more distal portions of such hyphae, can be separated from the autolysed hyphal content by a dense plug isolated behind a restrictive zone containing only small vesicula (Figure 4). The survival of rhizoids, suggested from their still dense, ribosomes-containing cytoplasm is interesting from a physiological point of view.

[8] The support of the Swiss National Research Fund is gratefully acknowledged.

Résumé. Les rhizoïdes d'*Allomyces arbusculus*, émis d'hyphes végétatifs et en conséquence nommés épihyphaux, sont riches en ribosomes quand ils sont jeunes; ils se remplissent par contre de vésicules et ne contiennent plus que quelques mitochondries peu développées dans les plus âgés.

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The Growth of Cell Membranes During Synchronized Cell Division of *Saccharomyces cerevisiae*

The study of biosynthesis of complex structures such as cellular membranes presents several problems like the growth of these membranes in dividing cells. In the present paper, the distribution of radioactivity in the cytoplasmic membranes of mature and young cells labelled by pulse chase technique has been investigated.

Buds and mature cells of yeast *Saccharomyces cerevisiae* strain IMI 140428 cultivated in Edinburgh Minimal Medium[1] with L-Leucine-^{3}H (10^5 cpm/ml) added during the last 30 min of incubation were used. Cells (about 30 g wet weight) taken from the culture in mid log phase were collected by centrifugation and washed twice with the same broth free from radioactivity. Sucrose density centrifugation (tube 80 ml capacity) according to MITCHINSON and VINCENT[2] method was employed to obtain synchronously growing cultures. The top layer ($\sim 5\%$) of cells was collected and incubated in new nonradioactive EMM broth. All the operations were performed at 25°C. Samples of the synchronized cell suspensions were collected on $0.025M$ cyanide during the first division cycle at 60, 75 and 90 min of incubation (in the phase preceding nuclear division) and at 115 min (after nuclear division; doubling time 125 min). The cells collected by centrifugation were drastically shaken in a Waring blendor for 30 sec. In this way, buds are detached from parent cells with a yield ranging from 10 to 25% (Table). The sample containing buds and parent cells was layered on sucrose gradient[2]. After centrifugation, the top layers containing the buds were separated from the other layers containing both the budding cells and large cells. Cells were checked for size distribution by phase contrast microscopy.

Membrane isolation was carried out both on buds and mature cells. For this purpose, the cells were transformed into protoplasts by the EDDY and WILLIAMSON[3] method, using a citrate phosphate buffer $0.005M$ pH 5.8 containing $0.55M$ rhamnose and 1.0 mg/ml freeze-dried snail enzyme (*Helix pomatia*). Membranes were then purified as described by MATILE[4]. Controls for the absence of hexo-

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Specific radioactivity of protein and lipids of purified membranes obtained from mature cells and buds of syncronized cultures of *Saccharomyces*

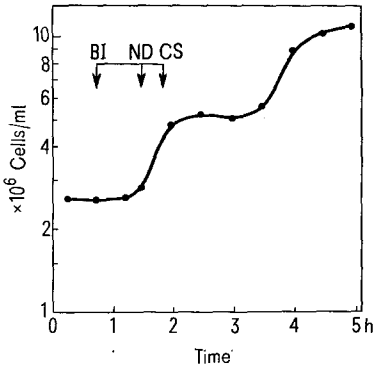
	Time (min)	Cell recovery[a] (% of total cells layered on the gradient)	Total protein (mg)	Total radioactivity (cpm)	Specific radioactivity (cpm/mg)	Relative specific radioactivity
			Leucine-^{3}H experiments			
Inoculum			3.92	17560	4479	1.00
Top layer (buds)	60	10	0.54	1670	3092	0.69
	75	16	1.09	2878	2641	0.59
	90	19	1.37	3390	2474	0.55
	115	25	1.94	4408	2272	0.51
Other layers (mature cells)	60	90	5.33	15936	2989	0.66
	75	84	5.38	14426	2681	0.59
	90	81	5.66	14619	2582	0.57
	115	75	6.22	14057	2259	0.50
			$^{32}PO_4^{\equiv}$ experiments			
Inoculum			3.65	1270	348	1.00
Top layer (buds)	90	19	1.46	284	195	0.56
Other layers (mature cells)	90	81	5.50	1105	201	0.58

Mature cells and buds were separated by centrifugation in sucrose density gradient. The data are the average of 3 experiments. [a] Cell count in haemocytometers.

kinase and the presence of Mg^{2+} dependent ATPase[5] were made. An aliquot of the preparations was used to measure protein content[6] and radioactivity was measured in a Beckman LS-100 liquid scintillation counter.

