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An olympian protozoan

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Ciliated protozoa of the genus Tetrahymena have provided a uniquely enabling platform for monumental discoveries in the molecular biology of the nucleus.

Introduction

Unicellular eukaryotes have contributed mightily to cell biology and we tend to immediately think of yeast, properly enough. But another group of single-cell organisms has played an exceptional role and continues to do so: ciliates of the genus Tetrahymena. This organism enabled the discovery of the telomeric DNA repeat, telomerase and self-splicing RNA, as well as figuring prominently in the recognition of histone modifications as an epigenetic mechanism. This brief essay summarizes these advances, in honor of those who have given Tetrahymena its place in the pantheon of model systems for the investigation of the nucleus—this its "special feature."

Roots

Tetrahymena was the central focus of a cellular physiology research group at the Carlsberg Laboratories in Copenhagen led by Erik Zeuthen, whose work began to attain international prominence after World War II. Tetrahymena had been cultured in the 1920s but Zeuthen's group refined its cultivation, developed methods for measuring its oxygen consumption in minute samples and also for synchronizing division, the latter work contributing importantly to the early cell cycle field.[1] The reach of Tetrahymena expanded with its adoption by others, many of whom had passed through the Carlsberg group, and it thus proved to be a scientific spore (if not literally a biological one). Amusingly, at a dinner party recently in Woods Hole, Massachusetts, it was suddenly recognized that, as chance would have it, all but one person at the table of seven had at some point worked on Tetrahymena.

Tetrahymena Emerges as a Genetic System

At the base of each cilium in Tetrahymena there is an intricate structure known as a basal body, a particular incarnation of the centriole. Pioneering work by Tracy Sonneborn in Paramecium and David Nanney and Joseph Frankel in Tetrahymena had revealed that the inheritance of ciliary row patterning was under cytoplasmic control. There was also a hint (and only that) from Feulgen staining that basal bodies might contain DNA. In the early 1960s efforts to isolate Tetrahymena basal bodies were underway in several laboratories. A study by an Antioch College (Ohio, US) undergraduate, Joan Argetsinger (later Steitz), during a summer visit to Joseph Gall's laboratory led to the conclusion that isolated Tetrahymena basal bodies do not have appreciable nucleic acid.[2] The notion that centrioles template their replication via a nucleic acid had some momentum at the time, though the evidence to date falls short.[3,4] In due course, methods were developed for Tetrahymena nuclear genetics and transformation, including the ability to independently manipulate the cell's two different nuclei (vide infra) to produce heterokaryons with two genotypes, taking this creature to new heights of importance as an experimental system.[5-8]

Exploiting the System: Unique Features Enable New Insights

As typical in ciliates, a Tetrahymena cell harbors two nuclei with out-of-phase cycles of replication and division.[9] The micronucleus harbors the full genome and functions as the germline. The macronucleus contains surgically redesigned chromosomes, involving elimination of substantial DNA and a typically ca. 45-fold amplification of the surviving fragments, with the expression of this reduced genome supporting vegetative growth. By this nuclear dualism, ciliates can carry ca. 20,000 genes in the macronucleus and yet retain, by virtue of the complete genome in the micronucleus, the ability to respond to a sudden, fortunate environment with a rapid increase in cell growth and division.

Among the macronuclear amplified chromosomes is one of special significance: a palindromic chromosome encoding the large ribosomal RNA. Its amplification (ca. 9,000 copies) produces twice that number of ends and this work by Joseph Gall set the stage for what was to come.[10] Elizabeth Blackburn arrived in the Gall laboratory having mastered Fred Sanger's method of DNA sequencing as a post-doc. Applying this to isolated macronuclear rDNA led to the revolutionary discovery of telomeric repeat DNA.[11] Subsequently, the use of Tetrahymena extracts led to the groundbreaking demonstration of telomeric repeat synthesis by the ribonucleoprotein reverse transcriptase telomerase.[12] Later, Tetrahymena was microinjected with constructs expressing altered telomerase RNAs to nail the case for de novo telomeric repeat addition at chromosome ends.[13]

The high transcriptional activity of rDNA in the Tetrahymena macronucleus appealed to new Assistant Professor Thomas Cech at the University of Colorado as a system for dissecting transcriptional regulation.[14] In prior work, again by Joseph Gall's laboratory, the facility of amplified rDNA isolation had enabled rRNA transcript mapping, which had revealed the presence of an intron.[15] Studies of this intron's removal by Cech and colleagues led to discovery of self-splicing RNA, a monumental breakthrough in the fields of gene expression and molecular evolution.[16]

The recognized nuclear separation of functions between the micro- and macronucleus in Tetrahymena also set the stage for early characterization of histone variants and modification enzymes. Acetylated histones had been reported earlier by the laboratories of Vincent Allfrey at Rockefeller University and James Bonner at Caltech but a link to function had not been made. As a graduate student in Hewson Swift's group at the University of Chicago, Martin Gorovsky took the important step of isolating Tetrahymena micronuclei vs. macronuclei.[17] His own group went on to make major contributions to appreciating the existence and biological significance of histone modifications.[18,19] More recently his group uncovered a pathway of small RNA-mediated heterochromatin formation during DNA elimination in the macronucleus.[20] This finding, in turn, has led to additional intriguing findings.[21,22] There is reason to believe that Tetrahymena will continue to play a key role in the next wave of advances in epigenetic mechanisms as well as RNA-mediated gene silencing, as it has in the recent past.

