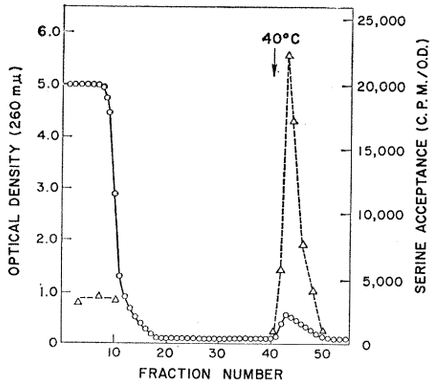


Fig. 2. Isolation of sRNA enriched in serine acceptance with a column containing polyU gel immobilized on plastic beads. A solution containing about 20 mg of sRNA from *Escherichia coli* was recirculated through the column at 0°C for 2 days and then was flushed out. At fraction 40, the column temperature was raised to 40°C. Portions from the collected fractions were tested for serine acceptance. Circles, optical density; triangles, specific activity for serine acceptance.

Table 1. Amino acid acceptor activity of sRNA isolated from polyU column. Reaction mixtures, in a total of 1.65 ml, were composed of 1 ml of sNRA solution containing about 0.5 O.D. unit (260 mμ, 10-mm light path) in the citrate buffer; and 0.65 ml of tris buffer (0.01*M*, *p*H 7.4) containing the following: adenosine triphosphate, 1.3 μmole; cytosine triphosphate, 0.25 μmole; phosphoenolpyruvate, 1.3 μmole; pyruvate kinase, 10 μg; amino acid activating enzyme, partially purified, from *E. coli* (*13*), 0.56 mg protein; $MgCl_2$, 5 μmole; KCl, 30 μmole; mercaptoethanol, 3 μmole; and one of C^{14}-labeled amino acids (specific activities from 13 to 360 mc/mmole), 1.0 μc. For the blanks, 1 ml of the citrate buffer was used to replace sRNA. The reaction was measured as radioactivity in the acid-insoluble fraction in comparison to that of blanks. After incubation at 37°C for 15 minutes, samples of 100 μl (in triplicate) were placed on Whatman No. 3 MM-paper discs to be washed with cold trichloroacetic acid (5 percent) five times and with ethanol twice (*14*). The washed discs were dried and counted with a scintillation spectrometer. OM, original mixture; RF, retained fraction.

C^{14}-Amino acid	μμmole of C^{14}-amino acid accepted by mμmole sRNA*		Total acceptance (%)	
	OM	RF	OM	RF
Serine	20.6	160.0	9.0	70.7
Leucine	80.0	47.7	35.2	21.1
Phenylalanine	12.0	0	5.2	0
Proline	20.5	4.5	9.0	1.9
Valine	20.0	0.7	8.8	0.3
Threonine	14.3	4.3	6.2	1.9
Glysine	8.5	1.2	3.7	0.5
Lysine	4.3	0.3	1.8	0.1
Isoleucine	3.0	0	1.3	0
Alanine	17.5	3.1	7.7	1.4
Tryptophan	0	0	0	0
Tyrosine	3.8	1.9	1.6	0.8
Arginine	7.9	0.9	3.4	0.4
Glutamic acid	4.3	1.4	1.8	0.6
Aspartic acid, plus methionine	10.5	0	4.6	0

* Molecular weight of 25,000 was assumed.

retained fractions showed increased ability to accept serine, they contained other species of sRNA because the acceptance of serine by these fractions varied when assayed individually. All the retained fractions were combined and examined for the ability to accept other amino acids (Table 1). There was a high specificity for serine (71 percent of total acceptance), but the material also accepted leucine (21 percent) and 14 other amino acids (less than 8 percent). Under the reaction conditions (Table 1), both the retained fraction and the original mixture showed the same total capacity to accept amino acids; about 23 percent of the RNA calculated from O.D. measurements was capable of accepting amino acids.

