the detection of the 1550-base transcript in 16- to 17-day testis RNA, we can conclude that the 2100-base α-tubulin transcript is absent even when the gel is overexposed. Since the sensitivity of detection of the pILαT1 cDNA clone is four times that of probe pRDαTT.3, we conclude that the number of 2100-base transcripts is increased by a factor of at least 132 between the prepuberal pachytene spermatocyte and the round spermatid. All of the α-tubulin RNA transcripts are much less abundant in elongating spermatids, and they consistently show a faster migration rate in agarose gels, as is also seen with other haploid mRNA's including one encoding a protamine (*12, 13*). Analysis of polysomal and nonpolysomal testicular RNA's (*13*) reveals that both the 2100- and 1550-base transcripts are present on polysomes (data not shown).

The absence of transcripts homologous to pRDαTT.3 in mouse brain suggests that the testicular and brain α-tubulins are from different genes. This is substantiated by the comparison of Southern blots probed with pRDαTT.3 and pILαTIII. Furthermore, pILαTIII hybridizes to neither the 2100- nor the 1550-base testicular transcripts. Multiple α-tubulin mRNA's are found in sea urchin testes (*15*), and a testis-specific β-tubulin is present in *Drosophila* (*16*). However, in contrast to the *Drosophila* tubulin, which is expressed throughout spermatogenesis, we detect transcripts from this mouse α-tubulin gene only in haploid cells. Electrophoretic studies of the polypeptides synthesized "in vivo" in purified mouse spermatogenic cells substantiate the synthesis of multiple tubulins in haploid cells (*17*).

The appearance of novel α-tubulin transcripts in round spermatids coincides with the formation of the manchette and flagellar axoneme. Thus, the haploid-specific tubulin mRNA may be involved in these structures, both of which are unique to spermatogenesis. It is unlikely that pRDαTT1 is involved in mitotic or meiotic spindles, neural cytoskeleton, or cytoskeletal structures, since it is absent from brain and from prepuberal and meiotic testicular cells.

The genetic implications of mRNA's transcribed in haploid spermatogenic cells are unclear because the haploid cells are interconnected by cytoplasmic bridges that could allow the interchange of haploid gene products. Nevertheless, the existence of genetic effects of haploid mRNA's is strongly suspected because of the unbalanced transmission of t alleles to the progeny of male mice (*18*). The gene products of mRNA's transcribed during the haploid phase of spermatogenesis could provide a means of selecting sperm of a particular genotype. The identification of specific haploid mRNA's (*12, 13*) marks a first step in understanding haploid gene expression at a molecular level.

ROBERT J. DISTEL
KENNETH C. KLEENE
NORMAN B. HECHT*

Department of Biology,
Tufts University,
Medford, Massachusetts 02155

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* To whom requests for reprints should be sent.

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Entamoeba histolytica: A Eukaryote Without Glutathione Metabolism

Abstract. Entamoeba histolytica *was found to grow normally without producing glutathione and the main enzymes of glutathione metabolism, indicating that glutathione is not essential for many eukaryotic processes. This parasitic amoeba is an unusual eukaryote whose special features may help define the crucial functions of glutathione in those eukaryotes that do use it. Since* Entamoeba histolytica *lacks mitochondria and the usual aerobic respiratory pathways, the finding that it grows without glutathione and other evidence support the hypothesis that a primary function of glutathione in eukaryotes involves protection against oxygen toxicity associated with mitochondria and suggest that eukaryotes may have acquired glutathione metabolism at the time that they acquired mitochondria.*

Glutathione (GSH) was once thought to be present in millimolar concentrations in all cells and to be essential for cell function (*1*), but the specific essential functions of glutathione have remained elusive since Rapkine first postulated that it served to regulate cell division (*2*). Mutants of *Escherichia coli* have been isolated that are deficient in the synthesis of GSH but which grow normally (*3*), and many species of bacteria grow without producing measurable GSH (*4*). Thus GSH does not have a general role in prokaryotic life. There is, then, a question of whether it has an essential function in eukaryotes. The eukaryotes that have been examined produce either GSH or its close relative homoglutathione (*5, 6*). Some mutants produce lower than normal amounts of GSH, but these residual amounts are usually significant (*7*). We examined the low molecular weight thiols of *Entamoeba histolytica*, a parasitic amoeba responsible for widespread amoebic disease (*8*), and found that this amoeba grows without producing or using glutathione. We believe this to be the first example in which a eukaryote has been shown to grow under standard laboratory conditions without producing gluthathione.

