oxygen consumption and did not ameliorate the damage to the mTAL induced by amphotericin (Table 1).

These results support the view that anoxic injury in cells of the mTAL is strongly conditioned by the rate of active ion transport. The traditional view of anoxic injury emphasizes the role of oxygen deprivation. However, in situations like those reported here, the consequences of anoxia may depend more on the rate of energy demand than on the degree of limitation of oxygen delivery. The polyenes did reduce oxygen delivery because of renal vasoconstriction (see Table 1). However, ouabain did not improve renal flow, and its protective effect was therefore presumably mediated entirely by a decrease in oxygen demand for active transport. In analogous experiments, we showed that mTAL injury produced by hypoxia or potassium cyanide in isolated perfused kidneys is prevented by decreasing active transport with ouabain or furosemide or by halting glomerular filtration (*12*).

A similar phenomenon in neurons has been described, in which synaptic activity potentiates anoxic damage (*16*). Persisting mitochondrial activity and continued electron flow in the absence of an oxygen sink may be associated with abnormal handling of charges in the process of energy transformation, leading to the increased formation of free radicals, as suggested for other cells (*17, 18*).

In summary, polyene toxicity is not a simple consequence of altered cell membrane permeability, since in isolated kidneys, polyene-induced injury to the mTAL depends on continued active transport. This injury appears to derive from an imbalance between limited oxygen availability and high oxygen demand (*19*).

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10. At 15 and 30 minutes of perfusion, while moderate to severe damage was already extensive, involving 95 ± 5 percent of mTAL's, other nephron segments showed only minimal to mild injury. Specifically, small apical vesicles were observed in proximal tubules that were otherwise normal. At 90 minutes, while damage was severe in 100 percent of mTAL's, limited cell injury was seen in some proximal tubules, involving brush border disarray and mitochondrial swelling in parts S_1 and S_2 of the proximal tubules and focal cell fragmentation in part S_3. Thus, although limited injury was apparent in the renal cortex, by far the most extensive and essentially irreversible damage was found in the mTAL's.
11. This contrasts with the paucity of morphological damage from amphotericin in vivo (*1*). Although drug concentrations, time periods, and modes of tissue fixation were different, a more likely explanation is the special conditions created by isolated perfusion. Thus, the small oxygen-carrying capacity of the perfusate (*6*) and the probable blunting of neurohumoral signals (for feedback regulation of glomerular filtration) may act in concert to expose the vulnerability of the mTAL to continued transport work during limited oxygen supply (*5, 6*). In vivo, decreased glomerular filtration and reabsorptive work would attenuate or prevent tubular necrosis from anoxia in this area of the kidney (*5, 12*).
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Haploid Expression of a Mouse Testis α-Tubulin Gene

Abstract. *A complementary DNA clone for an α-tubulin has been isolated from a mouse testis complementary DNA library. The untranslated 3′ end of this complementary DNA is homologous to two RNA transcripts present in postmeiotic cells of the testis but absent from meiotic cells and from several tissues including brain. The temporal expression of this α-tubulin complementary DNA provides evidence for the haploid expression of a mammalian structural gene.*

Spermatogenesis in mammals is a continuous process in which mitotic proliferation of spermatogonia is followed by meiosis and differentiation of haploid spermatids into mature spermatozoa (*1*). The major morphological changes resulting in the characteristic spermatozoan shape occur during the haploid phase (*1*).

As seen in other cellular morphogenic processes, the differentiating spermatogenic cells contain several distinct microtubular structures. These include the mitotic and meiotic spindles and two haploid structures, the manchette and the flagellar axoneme. These microtubular structures are assembled from heterodimers of α- and β-tubulin, evolutionarily conserved proteins that have both developmental and tissue heterogeneity (*2–4*).

To determine when specific tubulin genes are expressed during the differentiation of the spermatozoon, we looked for the appearance of tubulin messenger RNA (mRNA) transcripts in meiotic and postmeiotic testicular cells. We have isolated from a mouse testis complementary DNA (cDNA) library an α-tubulin cDNA clone, the 3′ end of which is homologous to at least two different α-tubulin RNA transcripts. These transcripts are detectable only during the haploid phases of spermatogenesis.