Physical forces influencing the retention of the serine-specific sRNA may be (i) van der Waals forces, (ii) hydrophobic bond formation, and (iii) complementary hydrogen bondings operating between the sRNA and the polyU gel. Among these forces, only the complementary hydrogen bondings would provide the selectivity needed for enriching a species of sRNA from mixtures. The complete sequence of alanyl sRNA (*9*) reveals that there are some oligonucleotide segments in the molecule available for intermolecular hydrogen bonding. The change in elution with temperature (Fig. 1) of the retained sRNA is consistent with the dissociation of a hybrid between some segment of the sRNA and the polyU gel. The observed melting temperature (T_m), 20°C, agrees with those T_m values (*10*) for dissociations of a hybrid between oligonucleotide and complementary polymer.

Subsequent to the completion of the work reported here, Erhan *et al.* (*11*) reported the retention of two species of charged sRNA's on polydeoxyoligothymidylic acid (poly-dT) and polydeoxyadenylic acid (poly-dA) columns. There was an apparent retention of C^{14}-phenylalanyl sRNA on a thymidylate column and of C^{14}-lysyl sRNA on an adenylate column at temperatures as high as 50°C. Only two "charged" species of sRNA were tested on each column. Therefore the heterogeneity of the retained material cannot be ascertained in their experiment because other C^{14}-aminoacyl sRNA's were not tested.

Grossman reported that two uracil photoproducts were formed when polyU was irradiated with ultraviolet light (*12*). A molecule of water may add at the uracil moiety, or a uracil-uracil dimer may be formed. When the irradiated polyU was compared with normal polyU in a cell-free system for protein synthesis, the hydrated form stimulated the incorporation of serine rather than phenylalanine. The formation of dimer resulted in the loss of incorporation of phenylalanine, which was not replaced by any other amino acid. Although it is attractive to associate the isolation of the serine-specific sRNA described above to Grossman's observation, such speculation must await further experimentation.

P. P. HUNG

Biochemical Research Department, Abbott Laboratories, North Chicago, Illinois

References and Notes

1. E. K. F. Bautz and B. D. Hall, *Proc. Natl. Acad. Sci. U.S.* **48**, 400 (1962); B. J. McCarthy and E. T. Bolton, *ibid.* **50**, 156 (1963); E. T. Bolton and B. J. McCarthy, *ibid.* **48**, 1390 (1962); B. Hoyer, B. J. McCarthy, E. T. Bolton, *Science* **140**, 1408 (1963).
2. P. T. Gilham, *J. Am. Chem. Soc.* **86**, 4982 1964); A. J. Adler and A. Rich, *ibid.* **84**, 3977 (1962); P. T. Gilham, *ibid.* **84**, 1311 (1962).
3. Potassium salt, $S_{20,\ w} = 8.4$; purchased from Miles Chemical Company.
4. Geon 101, Goodrich Co., Akron, Ohio.
5. R. J. Britten, *Science* **142**, 963 (1963).
6. Ultra-violet Products, Inc., Model SL-2537, South Pasadena, California.
7. B strain, purchased from General Biochemicals, Chagrin Falls, Ohio.
8. C. B. Klee and M. Staehelin, *Biochim. Biophys. Acta* **61**, 668 (1962).
9. R. W. Holley, J. Apgar, G. A. Everett, J. T. Madison, M. Marquisee, S. H. Merrill, J. R. Penswick, A. Zamir, *Science* **147**, 1465 (1965).
10. M. N. Lipsett, L. A. Heppel, D. F. Bradley, *J. Biol. Chem.* **236**, 857 (1961); E. K. F. Bautz and F. A. Bautz, *Proc. Natl. Acad. Sci. U.S.* **52**, 1476 (1964).
11. S. Erhan, L. G. Northrup, F. Leach, *Proc. Natl. Acad. Sci. U.S.* **53**, 646 (1965).
12. L. Grossman *ibid.* **50**, 657 (1963).
13. J. A. Carbon, *Biochem. Biophys. Res. Commun.* **15**, 1 (1964).
14. R. J. Mans and G. D. Novelli, *Arch. Biochem. Biophys.* **94**, 48 (1961).
15. I thank Miss L. Straube for technical assistance.

