Austin Journal of Environmental Toxicology

Open Access

Austin Publishing Group

Review Article

The *Tetrahymena* Paradigm: Genetic Insights and Ecotoxicity Assessment

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Received: September 19, 2023
Accepted: October 12, 2023
Published: October 19, 2023

Abstract

Tetrahymena, a ciliated protozoan, thrives in freshwater habitats across diverse climates. Its importance as a genetic model organism lies in its cellular complexity, with cells comparable to human cells and a genome size between yeast and human. Tetrahymena's germline genome undergoes unique chromosome breakage and DNA elimination, shedding light on genome rearrangement mechanisms. In 2020, the macronuclear genome was fully sequenced, offering improved insights into chromosome structure and gene models. Tetrahymena's molecular tools enable gene characterization, knockouts, and overexpression.

Beyond genetics, Tetrahymena serves as an ecotoxicity model, particularly Tetrahymena pyriformis. Changes in its morphology, food vacuole formation, and contractile vacuole response serve as toxicity indicators. Tetrahymena's unique nuclear dimorphism (micronucleus and macronucleus) adds depth to ecotoxicological research. Additionally, Tetrahymena's behavioral endpoints, including chemotaxis, phagocytosis, and motility, offer sensitive toxicity markers.

Researchers have developed a T-maze toxotactic assay, a quick tool for studying Tetrahymena's behavioral responses to different chemicals. Tetrahymena's phagocytosis and motility are influenced by exposure to toxicants, reflecting changes in ion channels. Tetrahymena's rapid movement makes it ideal for studying motility alterations caused by toxic substances.

AI-driven tools enhance Tetrahymena behavior studies, allowing for behavior classification, feature extraction, and predictive modeling. This interdisciplinary approach aids in understanding Tetrahymena's intricate behaviors, offering potential applications in gene discovery and pharmacological interventions.

In conclusion, Tetrahymena stands as an invaluable model organism for genetic and ecotoxicological research. Its advantages, including genetic tractability and behavioral sensitivity, contribute to our understanding of fundamental biological processes and chemical safety assessment, with AI playing a growing role in advancing this field.

Keywords: Tetrahymena; Genetic model; Genome features; Ecotoxicological model; Behavioral endpoints; Artificial intelligence; Chemical safety assessment; Research significance

Citation: Lee YR, Lai MC, Liu HH, Chen JR. The *Tetrahymena* Paradigm: Genetic Insights and Ecotoxicity Assessment. Austin J Environ Toxico. 2023; 9(1): 1044.

Key Points of this Review Article:

Tetrahymena as a Genetic Model: Tetrahymena, a freshwater protozoan, is a valuable genetic model organism with a complex genome, making it an alternative to human tissues for research.

Unique Genome Features: Tetrahymena's genome undergoes programmed chromosome breakage, offering insights into genome rearrangement mechanisms and gene regulation.

Ecotoxicological Model: Tetrahymena pyriformis is used in ecotoxicological studies, with changes in morphology, food vacuole formation, and contractile vacuole response as toxicity indicators.

Behavioral Endpoints: Tetrahymena's behaviors, including chemotaxis, phagocytosis, and motility, serve as sensitive toxicity markers.

AI Integration: Artificial intelligence enhances the study of Tetrahymena behaviors, enabling behavior classification, predictive modeling, and potential applications in gene discovery and pharmacology.

Overall Significance: Tetrahymena is a versatile model organism for both genetic and ecotoxicological research, contributing to our understanding of fundamental biological processes and chemical safety assessment.

Tetrahymena is a Protozoa Model for Genetic Study

A ciliated single-celled protozoan, Tetrahymena is a freshwater organism that inhabits streams, lakes, and ponds and can be found almost everywhere, in a range of climates. Tetrahymena ingests food particles through the process of phagocytosis and stores it in food vacuoles for the digestion. An oral apparatus is present at the anterior end of the Tetrahymena cell, to ingest food particles, also oral apparatus having four membranous structures from which this organism derives its name "Tetrahymena". *Tetrahymena thermophila* has been an important model system for biological research for many years.