A cDNA library was derived from mouse testis by priming polyadenylated [poly(A)$^+$] RNA with oligothymidilic acid. The resulting DNA fragments were cloned into the Sal I and Eco RI sites of plasmid pUC8 by means of linkers (*5*). After screening the cDNA library by colony hybridization (*6*) with a ^{32}P-labeled 1650–base pair (bp) insert of a clone containing the coding sequence and 3′ untranslated sequences from rat brain α-tubulin mRNA (designated pILαT1) (*7*), we obtained a colony containing an insert approximately 1000 bp long (called pRDαTT1). Plasmid pRDαTT1 proved homologous by Southern hybridization (*8*) to much of the rat brain clone pILαT1 but showed no detectable hybridization to the 3′ untranslated region of the rat α-tubulin sequence (the 3′ untranslated region of pILαT1 is hereafter called pILαTIII). On the basis of observations by others that the 3′ untranslated regions of tubulins hybridize to specific transcripts (*4, 9–11*), we sub-

cloned a 360-bp Sal I fragment containing the 3′ untranslated end of the mouse testicular pRDαTT1 DNA into the Sal I site of pUC8 (*5*) (the resulting plasmid is hereafter called pRDαTT.3). The remainder of pRDαTT1 was renamed pRDαTT.7.

Although pRDαTT.3 showed no homology to the rat tubulin clone pILαT1, both pRDαTT.7 and pRDαTT.3 proved homologous to genomic α-tubulin genes of mouse (Fig. 1). When hybridized to mouse sperm DNA previously digested with Pst I, pILαT1 and pRDαTT.7 yielded almost identical results, hybridizing to at least ten bands. This is consistent with the observation that α-tubulin is present in multiple copies in the genome of mammals (*3, 4, 7, 9–11*). A band of 1.4 kilobases (kb) (arrow) detected by pILαT1 was not seen with pRDαTT.7, presumably because pRDαTT.7 is missing its 5′ end. pRDαTT.3 is homologous to a subset of the α-tubulins seen when the translated region, pRDαTT.7, is used as a probe as well as to three new bands at 4.0, 1.8, and 1.3 kb. When we hybridized rat pILαTIII to Pst I–digested mouse DNA, we found homology to at least five α-tubulin bands but limited homology to those bands selected by pRDαTT.3, in agreement with our earlier observations. Similar results were seen when mouse sperm DNA was digested with restriction enzymes Bam HI or Ava I.

The tissue specificity of RNA homologous to pRDαTT.3 was analyzed by RNA blot hybridization (*12*). Plasmid pRDαTT.3 hybridized to transcripts of about 2100 and 1550 bases in poly(A)$^+$ RNA from mouse testis (Fig. 2). In contrast, no homologous transcripts were detected in 6 μg of poly(A)$^+$ RNA from mouse brain [hybridizing to ^{3}H-labeled polyuridylate [poly(U)] of 42,000 count/min (*12*)]. Since pRDαTT.3 can detect transcripts in testis RNA hybridizing to ^{3}H-labeled poly(U) at only 500 count/min, pRDαTT.3 must detect at least 84 times as many transcripts in testis as in brain. The filter was washed to remove the DNA probe (*13*), and then the same blot was rehybridized to pILαT1, which is the probe for the entire rat brain α-tubulin mRNA. RNA sequences of 2100, 1550, and 1650 bases were detected in poly(A)$^+$ RNA from mouse testis, whereas poly(A)$^+$ RNA from mouse brain contained two sequences of about 2200 and 1750 bases. Hybridization with the mouse plasmid, pRDαTT.7, produced identical results. Hybridization of 15 μg of total RNA from mouse kidney, spleen, ovary, lung, and liver with pRDαTT.3 did not detect the RNA's of lengths 2100 and 1550 bases, transcripts that are readily detectable in an equivalent amount of total testis RNA. Hybridization of up to 6 μg of poly(A)$^+$ and poly(A)$^-$ RNA from mouse testis or mouse brain with either the pILαT1 or pRDαTT.3 probe indicated that most of the α-tubulin transcripts are polyadenylated (data not shown).

