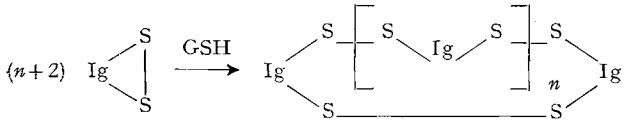
ses lipid the surface pressure would tend to rise and contract the lipoenzyme. When the latter contracts sufficiently into a reactive conformation it could trigger off a poised cellular reaction. Lipid compression (Figure 2b) would therefore be transient. Such a critical conformational change in a cellular surface molecule other than the Ig receptor provides a convenient, common pathway for lymphocyte stimulation. Any reaction with various membrane receptors which tended to indice comparable lipid compression could trigger off the same cellular response and synergistic effects would be possible. For triggering to occur antigens reacting with Ig must be multivalent, occupy substantial area, and liberate sufficient energy to induce adequate expansion of the 'barrier'. 'Tolerance' would occur if small multivalent antigens occupied the specific antibody receptors but induced insufficient expansion.

Since IgG contains only 1, but IgM contains 5, and IgA 2 subunits linked by disulphide bridges[22], IgG will be least effective for compressing a membrane film when reacting with antigen; IgG can form a ladder lattice only with multivalent antigen whereas the polymerized antibodies found in membranes have many more reactive sites. Glutathione (GSH) stimulates[23], but dehydroascorbic acid (DHA) inhibits cell division[23]. Stimulation of cells by hormones is preceded by a decrease in ascorbic acid. The appearance of GSH in high concentrations coincides with mitotic activity[23]. Oxidized GSH promotes membrane swelling but the disulphide hormones (e.g. oxytocin, vasopressin and insulin) are 10^7 times more effective[24]. Because the swelling reactions are enhanced by small concentrations of GSH, and other thiols which enhance disulphide exchange reactions, it is thought that the swelling arises from such exchanges[24]. Perhaps ascorbate reduces oxidized GSH, and GSH so liberated (perhaps with help from an enzymic disulphide hormone from T cells) then catalyses polymerisation of Ig subunits for incorporation into membranes to produce effective 'sweeps' on reaction with antigen:



By preventing reduction of oxidized GSH, DHA might inhibit such polymerization and so influence the immune response.

Under some conditions at 37°C Ig-antibody complex in lymphocytes moves along the membrane to form a cross-linked 'cap'[8]. This event shows that antigen-Ig complex can occupy a substantial area of the membrane. Such movements could arise from the surface pressure gradient generated by expansion of the lipoenzyme (Figure 2d). Antigen-Ig would then be swept from the membrane (pinocytosis) since antigen-Ig occupies more space than Ig.

Cooling to 0°C, or adding a metabolic inhibitor, prevents antigen-Ig movement but not formation of antigen-Ig complex[8]. If lipid compression tended to induce a marked rise in film rigidity because of the presence of cholesterol, such rigidity could be stabilized by a metabolic inhibitor which prevented contraction of the lipoenzyme, perhaps by suppressing removal of a metabolite bound to the latter.

Analogous principles probably apply in plasma membranes where different polypeptide hormones, inducing membrane conformational changes at separate receptors, via sulphide-disulphide exchange reactions[24], trigger c-AMP production at one common site[25]. Perhaps amocrine cells use similar principles to modulate information between various synapses via pressure changes in lipid.

The hypothesis suggests a number of experiments including the following. The postulated change in membrane surface pressure from antigen-Ig reaction, and the predicted influence on it of membrane stabilizers, labilizers and glutathione, should be observable in a surface balance. Spin labelling and microcalorimetry could be applied to study lipid mobility and entropy changes in lymphocyte reactions.

Zusammenfassung. Es wird vorgeschlagen, dass die Reaktion des Thiol/Disulpid Austausches zwischen Antigen oder Polypeptid-Hormon und dem Mambran-Antikörper oder Hormon-Rezeptor (in Lymphozyten oder Plasma Membranen), der im beweglichen Membran-Lipoid suspendiert ist, einen vergrösserten Oberflächen-Komplex bildet, der das Lipoid komprimiert und dadurch ein Enzym in eine kritische Konformation zusammendrückt, welch letztere die Immun-Reaktion oder c-AMP-Bildung auslöst.

D. E. Weiss

Division of Applied Chemistry, Forest Products Laboratory, Commonwealth Scientific and Industrial Research Organization, P.O. Box 310, South Melbourne (Victoria 3205, Australia), 4 July 1972.

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PRO EXPERIMENTIS

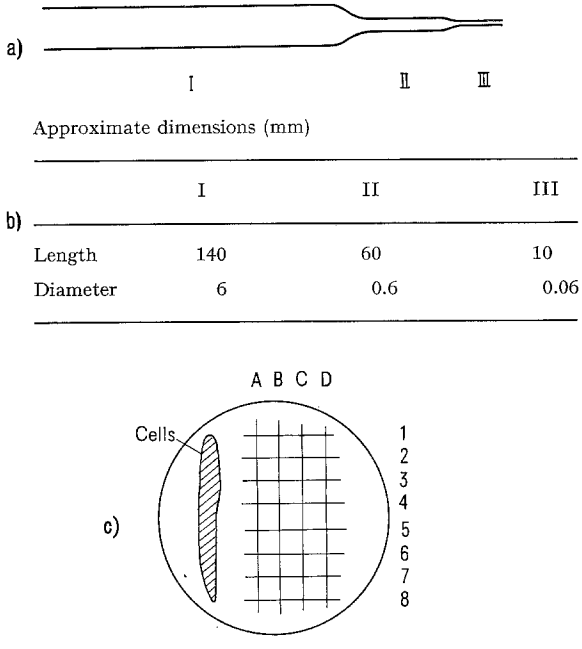
A Method for Dissecting Yeast Asci Without Micromanipulator