Tetrahymena thermophila cells are 40-50 um along the anterior-posterior axis and their complexity is comparable to human cells, making them a good alternative to human tissues [1]. The Tetrahymena genome size (roughly 220 Megabase pairs) is of the same order of magnitude as that of Drosophila-one order larger than yeast (Saccharomyces) and one smaller than human. The germline genome of the binucleated ciliate *Tetrahymena thermophila* undergoes programmed chromosome breakage and massive DNA elimination to generate the somatic genome. A complete sequence assembly of the germline genome and analyze multiple features of its structure and its relationship to the somatic genome, shedding light on the mechanisms of genome rearrangement as well as the evolutionary history of this remarkable germline/soma differentiation. The Micronuclear genome structure results strengthen the notion that a complex, dynamic, and ongoing interplay between mobile DNA elements and the host genome have shaped Tetrahymena chromosome structure, locally and globally. Non-standard outcomes of rearrangement events, including the generation of short-lived somatic chromosomes and excision of DNA interrupting protein-coding regions, may represent novel forms of developmental gene regulation [2].

Tetrahymena thermophila macronuclear genome has been fully completed in 2020. The first chromosome-level Tetrahymena macronuclear genome assembly, with approximately 300×long Single Molecule, Real-Time reads of the wild-type SB210 cells-the reference strain for the initial macronuclear genome sequencing project. All 181 chromosomes were capped with two telomeres and gaps were entirely closed. The completed genome shows significant improvements over the current assembly (TGD 2014) in both chromosome structure and sequence integrity. The majority of previously identified gene models shown in TGD were retained, with the addition of 36 new genes and 883 genes with modified gene models. The new genome and annotation have been incorporated into TGD. The new complete genome made possible for pursuit in previously underexplored challenging areas such as genome scrambling and chromosomal copy number [3].

Tetrahymena thermophila is a useful model organism for molecular research at all levels. Tetrahymena possesses many core processes conserved across a wide diversity of eukaryotes (including humans) that are not found in other single-celled model systems such as yeasts Saccharomyces cerevisiae and *Schizosaccharomyces pombe.* Conventional genetic analysis and molecular genetics tools for analysis of gene function have been developed for Tetrahymena. Homologues of human genes can be characterized, knocked out, and overexpressed [4].

Combining the power of forward and reverse genetics, the ciliate *Tetrahymena thermophila* is a model system whose study has led to important discoveries and insights into novel genes and processes in cell and developmental biology. Tetrahymena are evolutionarily divergent from the commonly studied organisms in the opisthokont, eukaryotes whose flagellate cells propel them with a single posterior flagellum, thus allowing for examination of both universally conserved and unique divergent biological processes. With the aid of molecular-genetic and genomic tools combined, Tetrahymena provides a system to couple gene discovery to mechanistic dissections of gene function in the cell, leading to fundamental biological insights covering the central dogma and beyond. The usefulness of Tetrahymena as a genetic model organism is manifested in terms of its short life cycle, easy and cost-effective handling, and its accessibility to both forward and reverse genetics.

Varieties of Tetrahymena species and strain can be obtained at American Type Culture Collection (ATCC, https://www.atcc.org/search#q=tetrahymena&sort=relevancy), Carolina Biological Supply Company, USA (https://www.carolina.com/browse/product-search-results?Nr=OR(product.type:Product),OR(product.catalogId:navigationCatalog,product.catalogId:teacherResourcesCatalog),OR(product.siteId:100001)&tab=p&Ntt=tetrahymena), Culture Collection of Algae and Protozoa (CCAP), UK (https://www.ccap.ac.uk/results2014.php), and Tetrahymena Stock Center Cornell University, (https://tetrahymena.vet.cornell.edu/).

Tetrahymena is as an Ecotoxicity Model

Tetrahymena pyriformis are frequently used and are considered as one of the representative environmental (aquatic) microorganisms in ecotoxicological studies to investigate the potential toxicity of various xenobiotics. Tetrahymena is a member of the heterotrophic free-living microfauna of aquatic ecosystems that occupies one of the first trophic levels. Together with other ciliates, it plays a key role in the transfer of matter and energy within the microbial loop and thus, is an early warning indicator of pollution. *Tetrahymena pyriformis* is a true eukaryotic cell, which can be easily cultured with a short generation time in axenic defined medium.