26 April 1965

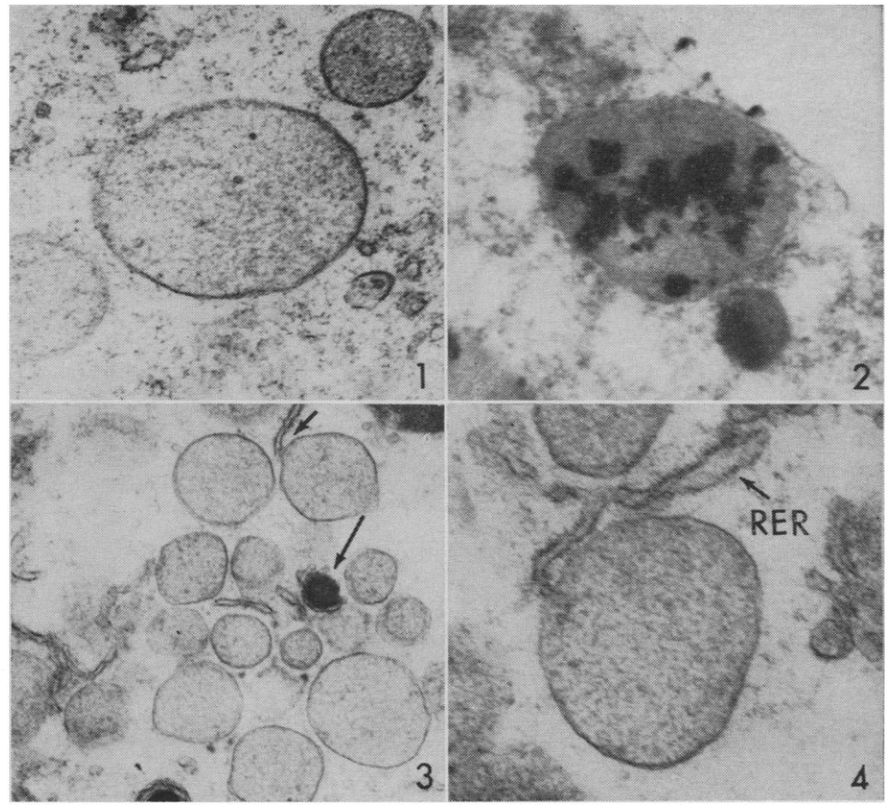
Primary Lysosomes in Tetrahymena pyriformis

Abstract. *Primary lysosomes have been identified in the protozoon* Tetrahymena pyriformis, *and some evidence from their ultrastructure is offered for their origin.*

The protozoon *Tetrahymena pyriformis* contains numerous, slightly dense, oval or spherical vacuoles of variable size (0.5 to 1.0 μ). In the absence of cytochemical evidence, these vacuoles were tentatively identified as lysosomes (*1*). According to de Duve

(*2*), primary lysosomes are those single-membraned vacuoles that are "conveyors of acid hydrolases which have not yet been involved in digestive events." The presence of one or more hydrolases is considered positive evidence for their identification. The study reported here was undertaken to determine the presence or absence of acid phosphatase in these vacuoles and to investigate their relation to the endoplasmic reticulum.

Ciliates (strain E) were grown axenically in 2 percent proteose-peptone (*p*H 7.4) at 25°C. When in the logarithmic growth phase (after 24 to 48 hours), they were concentrated by centrifugation into a soft pellet and fixed for 30 minutes either in 1 percent OsO_4 buffered with 0.14 Veronal acetate (*p*H 7.4) containing 45 mg of sucrose per milliliter, or in 3 percent cold cacodylate-buffered glutaraldehyde (*p*H 7.2) followed by 1 percent OsO_4 for 15 minutes. For acid phosphatase identification they were incubated for 20 minutes at 37°C in the sodium glycerophosphate medium of Gomori (*3*). Controls without substrate were maintained. They were dehydrated with graded ethanols and embedded in Epon 812 according to the method of Luft (*4*). The blocks were polymerized for 3 days at graded temperatures (37° to 60°C), sectioned with a Porter-Blum MT-2 microtome, and examined with an electron microscope (RCA EMU 3E).



