

of alkyltransferase than T cells or other human tissues and, after chemotherapy, rapidly proliferate to renew the progenitor compartment (7, 8, 11, 15), thereby increasing the risk of mutagenesis. Thus, inadequate amounts of alkyltransferase may be responsible for the development of secondary acute myeloid leukemias in patients who receive chloroethylnitrosourea- or procarbazine-based chemotherapy (27). In view of our findings with experimental tumors, a clinical strategy in which MGMT is introduced into hematopoietic precursors by gene therapy methods may merit consideration. Efficient expression of MGMT may increase bone marrow resistance to the cytotoxic and leukemogenic effects of nitrosoureas and potentially benefit patients receiving these agents.

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- The chimeric gene was constructed as follows. (i) The 340-bp avian β -actin promoter was isolated from pUC18 β -actin (28) and inserted into the unique Sal I site of plasmid Cla12N (28). (ii) The 702-bp MGMT cDNA was inserted into the Bam HI site of Cla12N. (iii) The 710-bp Sma I-Eco RI fragment from the bGH gene, which includes a portion of the fifth exon and the poly(A) region (19), was inserted into the Sma I-Eco RI sites of Cla12N. (iv) The 1903-bp Cla I fragment with all of these sequences was ligated into a Bluescript M13 plasmid containing a 2-kb Hind III fragment of the human CD2 locus control region [D. R. Greaves, F. D. Wilson, G. Lang, D. Kiousis, *Cell* 56, 979 (1989)]. The 3933-bp fragment containing the transgene was isolated after digestion with Apa I and Spe I. Purified DNA (50 ng/ml) was microinjected into single-cell embryos from pregnant (C57BL/6 x SJL)_{F1} mice 8 hours after fertilization. The embryos were then reimplanted into pseudopregnant mice. We predicted that the β -actin promoter would allow expression of the transgene in a number of tissues, that the bGH poly(A) region would confer mRNA stability (19), and that the CD2 locus control region would target expression to T cells in the thymus, as shown for the β -globin-CD2 chimeric gene.
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Terrestrial Soft-Bodied Protists and Other Microorganisms in Triassic Amber

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Protozoa, cyanobacteria, sheathed algae, sheathed fungi, germinating pollen or spores, and fungal spores have been found in amber 220 to 230 million years old. Many of these microorganisms can be assigned to present-day groups. This discovery of terrestrial, soft-bodied protists that can be referred to modern groups indicates that morphological evolution is very gradual in many protists and that both structural and probably functional stasis extend back at least to the Upper Triassic period.

The majority of organisms found in amber have been arthropods that accidentally fell into the sticky sap of resin-bearing trees (1-3). During an examination of Triassic amber from southern Germany, we discovered well-preserved microorganisms, which

formed a biocenose in association with the resin-bearing plant. These microorganisms represent the earliest known soft-bodied, terrestrial protists. Small pieces of amber were removed from layers of Raibler Sandstone on Mount Leiternose in Schliersee, Bavaria, Germany. These deposits belong to the Carnian stage of the upper Keuper succession of the Upper Triassic period and are dated at 220 to 230 million years old (4).

For microscopic observations, small pieces of the amber were crushed, mounted

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in glycerin on microscope slides, and examined with a Nikon optiphot microscope equipped with differential interference contrast. A variety of microorganisms were found in the amber, including ciliate protozoa, amoeba tests, sheathed bacteria, sheathed algae, spores or pollen grains of vascular plants, and fungal spores. Protozoan classification is based on the system by Lee *et al.* (5), and bacterial classification is taken from *Bergey's Manual of Systematic Bacteriology* (6), algal classification from

Bourrelly (7, 8), and fungal identification from Barnett (9).

Ciliate protists, allied to the family Cyrtolophosidae, were frequently observed in the amber matrix. These ciliates are ellipsoid in shape and vary from 45.0 to 55.0 μm in length. The cytostome is located in a small lateral vestibulum near the anterior end. The pellicle is distinct and contains cilia (Fig. 1, A and B). The fossil forms resemble in size, shape, and position of the cytostome representatives of the extant ter-

restrial ciliate genus *Cyrtolophosis*.

A second type of ciliate protist, larger and more ellipsoidal than the previous forms, was placed in the family Parameciidae. These ciliates are ellipsoid in shape, contain a centrally located oral groove, and vary from 136 to 146 μm in length. They resemble members of the present-day genus *Paramecium* (Fig. 1C) (10, 11). A third ciliate (Fig. 1D) represents a more oval morphotype and is 65 μm in length. It resembles smaller species of the genus *Nassula* in the family Nassulidae. The specimen figured appears to be in the process of ingesting a cyanobacterial filament, which extant members of this genus regularly do.

What appear to be tests of amoeba protists allied to the family Centropyxidae were rarely encountered (Fig. 1E). The specimen we found is 36 μm long and has a circular aperture (5.5 μm in diameter) near one end. A pair of short lateral spines appears on the portion of the body opposite the aperture. The structure of the test resembles extant representatives of the genus *Centropyxis*, but the fossil is not referable to any modern species of this genus.