Tetrahymena is generally pear-shaped, 50–60 µm in length, and 30 µm in width. Through a simple microscope, we can easily observe the changes in the morphology of Tetrahymena upon exposure of toxicants. Various studies have demonstrated that upon exposure of toxicants, Tetrahymena cells lost its shape from pear to round shape, decrease in size and bulging of the cell membrane observed [5-7].

The physiology and behavioral activities of Tetrahymena have proven to be effective parameters for studying the impact of different toxicants. The rate of food vacuole formation, influenced by the physiological state and various environmental factors such as pH, oxygen, and temperature, holds the potential to serve as a valuable indicator of the toxicity of various xenobiotics. By examining the capacity of food vacuole formation, researchers can gain insights into the toxic effects of different substances on Tetrahymena, allowing for a better understanding of their potential environmental impact [8,9]. In all freshwater protozoans, including Tetrahymena, the contractile vacuole plays a crucial role in maintaining the osmotic balance of the cells. This vacuole is particularly sensitive to changes in the cell's environment. A study investigating the effects of ethanol exposure on Tetrahymena cells revealed that the contractile vacuole enlarged in response to this substance. However, at higher concentrations of ethanol, a lack of contractile vacuole was observed. This highlights the importance of the contractile vacuole in responding to environmental changes and its potential as an indicator of cellular stress caused by various substances [10].

Tetrahymena possesses a unique nuclear apparatus known as nuclear dimorphism, where two distinct types of nuclei coexist within a single cell. This phenomenon is widespread among most Tetrahymena species and involves two well-defined nuclei: the Macronucleus (MAC) and the Micronucleus (MIC), each serving different functions and exhibiting structural and functional differences.

The germline Micronucleus (MIC) acts as a reservoir of genetic information for the sexual reproduction of the cells and is responsible for generating the macronucleus. It is diploid and contains five pairs of chromosomes while remaining transcriptionally silent. On the other hand, the Macronucleus (MAC) is a somatic cell nucleus that primarily governs the metabolic activities of the cell during vegetative replication. The MAC comprises approximately 300 chromosomes derived from the MIC chromosomes. The complete sequencing of the macronuclear genome has been accomplished, providing valuable insights into the intricate mechanisms of nuclear dimorphism in Tetrahymena [1,11,12].

Tetrahymena, a unicellular eukaryote, exhibits a range of distinctive biological features that establish it as a formidable model system for various aspects of molecular and cellular biology, particularly in the context of nuclear and chromatin biology. A notable characteristic is its nuclear dimorphism, wherein *Tetrahymena thermophila* harbors two distinct nuclei within a single cell. These nuclei, namely the germ-line micronucleus and the somatic macronucleus, display structural and functional differences. The germ-line micronucleus remains transcriptionally silent and stores genetic information for sexual reproduction, while the somatic macronucleus is transcriptionally active and primarily controls the cell's metabolism. This unique nuclear arrangement contributes to Tetrahymena's significance as a model for studying nuclear processes in eukaryotes [13]. In the Tetrahymena model, the application of proteomic approaches has significantly advanced our understanding of various nuclear events. These events encompass critical processes such as chromosomal replication, genome rearrangement, genome stability, transcription, and chromatin remodeling. Through the study of the proteome, researchers have gained valuable insights into the intricate molecular mechanisms that govern these essential nuclear processes in Tetrahymena [12].

Nuclear structural changes can serve as reliable toxicity markers for different xenobiotics, as alterations in gene levels are readily reflected at the protein level. For instance, a study on Tetrahymena pyriformis investigated the impact of the fertilizer melamine, and it was found that changes in the nuclear structure were linked to variations in protein expression levels. This correlation between nuclear alterations and protein expression highlights the potential of using nuclear structure as an indicator of toxicity in response to various environmental compounds [14].

