Quick guide

Tetrahymena thermophila

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What does it look like? *Tetrahymena thermophila* is a unicellular eukaryote which is larger than many mammalian cells (~30 × 50 μm). It swims in temperate freshwater environments, waving its coat of cilia (Figure 1, inset) and phagocytosing food propelled into its gullet. Hunger induces *Tetrahymena* cells to undergo a transformation, including growth of a long posterior cilium and more coordinated propulsion.

Why the specialized nuclei? Ciliates have two types of nucleus with distinct functions. In *Tetrahymena*, the diploid micronucleus is the germline. It replicates and segregates in a conventional cycle of S phase and closed mitosis. The ~120 Mb genome has ~27,500 predicted open reading frames, but is transcriptionally silent during vegetative growth. The ciliate-specific somatic macronucleus is a highly evolved gene expression machine. It differentiates from a mitotic sibling of the micronucleus in four steps: first, elimination of about 15% of the genome (transposons, centromeres and other repetitive elements); second, sequence-specific chromosome fragmentation; third, endoreplication; and fourth, selective amplification. The final tally comes to 45 copies of ~275 chromosomes and 9,000 copies of a unique palindromic chromosome encoding ribosomal RNA. Macronucleus chromosomes partition randomly followed by counting and readjustment of each chromosome's copy number.

How do they mate? Conjugation occurs between nutritionally starved, sexually mature cells of different mating types. Cell pairing induces micronuclei to undergo a program of division, migration, destruction and differentiation (Figure 1). Events analogous to meiosis, gametogenesis, pronuclear migration and fertilization, occur without any cell division, producing a zygotic genome that gives rise to a new micronucleus and macronucleus. DNA elimination occurs in the developing macronucleus, under epigenetic regulation from the parental macronucleus, which is later consumed internally. In subsequent vegetative growth, cells with a heterozygous micronucleus eventually become homozygous in the macronucleus. One pair of mated cells gives rise to progeny that express distinct combinations of macronucleus chromosomes, a process termed phenotypic assortment.

Is* Tetrahymena *amenable to molecular genetic analysis? Yes. Genes in the micronucleus and/or macronucleus can be targeted by homologous recombination. Strains can be generated with a lethal micronucleus genotype yet no phenotype, because of wild-type gene expression in the macronucleus. Such heterokaryons can be maintained indefinitely and induced to express the mutant genotype by mating. Cells can be transformed with a rescue plasmid during mating to assay for complementation. Gene targeting specific to the macronucleus initially affects only a few copies of the wild-type locus, facilitating analysis of deleterious mutations. The copy number of a targeted chromosome bearing a selectable marker can be increased by continued growth under selection. For non-essential genes, somatic knock-out strains are generated; for essential genes, wild-type chromosomes must be retained, so a somatic knock-down strain is generated instead.

What has* Tetrahymena *taught us? *Tetrahymena* has provided many insights about cilia, including the discovery of dynein, elucidation of the cellular principles of self-templated cortical patterning and genetic and cytological analysis of tubulins and tubulin post-translational modifications. Comparative analyses of the macronucleus and micronucleus have yielded histone variants, a

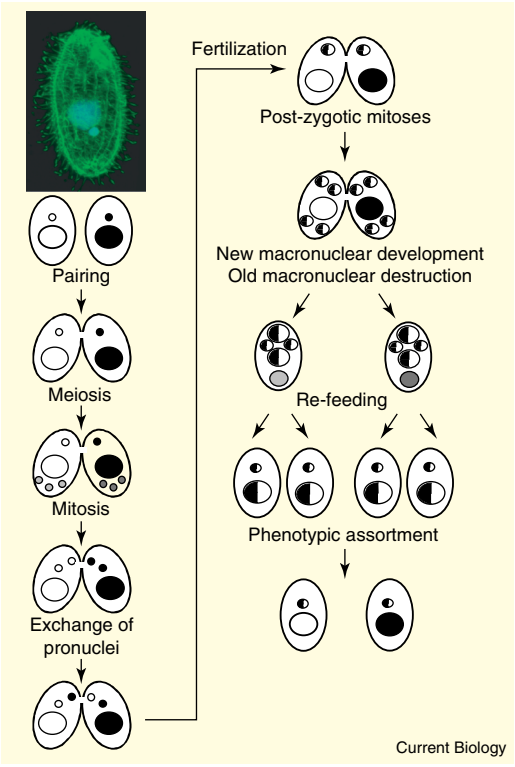

Figure 1. Nuclear dynamics during sexual reproduction of *Tetrahymena*.