Filaments of representatives of the class Thallobacteria were also found in the amber matrix. One type consisted of individual cells arranged end to end in branched filaments that extended 1 to 2 mm in length. There were indications of a sheath surrounding these cells. The individual cells ranged from 2.8 to 3.4 μm in length and were 1.6 μm in width (Fig. 1F). These organisms were identified as sheathed bacteria and resemble extant representatives of the genera *Crenothrix* and *Sphaerotilus*.

Other filaments could be identified as cyanobacteria belonging to the family Scytonemataceae. These filaments were thick, branched, and extended several millimeters in length. They were composed of a single inner row of cells enclosed within a thick tube. Cells within the inner portion ranged from 1.0 to 1.5 μm in diameter and 3.0 to 4.5 μm in length. The thalli were greatly thickened (4.0 to 11.0 μm in diameter) and were hyaline, homogeneous, and firm (Fig. 1G). Branching or pseudobranching was apparent; no heterocysts were observed. These organisms were identified as sheathed algae and resemble extant representatives of the genus *Scytonema*, especially those of the species *S. alata*.

A third filamentous morphotype resembled green algae in the family Trentepohliaceae. These branching filaments (6.0 to 10.0 μm wide) were encrusted with dense deposits (Fig. 1H). The morphology of these thalli resemble aerial green algae of the extant genus *Trentepohlia*. We interpreted the specimen in Fig. 1I to be a germinating spore or pollen grain on the basis of its size (20 μm in diameter), the

