Grund (Reichert, Berlin) on some of the theoretical problems associated with rapid freezing. It was shown that the present techniques preserve adequately only the surface layers of specimens unless the samples are thoroughly soaked in cryoprotective agents. In many future applications of cryoultramicrotomy the use of fixatives and anti-freeze agents may not be practicable, and several new techniques were discussed including the high pressure method proposed by Professor Moor.

The afternoon session dealt with the application of cytochemical method and autoradiography to cryoultramicrotome sections. Dr Appleton showed that it was possible to localize by X-ray microanalysis ions and electrolytes in frozen dried sections, and Dr R. Simard (University of Sherbrooke) demonstrated the rapid loss from the tissue of diffusable substrates in material which had been fixed before freezing and sectioning.

In summing up this most stimulating meeting, Dr Bernhard agreed that the cryomicrotome has proved itself capable of providing morphological information, but said that its use should now be directed to providing thin sections of undisturbed biological tissue for analysis by the sophisticated techniques of histochemistry, autoradiography, immunochemistry and X-ray microanalysis.