Figs. 1 to 4. Electron micrographs of cells fixed in osmium tetroxide (Figs. 1, 3, 4) or in glutaraldehyde (Fig. 2) and embedded in Epon 812. Fig. 1. A primary lysosome showing finely granular texture and the unit membrane. (× 23,500) Fig. 2. This cell was incubated in Gomori medium. The dense reaction products indicate the presence of acid phosphatase. (× 23,500) Fig. 3. A cluster of lysosomes with interspersed rough endoplasmic reticulum. The short arrow identifies one lysosome with closely associated rough endoplasmic reticulum. The structure indicated by the long arrow is probably a microbody. (× 12,500) Fig. 4. A lysosome showing continuity with rough endoplasmic reticulum (*RER*). (× 23,500)

At the ultrastructural level these vacuoles consist of fine granular material enveloped in a unit membrane (Fig. 1). When ciliates are incubated in Gomori medium (*3*) to demonstrate acid phosphatase activity, dense deposits of reaction products appear in these vacuoles (Fig. 2). They fulfill the description of primary lysosomes (*2*) and are therefore defined as such. They are easily distinguished from secondary lysosomes (autophagic vacuoles, phagosomes, or residual vacuoles) which are usually much larger and contain materials such as cellular particulates and partially digested food.

Primary lysosomes often appear in clusters (Fig. 3) and are always associated with rough endoplasmic reticulum and mitochondria. On rare occasions a vacuole, identical in structure to primary lysosomes, can be seen attached to rough endoplasmic reticulum (Figs. 3 and 4). The ribosomes apparently are lost from the region of the RER that gives rise to this vacuole. This is suggestive evidence that primary lysosomes arise from rough endoplasmic reticulum in *T. pyriformis*. Hydrolases synthesized at the ribosomes on the RER could then pass to the expanded region of the RER which pinches off as a primary lysosome. In mammalian secretory cells and neurons, primary lysosomes are thought to arise from Golgi vacuoles which are smooth endoplasmic reticulum (*5*).

It is generally agreed that enzymes are synthesized at the ribosomes of the RER and that the endoplasmic reticulum and Golgi apparatus are involved in their transport to active sites (*2*, *5*). The Golgi apparatus is usually described as smooth-surfaced, flattened sacs arranged in a typical pattern. These sacs package hydrolases and later release them into secondary lysosomes where they promote digestion (*2*). The Golgi apparatus has been reported in most cells examined, from protozoa to man. However, no such complex structures have been observed in *T. pyriformis* (*1*). Undoubtedly, hydrolases are produced in the same manner as in other cells but they may be packaged directly from rough endoplasmic reticulum. This would represent an alternative method for handling the transport of hydrolases. No evolutionary significance can be assigned to this observation since sarcodinids, flagellates, and sporozoa have well-defined Golgi apparatuses (*6*) which presumably function as they do in multicellular organisms.

ALFRED M. ELLIOTT
Department of Zoology,
University of Michigan, Ann Arbor

References and Notes

1. A. M. Elliott and I. J. Bak, *J. Protozool.* **11**, 250 (1964).
2. C. de Duve, *Federation Proc.* **23**, 1045 (1964).
3. G. Gomori, *Microscopic Histochemistry, Principles and Practice* (Univ. of Chicago Press, Chicago, 1952).
4. J. H. Luft, *J. Biophys. Biochem. Cytol.* **9**, 409 (1961).
5. A. B. Novikoff, E. Essner, N. Quintana, *Federation Proc.* **23**, 1010 (1964).
6. D. R. Pitelka, *Electron Microscopic Structure of Protozoa* (Macmillan, New York, 1963).
7. Supported by NIH grant AI 01416-13. I thank Gretchen Clemmons and Janis Work for technical assistance.

28 June 1965