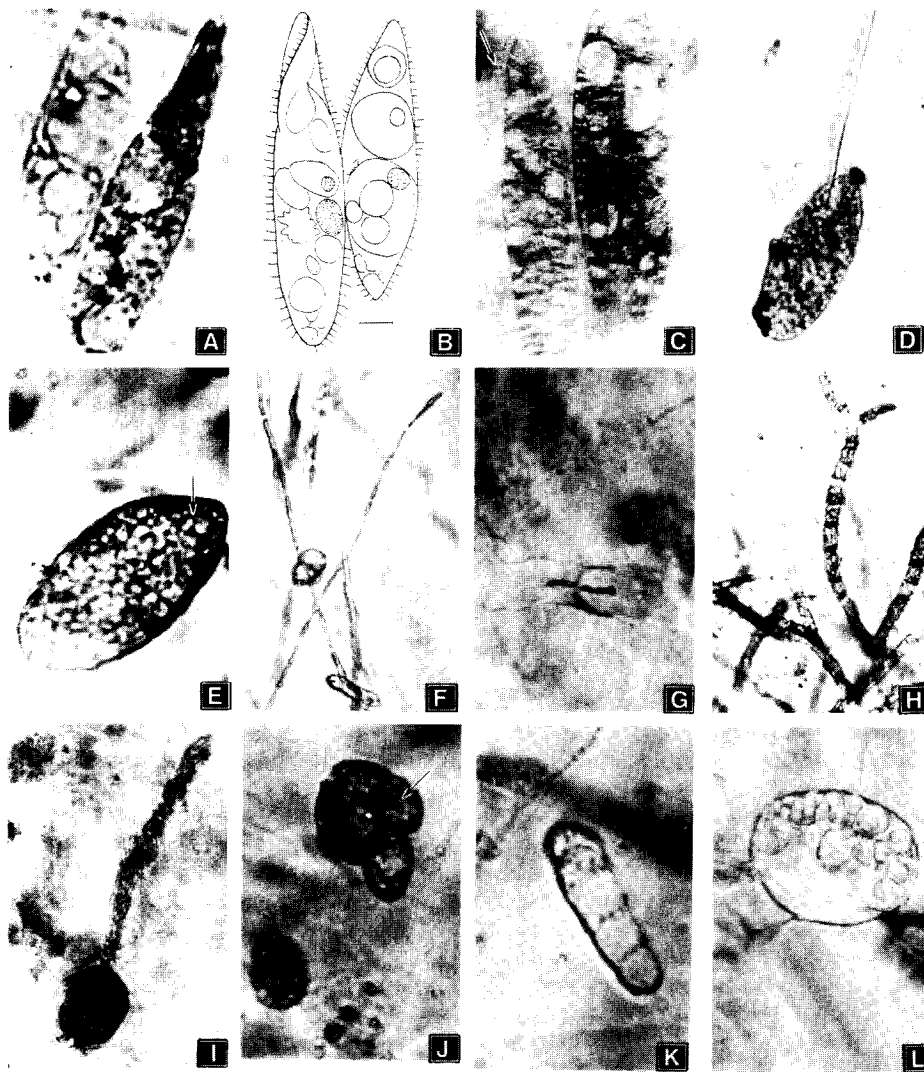


Fig. 1. (A) Two ciliates resembling members of the extant family Cyrtolophosidae (dorsal view). Panel width is 30 μm . (B) Reconstructed drawing of the ciliates in (A) (ventral view). Bar = 5 μm . (C) Three ciliates resembling extant members of the genus *Paramecium*; the arrow shows the region of cilia on one specimen. Panel width is 93.25 μm . (D) Oval-shaped ciliate resembling extant members of the genus *Nassula*. Note the cyanobacterial filament in the process of being ingested. Panel width is 82 μm . (E) Amoeboid test resembling those of extant members of the family Centropyxidae; the arrow points to a circular aperture. Panel width is 33.5 μm . (F) Filaments resembling extant forms of sheathed bacteria. Panel width is 30 μm . (G) Filaments resembling extant groups of sheathed algae. Panel width is 45 μm . (H) Filaments resembling extant forms of branching green algae. Panel width is 112 μm . (I) A possible germinating spore or pollen grain. Panel width is 56 μm . (J) A meiospore in which a protoplast has partially emerged. A second protoplast (arrow) remains in the spore. Panel width is 56 μm . (K) A possible multisepate fungal spore resembling representatives of the present-day Moniliales. Panel width is 33.5 μm . (L) A possible fungal or algal vesicle or oogonium. Panel width is 11.25 μm .

pattern on its thick outer (perhaps exine) wall, and its nonseptate germination tube (12). Another possible pollen grain or meiospore (25 μm in diameter) that contains two protoplasts is also shown (Fig. 1J).

A possible fungal spore (29 μm in diameter) is represented by the four septate, hyaline, ellipsoid structures in Fig. 1K. Similar spores are found in extant representatives of the genera *Dactylium* and *Hyaloflorae* in the Moniliales. Both of these genera contain saprophytic species. A possible fungal or algal vesicle (37 μm in diameter) with zoospores or an oogonium with oospheres (as in the extant genus *Saprolegnia*) is shown in Fig. 1L.

All of these fossils represent a biocenosis comprising a community of organisms that lived on the resin-bearing plant. Although the host plant could not be identified from analyses of the amber (13), it may have been the cycadeoid *Pterophyllum jaeegeri* because plant megafossils in the surrounding Raibler Sandstone were identified as belonging to this species. Some of the organisms shown here probably lived on the surface of the bark or leaves of the resin-producing plant (as do extant representatives of *Scytonema* and *Trentepohlia*). During prolonged periods of rainfall, stagnant water would have formed in bark crevices or branch bases long enough for populations of aquatic or semiaquatic microorganisms (such as ciliates, amoebas, and sheathed bacteria) to become established. Ciliates, especially larger forms, are indicators of eutrophic, often mesosaprobic environments (14, 15); we may presume a similar, nutrient-rich habitat for the fossil organisms. We speculate that these microhabitats were suddenly inundated with resin from the associated plant. The pollen grains and fungal spores could have fallen into the water source or have been blown against the sticky resin.

Bacteria, fungi, and algae are well known from marine rocks (16–20). Isolated bacterial cells have been observed in Tertiary amber (3, 21, 22), a ciliate resembling *Paramecium* was reported in Cretaceous amber (3, 23), and the test of the amoeba, *Prantlinina*, was reported from freshwater sediments of the Namurian (Carboniferous) of Czechoslovakia (24). Molecular studies have suggested that morphological evolution is slow or stationary in several protist groups; morphological stasis has been suggested in *Tetrahymena* (25) and in the amoebas *Acanthamoeba* and *Naegleria* (26) on the basis of deep protein or nucleic acid sequence differences among essentially identical species or strains. That the fossil ciliates and most of the other microorganisms reported here can be referred to modern groups confirms this morphological stasis as far back as 230 million years.

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Presentation of a Viral T Cell Epitope Expressed in the CDR3 Region of a Self Immunoglobulin Molecule

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Synthetic peptides corresponding to microbial epitopes stimulate T cell immunity but their immunogenicity is poor and their half-lives are short. A viral epitope inserted into the complementarity-determining region 3 (CDR3) loop of the heavy chain of a self immunoglobulin (Ig) molecule was generated from the Ig context and was presented by I-E^d class II molecules to virus-specific, CD4⁺ T cells. Chimeric Ig-peptide was presented 100 to 1000 times more efficiently than free synthetic peptide and was able to prime virus-specific T cells *in vivo*. These features suggest that antigenized Ig can provide an improved and safe vaccine for the presentation of microbial and other peptides.

Synthetic peptides can act as antigens for stimulating humoral and cell-mediated immunity. Some problems with the use of peptides as vaccines are short half-lives, poor immunogenicity, and a requirement for Freund's adjuvant (1, 2). The antigen-

binding or CDRs of Ig molecules represent an array of peptides that are as diverse as T cell epitopes and are also comparable in size. We explored the capacity of self Ig to present known microbial epitopes engineered into a CDR loop, given the additional evidence that self Ig molecules have long half-lives and might also be more efficiently internalized by Fc receptors for Ig on antigen-presenting cells (APCs).

We used the 5.5-kb DNA fragment encoding the heavy chain variable region (V_H) of the 91A3 antibody to arsonate (3) in a polymerase chain reaction mutagenesis

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