Table 1. Thiol components found in conditioned, glutathione-depleted growth medium and in trophozoites of the HM-1 strain of axenic *E. histolytica* grown in glutathione-depleted medium.

Thiol component	Total in medium* (m*M*)	Trophozoites (nmoles per 10^6 cells)	
		Thiol	Total*
Cysteine	0.5	5.1	6.8
GSH	< 0.01	< 0.025	< 0.08
Unidentified	0.4	1.3	3.7

*The sample was electrolytically reduced before deproteinization and labeling with monobromobimane.

Entamoeba histolytica strain HM-1 was grown on the TYI-S-33 medium of Gillin and Diamond (*9*). This medium contains yeast extract that is rich in glutathione and, since *E. histolytica* incorporates substantial quantities of medium through endocytosis (*10*, pp. 11–17), cells grown on this medium contain

significant amounts of glutathione. Medium specifically depleted of glutathione was obtained by addition of γ-glutamyl transpeptidase (1 U/ml) and incubation for 3.5 hours at 37°C before autoclaving. The growth rate of *E. histolytica* in glutathione-depleted medium was equal to that of controls for at least 7 or 8 generation times, at which time cells were harvested for analysis.

Thiol components were analyzed by fluorescent labeling of the thiol with monobromobimane and separation of the resulting derivatives by high-performance liquid chromatography (HPLC) (*5, 11*). The thiol components in the growth medium were present predominantly as oxidized forms that could be electrolytically reduced in 0.1*M* methanesulfonic acid by a system similar to that described by Saetre and Rabenstein (*12*) operated at a current of 6 mA for 15 minutes. *Entamoeba histolytica* (~ 4×10^6 cells) was extracted in 0.5 ml methanesulfonic acid (0.2*M*) plus 0.5 ml sodium methanesulfonate (4*M*), the precipitate removed by centrifugation, and the *p*H adjusted to 8 in the presence of 2 m*M* monobromobimane. To determine total thiol components, the sample was electrolytically reduced before addition of monobromobimane.

Chromatograms (Fig. 1) were obtained from electrolytically reduced samples of the conditioned growth medium and of acid extracts of *E. histolytica*. Cysteine is the main component in both, and a series of unidentified thiol derivatives, labeled 1 through 5, occur in similar ratios in the medium and in the cells. Components 1 and 5 were shown to occur in the Trypticase used to prepare the growth medium, and the remaining components were found to be formed during autoclaving of the medium. Glutathione, which is eluted about 2 minutes after component 2, is not detectable in conditioned medium or in cells. To show that thiols are not lost or degraded during the extraction process, glutathione, γ-glutamylcysteine, cysteinylglycine, homocysteine, and pantetheine (1 μmole/g), along with ergothioneine (10 μmole/g), were added to a cell sample during the extraction. Subsequent analysis showed 80 to 100 percent recovery of these components.

Quantitative data from the thiol analyses are given in Table 1. Neither GSH nor γ-glutamylcysteine, which is eluted at 12.5 minutes (Fig. 1), were present at significant amounts in *E. histolytica*. This is not the result of a deficiency in required precursors for GSH synthesis since the medium contains more than 40 m*M* of glutamic acid and more than 10 m*M* of glycine (*9*), and the cells contain abundant cysteine (Table 1) and ~ 0.7 m*M* of adenosine triphosphate (*13*). The enzymes of GSH synthesis thus appear to be absent or inhibited. Synthesis of such enzymes might be repressed or their activity inhibited during growth on medium containing glutathione, but why this should occur during extended growth on medium deficient in glutathione is not clear. A more probable explanation is that *E. histolytica* is not able to produce the enzymes for GSH synthesis.

The central enzymes that use GSH also appear to be absent in *E. histolytica*. Cells grown on normal TYI-S-33 medium were found to contain about 0.7 nmole of total glutathione per 10^6 cells, but only one-third of this was in the reduced form. Thus, *E. histolytica* does not efficiently reduce the glutathione acquired from the growth medium. Assay of cell lysates for glutathione reductase and γ-glutamyl transpeptidase failed to detect measurable activity (*14*). It was previously reported (*15*) that glutathione peroxidase activity is very low in *E. histolytica*.