Tetrahymena contains typical cytoplasmic organelles, including mitochondria, lysosomes, ribosomes, the Golgi complex, peroxisomes, and the endoplasmic reticulum. The structural and physiological characteristics of these organelles can serve as sensitive biomarkers for studying the toxicity of various xenobiotics. Changes in the structure of these organelles can reflect the physiological state of the cells. For instance, during environmental stress or starvation, mitochondria and peroxisomes may become electron-dense, and unusual structures not typically present in cells might emerge, indicating alterations in the metabolic state of the cells. By observing such changes in organelle structure, researchers can gain valuable insights into how environmental factors and toxicants affect cellular health and function in Tetrahymena [15]. Observations revealed the presence of lipid bodies and inorganic materials, mainly containing potassium, magnesium, calcium, and phosphorous, within Tetrahymena cells. As cells transition from the stationary phase to new growth media, they become devoid of granules. This suggests that granules may accumulate toxic and heavy metals, such as cadmium, copper, and zinc. The presence or absence of these granules can serve as indicators of potential toxic exposure and heavy metal accumulation in Tetrahymena, providing valuable insights into the impact of environmental contaminants on the organism's cellular health [16]. These structures play a crucial role in cellular detoxification. Notably, it has been demonstrated that cells forming granules exhibit increased resistance to high concentrations of metals. This indicates that the granules contribute to the defense mechanism of the cells against metal toxicity, providing a protective effect. Understanding the function and significance of these granules in the detoxification process of Tetrahymena cells can offer valuable insights into how the organism responds to environmental stressors and pollutants [17].

Research studies have confirmed that *Tetrahymena pyriformis* serves as a viable and alternative cellular model for conducting *in vitro* toxicological assessments of diverse chemicals, ranging from pesticides and heavy metals to pharmaceutical drugs and organic compounds. By utilizing *Tetrahymena pyriformis* in toxicological studies, researchers can obtain valuable data on the potential adverse effects of these substances on cellular health and function. The organism's sensitivity to various toxicants and its ease of culture make it a reliable and practical tool for evaluating the toxicological impact of different chemicals in a controlled laboratory setting. This has significant implications for understanding the potential risks posed by environmental pollutants and other hazardous substances on aquatic organisms and ecosystems [18-22]. Researchers have successfully developed a simple and rapid test using *Tetrahymena pyriformis* to screen and assess the cytotoxicity of xenobiotics. This method involves estimating the activities of nonspecific esterases within the cell by concentrating a specific amount of fluorescence associated with fluorescein dye. The study presents the 1-hour median Effective Concentration (EC_{50}) values of 10 inorganic and eight organic substances, comparing them with the EC_{50} values obtained from three other bioassays: the conventional *T. pyriformis* proliferation rate with 9-hour median inhibitory concentrations, the Microtox(R) assay with 30-minute EC_{50}s, and the *Daphnia magna* assay with 4-methylumbelliferyl beta-D galactoside 1-hour EC_{50}s. This novel approach offers a valuable and efficient tool for quickly assessing the potential cytotoxic effects of various xenobiotics, contributing to our understanding of their impact on cellular health and providing relevant data for toxicological evaluations [23]. Behavioral studies in toxicology offer several advantages. Organismal behavior is highly sensitive to environmental changes, making it a powerful indicator of potential toxicity. Even at low concentrations, behavioral effects can manifest rapidly, providing valuable insights into the impact of toxic substances. Observing behavior in real-time allows for non-invasive and continuous monitoring of living organisms, while repeated measurements enable the examination of individual cells over time. By focusing on behavioral parameters, toxicologists can gain a deeper understanding of how xenobiotics and pollutants affect living organisms, contributing to more accurate and comprehensive toxicological assessments [24]. Behavioral endpoints have proven to be more sensitive than survival bioassays in toxicity studies. In the case of Tetrahymena, behavior research primarily centers on chemotaxis responses, motility, and phagocytosis activity. Chemotaxis responses, in particular, serve as valuable indicators for evaluating environmental contamination. By observing the behavioral changes in Tetrahymena in response to various stimuli, researchers can gain valuable insights into the presence and impact of contaminants in the environment. This focus on behavioral endpoints enhances the accuracy and reliability of toxicity assessments, making Tetrahymena an important model organism for ecotoxicological studies [25].