In its enablement of these discoveries Tetrahymena has greatly accelerated our molecular understanding of the nucleus. This olympian protozoan has been graced on two occasions by the stardust of Stockholm. As has been proven so often, deep insights often emerge from exploiting model systems that may at first appear strange and different but in their simplicity of use, and often an exaggerated presentation of the problem at hand, offer great promise for fundamental advances. This article celebrates this organism, but it also salutes those who have pioneered advances in molecular and cell biology with this collaborative and catalytic cell. In *Hamlet* the principal says "There are more things in heaven and earth, Horatio, than are dreamt of in your philosophy." Thus do we applaud the endless frontier that any one of life's forms can surprisingly reveal, as Tetrahymena has—and will likely do more than once again.

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References

1. Scherbaum O, Zeuthen E. Induction of synchronous cell division in mass cultures of *Tetrahymena pyriformis*. Exp Cell Res 1954; 6:221-7.
2. Argetsinger JE. The isolation of ciliary basal bodies (kinetosomes) from *Tetrahymena pyriformis*. J Cell Biol 1965; 24:154-5.
3. Marshall WF, Rosenbaum JL. Are there nucleic acids in the centrosome? Curr Topics Dev Biol 2000; 49:187-205.
4. Pederson T. The centrosome: built of an mRNA? Nat Cell Biol 2006; 8:652-4.
5. Bruns PJ, Brussard TE. Nullisomic Tetrahymena: eliminating germinal chromosomes. Science 1981; 213:549-51.
6. Orias E, Bruns PJ. Induction and isolation of mutants in Tetrahymena. Meth Cell Biol 1976; 13:247-82.
7. Tondravi MM, Yao MC. Transformation of *Tetrahymena thermophila* by microinjection of ribosomal RNA genes. Proc Natl Acad Sci USA 1986; 83:4369-73.
8. Kahn RW, Andersen BH, Brunk CF. Transformation of *Tetrahymena thermophila* by microinjection of a foreign gene. Proc Natl Acad Sci USA 1993; 90:9295-9.
9. Collins KC, Gorovsky MA. *Tetrahymena thermophila*. Curr Biol 2005; 15:317-8.
10. Gall JG. Free ribosomal RNA genes in the macronucleus of Tetrahymena. Proc Natl Acad Sci USA 1974; 71:3078-81.
11. Blackburn EH, Gall JG. A tandemly repeated sequence at the termini of the extrachromosomal ribosomal RNA genes in Tetrahymena. J Mol Biol 1978; 120:33-53.
12. Greider CW, Blackburn EH. Identification of a specific telomere terminal transferase activity in Tetrahymena extracts. Cell 1985; 43:405-13.
13. Yu G, Bradley JD, Attardi LD, Blackburn EH. In vivo alteration of telomere sequences and senescence caused by mutated Tetrahymena telomerase RNAs. Nature 1990; 344:126-32.
14. Cech TR. Nobel lecture. Self-splicing and enzymatic activity of an intervening sequence RNA from Tetrahymena. Biosci Rep 1990; 10:239-61.
15. Wild MA, Gall JG. An intervening sequence in the gene coding for 25S ribosomal RNA of *Tetrahymena pigmentosa*. Cell 1979; 16:565-73.
16. Kruger K, Grabowski PJ, Zaug AJ, Sands J, Gottschling DE, Cech TR. Self-splicing RNA: autoexcision and autocyclization of the ribosomal RNA intervening sequence of Tetrahymena. Cell 1982; 31:147-57.
17. Gorovsky MA. Studies on nuclear structure and function in *Tetrahymena pyriformis* II. Isolation of macro- and micronuclei. J Cell Biol 1970; 47:619-30.
18. Allis CD, Glover CVC, Gorovsky MA. Micronuclei of Tetrahymena contain two types of histone H3. Proc Natl Acad Sci USA 1979; 76:4857-61.
19. Allis CD, Glover CV, Bowen JK, Gorovsky MA. Histone variants specific to the transcriptionally active, amitotically dividing macronucleus of the unicellular eukaryote, *Tetrahymena thermophila*. Cell 1980; 20:609-17.
20. Mochizuki K, Fine NA, Fujisawa T, Gorovsky MA. Analysis of a piwi-related gene implicates small RNAs in genome rearrangement in Tetrahymena. Cell 2002; 110:689-99.
21. Yao MC, Fuller P, Xi X. Programmed DNA deletion as an RNA-guided system of genome defense. Science 2003; 300:1581-4.
22. Aronica L, Bednenko J, Noto T, DeSouza LV, Michael Siu KW, Loidl J, et al. Study of an RNA helicase implicates small RNA-noncoding RNA interactions in programmed DNA elimination in Tetrahymena. Genes Dev 2008; 22:2228-41.