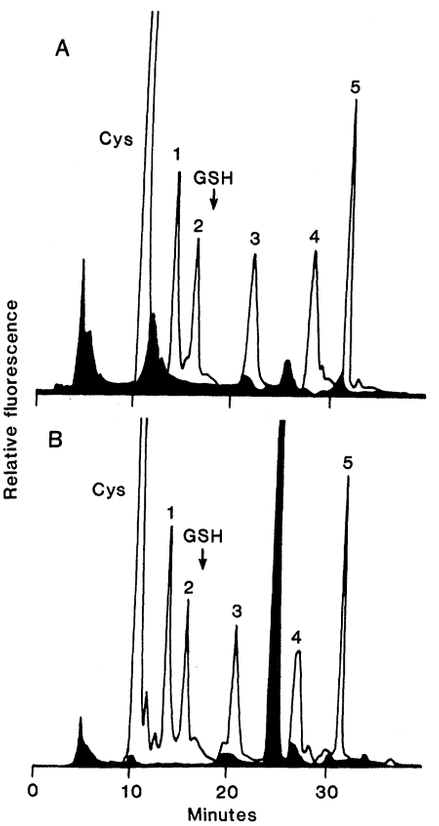

Fig. 1. High-performance liquid chromatrophy chromatogram of monobromobimane-labeled thiols obtained from electrolytically reduced samples of (A) conditioned growth medium (glutathione-depleted) and (B) trophozoites of *E. histolytica* grown on glutathione-depleted medium. Filled portion represents the chromatograms obtained in control experiments in which the sample was treated with 2-pyridyl disulfide before reaction with monobromobimane and thus represents fluorescence due to nonthiol components. A water-methanol solvent system was used in conjunction with a C-18 reversed-phase column (*5*) and only the first 35 minutes of the chromatogram is shown. Abbreviations: Cys, cysteine; GSH, glutathione; 1 to 5, unidentified components.

Cysteine is the main thiol component in *E. histolytica* and is present largely in the thiol form (Table 1). Components 1 through 5 occur mainly in nonthiol forms indicating that they, like glutathione, are not efficiently reduced by *E. histolytica*. Other thiol components in electrolytically reduced extracts included coenzyme A (~ 0.4 nmole per 10^6 cells), which is eluted late as a broad peak and was best analyzed by ion-pairing HPLC methods (*5*), and pantetheine (~ 0.07 nmole per 10^6 cells). Hydrogen sulfide was not found in acid extracts, being largely lost during the extraction, but when cells were lysed in *N*-ethylmorpholine buffer (*p*H 8) containing 2 m*M* of monobromobimane and then deproteinized with acid (*5*), a very large peak was observed at 72 minutes, corresponding to hydrogen sulfide at a concentration of ~ 5 nmole per 10^6 cells. This value is comparable to that reported earlier for the sulfide content of *E. histolytica* and is presumed to derive from the high level of iron-sulfur protein in this organism (*16*).

Since *E. histolytica* absorbs nutrients, carries out protein and nucleic acid synthesis, and undergoes cell division—all without producing or using GSH—glutathione does not have a specific and essential role in these processes. Because *E. histolytica* is an atypical eukaryote, its unusual characteristics help define the crucial function of GSH in eukaryotes that do use it. *Entamoeba histolytica* lacks mitochondria and the usual aerobic respiratory pathways (*17*). Mitochondria have been shown, on the basis of RNA and protein sequence data (*18, 19*), to be most closely related to purple photosynthetic and related bacteria (*19*), a group that includes those Gram-negative facultative anaerobes and aerobes that produce glutathione (*4, 5*). This suggests that eukaryotes may have acquired glutathione metabolism at the same time that they acquired mitochondria and that GSH may be essential to mitochondrial function in eukaryotes. In accord with this, Meredith and Reed (*20*) reported that isolated rat hepatocytes recover from drug-induced depletion of cytoplasmic GSH but do not survive depletion of the separate mitochondrial GSH pool. Meister and Griffith (*21*) found that treatment of rats with drugs which deplete both cytoplasmic and mitochondrial GSH pools in the liver is lethal, whereas treatment that depletes only cytoplasmic

GSH is not. Meredith and Reed (*20*) also reported that depletion of mitochondrial GSH is accompanied by increased lipid peroxidation, indicating that the antioxidant function of GSH in mitochrondria may be its most critical role.