A T-maze toxotactic assay was developed using Tetrahymena thermophila as the test organism, and spring water served as the control, while sodium chloride and guaiacol were used as reference toxicants. The results showed that Tetrahymena thermophila exhibited a positive response towards sodium chloride, indicating attraction, while it displayed a negative response towards guaiacol, indicating avoidance. However, there was no preferred response observed with spring water, suggesting that *Tetrahymena thermophila* did not show any specific attraction or avoidance towards it. This T-maze assay provides a valuable tool for studying the behavioral responses of *Tetrahymena thermophila* to different chemicals and assessing their potential toxicity [26].

Phagocytosis in Tetrahymena species can be easily observed using a light microscope. Numerous studies have demonstrated that various contaminants can influence the grazing ability of Tetrahymena. When exposed to different toxicants at various concentrations, Tetrahymena displayed both an increase and a decrease in phagocytosis activity. This suggests that the impact of toxicants on the phagocytosis process is diverse and can vary depending on the specific contaminant and its concentration. Studying the effects of toxicants on phagocytosis in Tetrahymena provides valuable insights into the potential risks of environmental contaminants on the feeding behavior of these microorganisms [27,28]. Researchers have explored the phagocytosis activity of Tetrahymena using different techniques, such as employing latex beads in the presence of various metals (zinc, copper), a protein inhibitor (cycloheximide), and a detergent (Triton X-100) [27]. They have also employed automated image analysis [5], and genetically modified Green Fluorescent Protein (GFP) tagged bacteria along with fluorometry to assess the ingestion rate of protozoans [29]. These approaches allowed them to investigate and better understand the process of phagocytosis in Tetrahymena.

Motility serves as a sensitive indicator of toxic stress caused by environmental contaminants. The ciliary movement of Tetrahymena is regulated by Ca2+, K+, and Na+ ion channels, which play crucial roles in this process. The rate and direction of ciliary beats are influenced by the permeability of these ion channels, resulting in changes in the membrane potential. These factors are essential for understanding how toxic substances can impact the motility and behavior of Tetrahymena as a response to environmental changes [30,31]. In laboratories, researchers have investigated the correlation between ciliary movement and exposure to various substances such as pesticides (acephate), metals (lanthanum, zinc), plant lectin (concanavalin A), and carbon nanotubes in ciliates. By studying how these substances affect ciliary movement, scientists gain insights into their potential toxic effects on the organisms and the environment [32-34].

Exposure to toxicants can lead to alterations in ion channels, which, in turn, affect the swimming behavior of protozoans like Tetrahymena. These changes in motility serve as indicators of membrane stability. Tetrahymena's rapid movement makes it easy to observe changes in motility under a microscope, making locomotive activity a sensitive measure for assessing the potential toxicity of various xenobiotics.

In a specific study, it was found that high concentrations of lanthanum metal caused changes in Tetrahymena's movement pattern. The cell's motility decreased, and a rocking movement was observed. This alteration was attributed to the interference of the metal with calcium channels that are crucial for ciliary activity. The inhibition of calcium entry resulted in the immobility of the cells, highlighting the impact of the toxicant on Tetrahymena's movement behavior [35].

Locomotion Tracking in Tetrahymena

In their research, Frank Pennekamp, Jean Clobert, and Nicolas Schtickzelle delved into understanding the intricate relationship between movement, morphology, and dispersal in Tetrahymena ciliates. They investigated these connections across 44 different genotypes of Tetrahymena thermophila. To do so, they employed two-patch populations to analyze individual movement trajectories, activity, morphology, and dispersal rate. The researchers observed that variation in movement behavior both among and within genotypes could be attributed to differences in morphology. Notably, genotypes exhibited distinct characteristics in terms of movement speed and linearity. These differences in movement were partly explained by morphological features, such as cell size and shape. Larger cells consistently displayed higher movement speed and higher linearity in their trajectories.

