PART I

Introduction
CHAPTER 1

Perspectives on the Ciliated Protozoan
Tetrahymena thermophila

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Abstract

In biology, scientific discoveries are often linked to technical innovations made possible by an inspired choice of model organism. Ciliate species, especially Tetrahymena thermophila, have had historically significant roles as uniquely enabling experimental systems. More importantly, as the chapters in this volume attest, ongoing efforts of the T. thermophila model organism community have created a knowledge and resource infrastructure for systems-level studies across a whole genome or proteome, setting the stage for understanding the fundamental biology underlying the sophisticated life cycle and environmentally responsive behaviors of this free-living, single-celled eukaryote. One hope is that these developments will stimulate the integration of ciliates into phylogenomic comparative analyses and also encourage the experimental use of T. thermophila by a broader scientific community. This early branching yet highly gene-rich eukaryote has much to offer for future studies of human-relevant basic biology.
I. Introduction

In context of the eukaryotic family tree, ciliated protozoa launched their evolutionarily successful lineages long before the successful radiation of plants, fungi, or animals. What about the ciliates gives them the necessary advantages of growth, reproduction, and adaptability in the face of changing environmental conditions? Although these questions merit real answers from future studies, one can speculate based on features that are common to modern-day ciliates but are distinctive when ciliates are compared to other extant life. In addition to their defining characteristic of cilia-driven motility, ciliates share the properties of a large cell size, specialization of germline versus somatic nuclei within the same cytosol, and relatively high expressed gene content (comparable to mammals). Large cell size has been proposed to contribute to better feeding (ciliates are indiscriminate omnivores). The specialization of germline versus somatic nuclei allows remarkable en masse sampling of germline genotype allele combinations in the asexual phase of population growth and also an elegant mechanism for epigenetic influence of growth history on transmission of adaptive traits to sexual progeny. Streamlining of the expressed, somatic, macronuclear genome by large-scale elimination of repetitive DNA from the silent, germline, micronuclear genome could support the chronologically high rate of gene duplication and divergence that gives *T. thermophila* parity of gene number with animal genomes. These speculations have their origins in the literature summarized in the chapters that follow, which together provide an opportunity to integrate broadly across lessons learned from diverse research areas spanning from field studies of cell communities in their native habitat to reverse genetics of inbred strains maintained under controlled laboratory conditions.

II. Historical Contributions

Numerous useful features of *T. thermophila* account for its history of contributions as a model organism (Collins and Gorovsky, 2005; Orias et al., 2011; Pearson and Winey, 2009; Turkewitz et al., 2002). *T. thermophila* has a large size that is nonetheless modest among ciliates (≈30 × 50 μm) and a rapid doubling time (about 2 h at 37 °C) made possible by a highly organized cortical architecture and a somatic nucleus streamlined for Herculean transcriptional output. Large size enables poking the cell with electrodes or with a needle to inject or ablate, the resolution of subcellular compartments by live or fixed whole-cell imaging, and obtaining lots of extract for biochemical studies from the more than 10^6 cells/mL that can be cultured in simple media. Combining conventional genetics (Chapter 10) and molecular genetics (Chapter 11) with the ease of biochemical analyses and purifications (Chapters 12 and 14) and advantages for cytology (Chapter 13), there is a wealth of opportunity for systems-level investigations to address complex mechanisms of cellular communication and behavior (Chapter 15). The ease of culture (Chapter 8), annotated genome contents (Chapter 4), and phenotypic and genotypic
strain diversity (Chapters 2–5 and 9) lend themselves to student training opportunities that yield new findings and publications (Chapter 16).

Historical highlights of discoveries enabled by use of *T. thermophila* include the histone composition and modification differences between euchromatin and heterochromatin, which are readily detected by comparison of the macronucleus and micronucleus (Chapter 3). Much insight has also been gained about the process of DNA palindrome formation, which occurs during formation of the small macronuclear chromosome encoding large ribosomal RNAs (the rDNA chromosome) from its single-copy locus in the micronucleus (Tanaka and Yao, 2009). Some effort has been devoted to defining the principles of macronuclear chromosome counting (Donti et al., 2009) and the conditionally essential, checkpoint-monitored processes of micronuclear mitosis and meiosis (Chapter 7). Pioneering discoveries exploiting *T. thermophila* also include the Nobel prize-winning self-splicing activity of the group I intron within the large ribosomal RNA precursor (Cech, 2004) and the simple-sequence repeat nature of chromosome telomeres and telomeric-repeat synthesis by telomerase (Blackburn, 2010; Greider, 2010), with additional seminal discoveries of microtubule motors, post-translational modifications, and dynamics (Chapter 5).

### III. Compelling Opportunities

In addition to the established fields of study among researchers currently using *T. thermophila*, improved ciliate genome annotations and new methods (such as high-resolution imaging, deep sequencing, and quantitative proteomics) beg for expansion of ciliate model-system applications to new fields of study. Among these would be the biology of organellar biogenesis, remodeling, and function (see Chapter 5); membrane specialization, vesicle traffic, and regulated secretion (see Chapter 6); and different types of autophagy induced on massive scale to accomplish programmed nuclear death during sexual reproduction (Akematsu et al., 2010) or to recycle cytosolic compartments and components of translation machinery when cells enter a state of growth arrest (Andersen and Collins, 2012; Nilsson, 1984). Also worthy of revisiting is the use of *T. thermophila* to characterize differential ribosome compositions that may reprogram translation (Hallberg and Sutton, 1977; McMullin and Hallberg, 1986) and to investigate stress-responsive regulation of translation in general (Calzone et al., 1983).

Mechanisms that govern the selectivity of nuclear import have recently begun to be defined by directly exploiting nuclear dualism (Iwamoto et al., 2009; Malone et al., 2008; Orias et al., 2011). There are also early hints that *T. thermophila* can provide new insights into principles of higher order chromatin organization, for example, the basis for clustering of rDNA chromosomes or condensin-dependent chromosome segregation (Cervantes et al., 2006). Principles of developmentally induced genome remodeling are an obvious direction for continued study, including elucidation of the machinery that directs chromosome breakage and joining,
chromosome breakage coupled to new telomere addition, and site-specific recombina
tion (Chalker and Yao, 2011; Orias et al., 2011). Also the roles of \textit{T. thermophila}
Piwi-protein RNPs in small RNA-mediated epigenetic regulation are just beginning
to be understood (Couvillion et al., 2009, 2010; Schoeberl and Mochizuki, 2011).

IV. Chapter Logic

The early Chapters 2–7 are knowledge summaries and systems perspectives. The
later chapters 8–16 provide detailed methodological guidance, as well as general
operating principles to enable extensions beyond established protocols. To supple-
ment and update the compendium of this volume, which represents the cumulative
expertise of the model organism community through 2011, the community is building
updatable inventories of strains, plasmids, methods, and gene curations through the
\textit{Tetrahymena} genome database (http://ciliate.org/index.php/home/welcome),
\textit{Tetrahymena} functional genomics database (http://tfgd.ihb.ac.cn/), and \textit{Tetrahymena}
stock center (http://tetrahymena.vet.cornell.edu/). Beyond these resources, a ciliate
list-serve allows queries for reagents and advice to be distributed across the model
organism community (http://listserv.uga.edu/archives/ciliatemolbio-l.html).

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commitment to collaborative generation of shared resources.

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