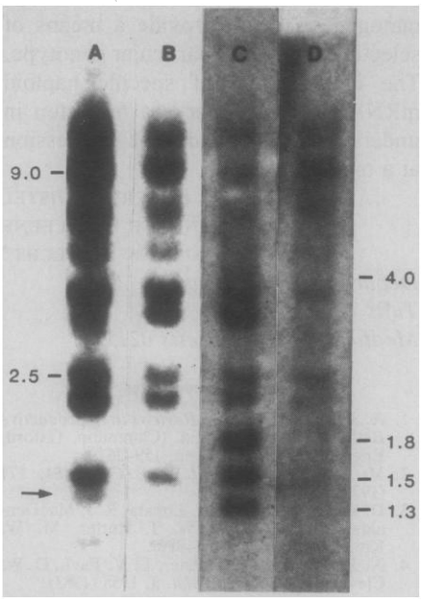

Fig. 1. Hybridization of Pst I–digested mouse sperm DNA with α-tubulin cDNA clones. Mouse sperm DNA was isolated from epididymal sperm and digested with Pst I. DNA (10 μg per lane) was subjected to electrophoresis in a 1 percent agarose gel; transferred to nitrocellulose filters (*8*); hybridized in 0.4*M* sodium phosphate buffer (*p*H 7.0), double-strength Denhardt's solution, and salmon sperm DNA (100 μg/ml) at 65°C for 16 hours; washed several times in fresh 0.4*M* phosphate buffer and 1 percent sodium dodecyl sulfate (SDS) at 65°C; and then washed for 1 hour in 0.04*M* phosphate buffer and 1 percent SDS at 65°C. Each lane was hybridized to a different ^{32}P-labeled (*12*) α-tubulin probe: (A) pILαT1, (B) pRDαTT.7, (C) pRDαTT.3, and (D) pILαTIII, consisting of 195 bp of the 3′ end of pILαT1 derived by digestion with Mbo II (*9*).

To determine when during spermatogenesis the testicular α-tubulin transcripts were present, we isolated RNA from pachytene spermatocytes (meiotic cells), round spermatids (early haploid cells), and elongating spermatids (later haploid cells) that had been purified by unit gravity sedimentation in bovine serum albumin and centrifugation in Percoll (*12*). RNA homologous to pRDαTT.3 was detected in round spermatids and elongating spermatids, but not in pachytene spermatocytes (Fig. 2). Round spermatids contained detectable amounts of both the 2100- and 1550-base transcripts and an RNA that comigrated with the poly(A)$^-$ form of the 2100-base RNA. Elongating spermatids contained reduced amounts of the RNA's. Rehybridization of this blot with pILαT1 and its overexposure confirmed that the 2100-base transcript was absent in pachytene spermatocytes and present in round spermatids; an additional 1650-base transcript was present throughout spermatogenesis.

To confirm the absence of the 2100- and 1550-base α-tubulin transcripts in meiotic cells, we examined testicular poly(A)$^+$ RNA from 16- to 17-day-old mice. In these animals, spermatogenesis had proceeded only as far as the pachytene spermatocyte (*14*). When the probe pRDαTT.3 was hybridized to 6 μg of prepuberal testis poly(A)$^+$ RNA [hybridizing to ^{3}H-labeled poly(U) of 66,000 count/min], neither the 2100- nor the 1550-base transcripts were detected (Fig. 2). Since 25 percent of this RNA is from pachytene spermatocytes, we conclude that the 2100- and 1550-base transcripts increase by a factor of at least 33 between the prepuberal pachytene spermatocyte and round spermatid. Rehybridization with pILαT1 clearly showed an abundance of α-tubulin transcripts of 1650 bases in prepuberal testis poly(A)$^+$ RNA. Although this obscures

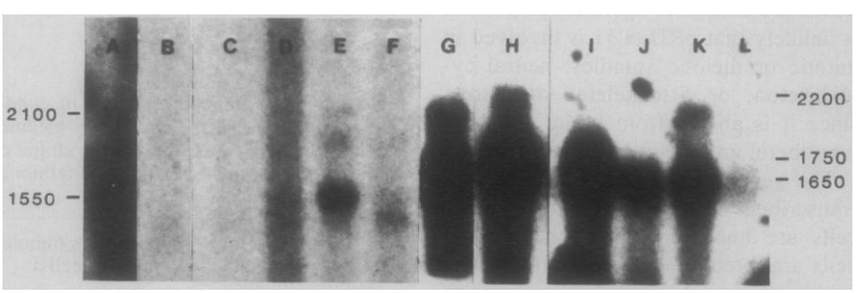

Fig. 2. Size estimate of α-tubulin mRNA recognized by mouse testicular and rat brain cDNA probes. RNA (6 μg per lane) was denatured with glyoxal, subjected to electrophoresis in a 1.5 percent agarose gel, and transferred to nitrocellulose (*12*). Filters were hybridized to pRDαTT.3 as described in Fig. 1, washed (*13*), and rehybridized to pILα1. The sources of RNA, all from mouse, were (A) total testis poly(A)$^+$, (B) brain poly(A)$^+$, (C) 16-day-old prepuberal testis poly(A)$^+$, (D) pachytene spermatocyte, (E) round spermatid, and (F) elongating spermatid. Lanes A to F were hybridized to pRDαTT.3; lanes G to L were rehybridized to pILαT1.

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.

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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