Moreover, the study identified marked disparities in movement between resident and dispersing individuals, with these differences being mediated by the genotype of the Tetrahymena. The findings underscored the close connection between movement and dispersal on multiple levels, highlighting the importance of understanding movement behavior for making accurate predictions about dispersal patterns [36].

A computerized *in vitro* test known as the Motility Assay (MA) was created as a substitute for the rabbit eye irritancy test (Draize) to assess chemical toxicity. The assay focuses on the swimming patterns of *Tetrahymena pyriformis*, a motile biological cell, and utilizes automated scoring to measure how these cells respond when exposed to potentially harmful chemicals.

During the test, the motile cells of *Tetrahymena pyriformis* either slow down or display altered swimming patterns in the presence of the chemicals being tested, indicating their potential toxicity. The MA offers comparable or even improved detection limits when compared to traditional in vivo animal tests for eye irritancy. Thus, the results obtained from the Motility Assay are comparable to or better than those from the Draize test in identifying potential eye irritants.

By providing an effective alternative to animal testing, the Motility Assay contributes to the development of more ethical and reliable methods for evaluating chemical toxicity. By utilizing the unique swimming behavior of *Tetrahymena pyriformis*, this test offers valuable insights into the potential harmful effects of chemicals without the need for animal experimentation [37].

Unraveling Tetrahymena Behavior with AI

This concise piece delves into the synergy of molecular biology and Artificial Intelligence (AI) applied to Tetrahymena behavior studies. Tetrahymena, a well-established model organism, offers unique insights into cellular decision-making processes. Integrating AI into this field presents novel opportunities.

Current Tetrahymena research centers on behaviors like chemotaxis, phototaxis, and mechanosensation [38]. AI brings valuable tools to the table: it can classify behaviors, extract key features, predict responses, and fuse behavioral data with molecular insights. This combination allows for a more comprehensive understanding of Tetrahymena's intricate behaviors.

The future promises AI-driven neural network models simulating Tetrahymena behavior [39], high-throughput screens for gene discovery, multi-omics integration, evolutionary studies, and potential pharmacological interventions. Collaborative interdisciplinary efforts will unlock the molecular secrets guiding Tetrahymena behaviors, with implications extending to broader biological and medical contexts [40].

Conclusion and Perspective

Tetrahymena, as a model organism for biological research, offers numerous advantages that greatly facilitate the study of essential cellular processes [41,42]. Its characteristics as a single-celled organism with a substantial size and straightforward culture requirements make it an ideal candidate for investigating fundamental biological phenomena. Additionally, its short life cycle and ease of genetic manipulation enable researchers to swiftly collect data and conduct experiments. Moreover, Tetrahymena's well-characterized genome, preservation of vital biological processes, and sensitivity to stimuli make it a highly appealing subject for scientific research [43]. As a result, Tetrahymena has played a pivotal role in advancing our understanding of fundamental biological mechanisms and has become a cornerstone in the progress of molecular and cellular biology.

In the contemporary world, there is a growing focus on ensuring the safety of chemicals used in various industries. Private industries and government regulatory agencies are increasingly concerned about the potential hazards posed by different chemical substances. To safeguard human health, the environment, and comply with safety regulations, rapid and accurate chemical safety assessments are crucial. In this regard, *in silico* computer-based techniques, such as computer simulations and modeling, present practical alternatives for assessing chemical safety in an environmentally friendly and cost-effective manner. By leveraging computational algorithms, these *in silico* techniques predict the properties and behaviors of chemicals, including their potential hazards. This approach is particularly valuable for assessing the properties of certain organic chemicals, such as those that are persistent, bioaccumulate, and toxic, which are of significant concern due to their potential adverse effects on ecosystems and human health. *Tetrahymena pyriformis* is commonly used as a model organism in toxicity testing to evaluate the harmfulness of substances to living organisms [44]. Moreover, information gain analysis is employed to identify the most relevant substructure patterns in chemicals associated with *Tetrahymena pyriformis* toxicity, aiding in the development of precise predictive models [45]. Looking to the future, the rapid advancements inA rtificial Intelligence (AI) and automation hold great promise for the field of chemical safety assessment.

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