The inset shows *Tetrahymena* fixed and co-stained with DAPI to detect the large macronucleus and small micronucleus (blue), and with anti-α-tubulin antibodies to detect microtubules in the numerous cilia (green). In the schematic part of the figure, small circles are micronuclei, large circles and macronuclei and gray circles are nuclei undergoing destruction. White and black shading indicate distinct or mixed genotypes. (Inset courtesy Dorota Wloga and Jacek Gaertig; anti-α-tubulin antibodies from Joseph Frankel.)

histone code of post-translational modifications and other properties of chromatin now recognized to be general features of eukaryotic biology. Other credits include the discovery of self-splicing RNA, the first sequencing of telomeric repeats and the discovery of telomerase. More recently, macronucleus differentiation by DNA elimination revealed a role for an RNAi pathway in heterochromatin formation. Further studies have described avoidance behavior, surface antigen variation and a stunning complexity of microtubule and membrane systems.

Why work with* Tetrahymena*? Robust methods for strain storage, gene targeting and designer genetic crosses have been developed, complementing the long-standing ease of cytology, micromanipulation and biochemistry. *Tetrahymena* entered the model organism pantheon with the recent sequencing and assembly of the macronucleus genome coordinated from The Institute for Genomic Research. Genome sequence, open reading frame predictions, EST sequences and gene indices are free online (http://www.tigr.org/tdb/euk/).

Where can I learn more? The *Tetrahymena* Genome Database (TGD) coordinated from Stanford University (http://www.ciliate.org/) contains a wealth of information, including ciliate literature compiled for text search by keyword or other features. For additional methods and discussion of many fascinating aspects of *Tetrahymena* and ciliate biology, we recommend: *The Molecular Biology of Ciliated Protozoa* (J.G. Gall, Editor, Academic Press, 1986); and *Methods in Cell Biology. Volume 62:* Tetrahymena thermophila (D.J. Asai and J.D. Forney, Editors, Academic Press, 2000).

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All phaged out

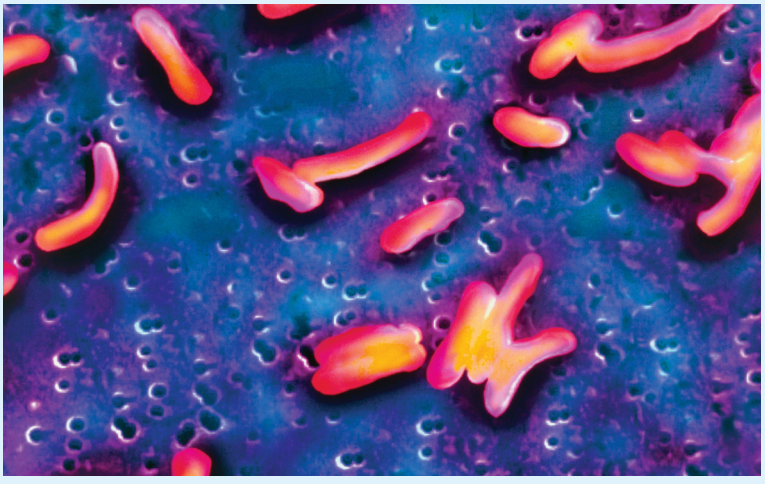
One of the puzzling aspects of devastating cholera outbreaks in areas where the disease is endemic is that they generally appear to fizzle out naturally. In Bangladesh, outbreaks usually occur twice a year, with the highest number of cases just after the summer monsoon and a somewhat smaller number of cases in the spring. It appears that a number of biological and physical factors may affect the survival and abundance of the disease-causing bacteria, *Vibrio cholerae.* But the disease is only carried by the strains O1 and O139 of the bacteria and researchers have puzzled how environmental factors might affect these strains.

Recent work has suggested that predation of *V. cholerae* O1 by bacteriophages may influence this seasonal pattern of epidemics, as it is known that this disease-causing strain is sensitive to a particular lytic bacteriophage, called JSF4. It has, however, been difficult for researchers to get quantitative, dynamic data on the presence of the toxic strain and bacteriophages both in the environment and in patients during the course of an epidemic.

But a team led by Shah Faruque at the International Centre for Diarrhoeal Disease Research in Dhaka, and John Mekalanos at Harvard Medical School in Boston now report a study in the Proceedings of the National Academy of Sciences (published online), describing a quantitative estimate of a pathogenic *V. cholerae* O1 and the lytic bacteriophage JSF4 in environmental water samples and from faeces of patients through the course of an epidemic in Dhaka last year.

The team exploited the fact that the toxigenic *V. cholerae* O1 causing recent epidemics is resistant to multiple antibiotics, including streptomycin. So the team tested for the presence of this strain in samples by selecting for it on culture plates containing streptomycin which would kill non-resistant strains of the bacteria. The team also used this system to monitor the changing prevalence of this strain in relation to that of the JSF4 lytic bacteriophage during the course of the epidemic.

The researchers tested the sensitivity of their assay to detect the pathogenic *V. cholerae* O1 amongst the many other non-pathogenic *V. cholerae* bacteria in their samples by comparing cultures on media containing or not containing streptomycin. Selection on streptomycin plates



Under pressure: The growth in numbers of disease-causing *Vibrio cholera* bacteria strains may be quickly followed by increased numbers of lytic bacteriophages that can drastically reduce their number in field situations. (Picture: Science Photo Library.)