Entamoeba histolytica is also unusual in that it uses a more primitive form of cell division than higher eukaryotes and appears to lack microtubules (*10*, chapter 2). Thus, we cannot exclude the possibility that GSH plays a specialized role in microtubule function or mitosis in some higher eukaryotes (*22*). In many respects *E. histolytica* resembles anaerobic or microaerophilic bacteria more than it resembles most other eukaryotes. It does not tolerate normal oxygen tensions (*23*), has a high level of iron-sulfur protein (*14*), and does not produce glutathione. It meets many of the criteria outlined by Margulis (*24*) as characteristics of protoeukaryotes, the primitive anaerobes that served as cytoplasmic host in the symbiotic acquisition of mitochondria (*22*). Data from RNA and protein sequence studies may help us understand how *E. histolytica* is related to prokaryotes and to higher eukaryotes.

ROBERT C. FAHEY*
GERALD L. NEWTON
Department of Chemistry, University of California, San Diego, La Jolla 92093

BRADLEY ARRICK
TOBIE OVERDANK-BOGART
STEPHEN B. ALEY†
Laboratory of Cellular Physiology and Immunology, Rockefeller University, New York 10021

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* To whom requests for reprints should be sent.
† Present address: Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md. 20205.

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Schwann Cell Galactocerebroside Induced by Derivatives of Adenosine 3′,5′-Monophosphate

Abstract. *Indirect immunofluorescence was used to show the presence of galactocerebroside (galC), a lipid found in myelin, on the surface of about half of the Schwann cells isolated from neonatal rat sciatic nerves and cultured for 1 day without neurons. By day 4 in vitro, the Schwann cells had all lost their surface galC. Three days after beginning treatment with 10^{-3} molar 8-bromo-adenosine 3′,5′-monophosphate (8-bromo cyclic AMP) or $N^6,O^{2'}$-dibutyryl adenosine 3′,5′-monophosphate (dibutyryl cyclic AMP), galC reappeared on the Schwann cells, and 2 days later 48 percent of the cultured Schwann cells showed surface galC. Tritium from tritiated D-galactose was incorporated into galC by the 8-bromo cyclic AMP– and dibutyryl cyclic AMP–treated cultures at a rate 15 times the control rate.*

Schwann cells synthesize myelin, a differentiated, galactolipid-rich form of plasma membrane, only when they are in contact with appropriate axons (*1*). The nature of the axonal signal that triggers Schwann cell myelin synthesis is not known.

Derivatives of adenosine 3′,5′-monophosphate (cyclic AMP) in millimolar concentrations stimulate biosynthesis of

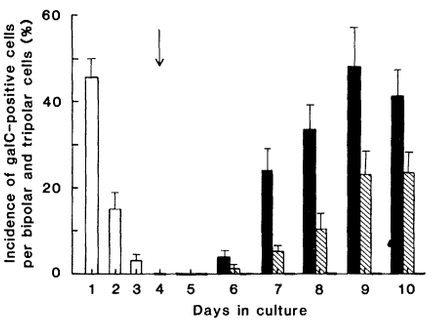

Fig. 1. Time course of disappearance of surface galC from cultured neonatal rat Schwann cells and of reappearance of this lipid upon treatment of the cultures with $10^{-3}M$ 8-bromo cyclic AMP (solid bars) or $10^{-3}M$ dibutyryl cyclic AMP (striped bars). The cyclic AMP derivatives were added to the medium on day 4 of culture (indicated by arrow). The incidence of galC-positive Schwann cells in control cultures without treatment is indicated by open bars. Results were expressed as the mean ± standard deviation of 5 to 10 determinations. Cover slips were processed at the times indicated. Each cover slip was rinsed with phosphate-buffered saline (PBS) ($1.4 \times 10^{-1}M$ NaCl, *p*H 7.4) then exposed to a 1:20 dilution of rabbit antiserum to galC for 20 minutes at 25°C. After being rinsed with PBS, the cover slips were exposed to a 1:50 dilution of rhodamine-conjugated goat antiserum to rabbit immunoglobulin G (Cappel) for 20 minutes at 25°C. The cover slips were exposed to 5 percent acetic acid in ethanol at 5°C for 5 minutes, then mounted in glycerol.