The same experiment from growth of *Saccharomyces* to membrane isolation was repeated, except that cells were grown in the presence of $^{32}PO_4$ (10^5 cpm/ml) to label the lipid phase and collected after 90 min of incubation (in the first synchronized division cycle). Isolated membranes were extracted 3 times with chloroform-methanol, 2:1[7], and the extracts were assayed for lipid extractable phosphorus[8] and for radioactivity in D47 Nuclear Chicago flow counter.

The Figure shows the growth curve of a synchronous culture and the Table gives average data from 3 experiments. It is evident that the specific radioactivity for both leucine-^{3}H and $^{32}PO_4$ of membranes from buds and parent cells separated by sucrose gradient centrifugation is about half that of cells present in the inoculum. This fact suggests that macromolecules present in the membranes of inoculum cells are randomly diluted both in parent cells and buds. The results given in the Table (obtained by following the change of specific radioactivity in membranes purified from buds and mature cells at the indicated times) also show that there is no difference in mature cells and buds in rate of membrane growth.



Growth curve of synchronous cultures of *Saccharomyces cerevisiae*. CS, cell separation was determined by particle count in haemocytometers; ND, nuclear division was determined by HARTWELL[9], modification of the technique by ROBINOW and MARAK; BI, budding was determined by visual inspection in phase-contrast microscope.

Further, the behaviour of the change rate of specific activity in buds and mature cells excludes the possibility that what is transferred from parent cells to newly forming buds is a pool of membrane subunits. The experiments in which cells were incubated in the presence of $^{32}PO_4$ show that membrane lipid synthesis followed the same pattern (i.e. the dilution pattern) as membrane protein synthesis. The fact that the dilution effect is observed for both protein and lipid synthesis, strongly suggests that all the components of *Saccharomyces* membranes follow the same dilution pattern during cell division.

Riassunto. Cellule di *Saccharomyces cerevisiae* venivano coltivate in brodo centenente L-Leucina-^{3}H o $^{32}PO_4^{=}$ raccolte in fase log e quindi coltivate in modo sincrono in nuovo terreno privo di radioisotopi. Le cellule e le gemme venivano separate per centrifugazione su gradiente di saccarosio, quindi si isolavano le membrane. La radioattività specifica nelle membrane purificate risultava circa il 50% di quella presente inizialmente nelle cellule usate per la coltura sincronizzata.

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STUDIORUM PROGRESSUS

Localization of Ions in Cells of *Potamogeton lucens* L.[1]

Determination of ion content in various organelles and the relative distribution of ions within various cell-compartments is of great interest to biologists and has been the topic of numerous investigations (ARNOLD[2]; ARISZ[3]; OSMOND[4]; OSMOND et al.[5], LÄUCHLI and SCHWANDER[6], ZIEGLER and LÜTTGE[7]; LÄUCHLI and LÜTTGE[8], JENNINGS[9–11], LARKUM[12], NOBEL[13], WAISEL et al.[14], WAISEL and ESHEL[15], etc.) Undestanding of physiological phenomena like the ionic requirements for enzyme activity, into osmotic adaptation of cells, into symplast transport etc., depend very much on verification of the exact content of ions at the site of operation. Thus, such information is of great significance for the study of various processes which take place within the cells.

However, in spite of the use of several methods, i.e. autoradiography, specific dyes, electron microscopy, etc. (cf. JENSEN[16]; LÜTTGE[17,18]; LÄUCHLI[19,20]; VAN STEVENINCK and CHENOWETH[21] etc.), the precise localization and content of ions in subcellular compartments remains a matter of speculation.

Compartmental analysis had contributed a great deal to a better estimation of ion content inside various subcellular compartments. Nevertheless, being based on certain unmeasurable estimated values, its results are still open to criticism.

The recent application of X-ray microanalysis to biological material provides a better tool for determination of ion distribution within cells, and offers a direct method for localization of most of them in some subcellular compartments.

A stock of *Potamogeton lucens* plants was grown outdoors for several months in tap water. Leaves and branches were detached from those mother plants and transferred into tap water to which 100 m*M* NaCl were added. The material was kept there for 24 h under continuous light and controlled temperature (27° ± 2°C). Following such a period of adjustment, samples of 1 cm² each were taken from the midrib region of mature leaves for examination. The samples were placed on top of a cold (−70°C) microtome block-holder. Distilled water were used for