Saccharomyces cerevisiae is an organism suitable for genetical studies since, amongst other reasons, it is amenable to tetrad analysis. The dissection of yeast asci is generally accomplished with the aid of a micromanipulator (Winge and Laustsen[1]). An important improvement in technique resulted from the introduction of snail digestive extract for the enzymatic digestion of the ascus wall (Johnston and Mortimer[2]). In this report I wish to describe a method, amenable to modifications, which allows the dissection of yeast asci without the aid of a micromanipulator.

Two simple instruments are used. Firstly a micropipette is made from glass tubing, the form and approximate dimensions of which are indicated in the Figure (a, b). To the large end of the pipette, a piece of transparent plastic

[1] Ö. Winge and O. Laustsen, C.r. Trav. Lab. Carlsberg *22*, 99 (1937).
[2] J. R. Johnston and R. K. Mortimer, J. Bact. *78*, 292 (1959).

tube is fitted, approximately 40 cm in length. The tip is held in ethanol and cleaned by repeatedly blowing air through the system. Secondly, from glass rod, a microneedle is made having the same form and dimensions as the pipette, the tip being rounded in a small flame. Plates with particle-free agar, which were poured in an exactly horizontal position, are prepared by cutting lines in the surface, as indicated in the Figure (c), with the aid of a sterile needle or scalpel etc. The age of the agar plates is not very critical although neither freshly poured nor old dry plates should be used. The spores will be placed at the intersections. In the diagram the plate is prepared for 8 asci. A loop of sporulated cell material is transferred to an enzyme solution (e.g. 'Glusulase', Endo Laboratories, Inc., Garden City, N.Y. 11530; 0.02 ml enzyme preparation in 5 ml distilled water, 20–30 min at room temperature). If necessary the titer of this suspension is adjusted as to generate a reasonable average 'nearest neighbour' distance (e.g. 20 × cell diameter) between cells and asci on the plate. A loop of the suspension is then streaked on the left side of the plate (Figure c). The microscopic magnification is chosen such that a workable distance between agar and objective is maintained and that asci can be recognized with ease (e.g. objective 10×, eye pieces 10×). Also an armrest is necessary for supporting the hand which holds the pipette.



Form a) and approximate dimensions b) of glass micropipette. Prepared agar plate (c).

An isolated four-spored ascus is identified under the microscope. The free end of the plastic tube is held in the mouth and the tip of the pipette is brought into the field. A cell-free area on the surface is touched repeatedly thus filling the tip with liquid by capillary action. Then the ascus is touched and thereby picked up. The pipette is lifted slightly, and, with the other hand, the plate is moved so as to bring the first intersection 1A into focus (Figure c). The pipette is lowered and the ascus blown out. The success or failure of the manoeuvre has to be checked. The tip is then cleaned in ethanol and further asci are accordingly placed at the other intersections 2A to 8A. The second step consists in separating the 4 adhering spores of an ascus by rubbing it with the microneedle. This step may be facilitated by keeping the plate at 4 °C over night. Finally, in the third step, 3 spores of each ascus are transfered with the pipette to the corresponding intersections B, C and D, using the same technique as for the initial ascus isolation.

It is my experience that with sufficient practice yeast asci can be dissected by free hand no less efficiently than with the micromanipulator. The free hand method can also be used, with modifications, for 1. work with other yeasts such as *Schizosaccharomyces pombe*, 2. single cell isolations (standard cloning, zygote isolation), 3. cell to cell pairings and 4. pedigree analyses[3].

Zusammenfassung. Eine Methode wird beschrieben, die es erlaubt, einzelne Hefezellen freihändig, d.h. ohne Zuhilfenahme eines Mikromanipulators, zu isolieren. Diese Methode mag Anwendung finden bei Einzelzellisolationen, Stammbaumanalysen, Tetradenanalysen und Zell-zu-Zell-Paarungen.

P. MUNZ

Institute for General Microbiology, University of Bern, Altenbergrain 21, CH-3013 Bern (Switzerland),
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A Modified Technique of Tissue Incubation for Insect Fat Body

Protein synthesis in insect fat body has frequently been studied in vitro by the incorporation of labelled amino acid from an incubation medium[1–3]. Previous reports have described the incubation of pooled fat bodies for periods of up to 5 h, using small flasks or boiling tubes mounted vertically on shaking machines achieving rapid agitation. A modification of this procedure has been developed to optimize the aeration of incubated tissues and facilitate the separate use of individual fat bodies.

5-day-old adult males of *Locusta migratoria migratorioides* (R. & F.) were decapitated and each carcass opened in the mid-ventral line. After removal of the gut and testis, the visceral fat body was dissected under sterile Ringer's solution and floated out as a single sheet. Each fat body was collected on a stainless steel spatula and introduced into a tissue culture tube containing a sterile aliquot of STEVENSON and WYATT's 'A' medium[2], modified by the inclusion of leucine (0.2 m*M*) and a mixture of unlabelled and U-^{14}C-valine. Each fat body was stranded on the side of a culture tube in a position where the medium would wash over it periodically during the incubation. The tubes were fitted onto a Matburn

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