Letter to the Editor

Proposed Genetic Nomenclature Rules for *Tetrahymena thermophila*, *Paramecium primaurelia* and *Paramecium tetraurelia*

The Seventh International Meeting on Ciliate Molecular Biology Genetics Nomenclature Committee: Sally Lyman Allen,* Marsha I. Altschuler,† Peter J. Bruns,‡ Jean Cohen,§ F. Paul Doerder, Jacek Gaertig,†† Martin Gorovsky,‡‡ Eduardo Orias§§ and Aaron Turkewitz*****

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ABSTRACT

The genetics of the ciliated protozoa *Tetrahymena thermophila* and certain species of Paramecium (*P. primaurelia* and *P. tetraurelia*) have reached a level of maturity such that rules for genetic nomenclature for micronuclear and macronuclear genetics need to be clarified for workers in the field as well as for other geneticists. After a short introduction, the rules follow.

THE organisms *Tetrahymena thermophila*, *Paramecium primaurelia*, and *Paramecium tetraurelia* belong to the ciliated protozoa and are freshwater organisms that inhabit streams, lakes, and ponds. The species specified are those that have been used the most often in genetic studies; however, the rules should apply to other species of Tetrahymena and Paramecium. *T. thermophila* was originally designated variety 1, then syngen 1, of *T. pyriformis*, and *P. primaurelia* and *P. tetraurelia* were first referred to as varieties, then syngens of *P. aurelia*. Current species names were given to the *T. pyriformis* complex by Nanney and McCoy in 1976 and to the *P. aurelia* complex by Sonneborn in 1975. Tetrahymena and Paramecium are unicellular eukaryotes with various complex and specialized cell structures. The cells are large, 40–50 μm along the anterior-posterior axis for Tetrahymena, 105–125 μm for *P. tetraurelia*, and ~150 μm for *P. primaurelia*. The genome size of Tetrahymena is ~220 megabase pairs, roughly the same order of magnitude as that of Drosophila. The genome of *P. tetaurelia* is similar in size (Preer and Preer 1979).

A number of inbred strains were developed for *T. thermophila* (Allen and Gibson 1973; Allen *et al.* 1984), designated A, B, B2, B3, C2, C3, D, and F, and derived from different combinations of natural isolates; these strains are available from the American Type Culture Collection (ATCC) from Dr. Thomas Nerad. By prior agreement among Tetrahymena workers, inbred strain B, which is genetically the most stable, is designated as the reference strain, and laboratory mutants have been isolated in the genetic backgound of this strain. DNA polymorphisms between inbred strains B and C3 have been used to genetically map the genome (Orias 1996, 1998a). Because Paramecium species undergo autogamy, the various natural isolates (also called stocks) are homozygous for all loci. Although certain stocks are more commonly used (*e.g.*, stocks 156 and 168 of *P. primaurelia* and stocks 51 and d4-2 of *P. tetraurelia*), no reference strain is designated for Paramecium. The Sonneborn collection of Paramecium stocks resides at the ATCC, and stocks may be obtained from Dr. Nerad.

Nuclear dimorphism: The nuclear apparatus of Tetrahymena and Paramecium is composed of two structurally and functionally differentiated types of nuclei. The micronucleus (MIC) is the germline, that is, the store of genetic information for the sexual progeny. It is diploid and contains five pairs of chromosomes in Tetrahymena and ~50 pairs of chromosomes in Paramecium. The MIC is capable of undergoing meiosis during conjugation. There is one MIC in Tetrahymena and two MIC in Paramecium. There is a single macronucleus (MAC) in both Tetrahymena and Paramecium. The MAC is the

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somatic nucleus, that is, the nucleus actively expressed during vegetative multiplication, starvation, and conjugation, until the later stages. A new MAC is derived from the MIC during conjugation as the old MAC (from the previous sexual generation) is destroyed. Several types of programmed rearrangements occur during the development of a new MAC: (1) fragmentation of the MIC chromosomes into smaller MAC chromosomes (called autonomously replicating pieces, or ARPs, by Orias 1998a); (2) addition of new telomeres to the ends of the new chromosomes; (3) deletion of internal DNA segments; and (4) amplification of most of the MAC chromosomes to an average level of 45 copies in Tetrahymena and 800 copies in Paramecium. The MAC then replicates amitotically. In Tetrahymena each of the MAC chromosomes assorts randomly. Both the MIC and MAC have genetic systems that can be studied with a variety of techniques. For references on the details of MIC and MAC genetics, see the following: Bruns and Cassidy-Hanley 1993; Steele *et al.* 1994; Bleyman 1996; Coyne *et al.* 1996; Meyer and Keller 1996; Orias 1996, 1998a,b; Yao 1996; Klobutcher and Herrick 1997; Meyer *et al.* 1997; and Preer 1997.

DNA-mediated transformation: Both Tetrahymena and Paramecium can be transformed. Efficient DNA-mediated transformation of Tetrahymena cells has been accomplished by three methods: (1) microinjection, (2) electrotransformation, and (3) biolistic bombardment. Both the germline nucleus (MIC) and the somatic nucleus (MAC) can be transformed. In Paramecium, microinjection has been successfully used to transform the MAC, where rescue of mutants and cloning genes by functional complementation is possible. For references see Bourgain and Katinka 1991; Cassidy-Hanley *et al.* 1997; Gaertig and Gorovsky 1992; Gaertig *et al.* 1994; Godiska *et al.* 1987; Gorovsky 1997; Hai and Gorovsky 1997; Haynes *et al.* 1996; Kanabrocki *et al.* 1991; Skouri and Cohen 1997; Tondravi and Yao 1986.

Nomenclature: Genetic analyses of Tetrahymena and Paramecium have reached a level of maturity such that rules for genetic nomenclature need to be clarified so that workers in the field can communicate more effectively with each other and with other geneticists. This is the purpose of the following rules.

MICRONUCLEAR GENETICS

General

1. Previously named genes need not be renamed.
2. All genes are given symbols that will have three letters in italics. All loci are numbered immediately following the gene symbol. All wild-type alleles are written entirely in uppercase. Example: *BTU2*.
3. In *Paramecium primaurelia* and *Paramecium tetraurelia*, families of numerous, almost identical, genes are frequent. Genes in a family are designated by the same gene symbol, followed by a three-digit number to indicate family membership. Examples: TUB101 and TUB102 are beta-tubulin loci in the same family, whereas TUB201 is in a different family.
4. Unlinked genes are separated by semicolons. Alleles are separated by a slash, Linked genes can be grouped on the same side of a slash, separated by commas. Example: *BTU2*/*BTU2*; *CHX1*, *EST1*/*CHX1*, *EST1*.
5. Alleles altered by induced mutation or *in vitro* manipulations should be written entirely in lowercase. All alleles other than wild type are given a hyphenated number. Example: *chx1-1*.

Strain origins

1. For *Tetrahymena thermophila*:
   a. Strain B serves as the reference strain; loci from inbred strain B need no further identification.
   b. Genes from all other inbred strains and natural isolates have the strain identified by an uppercase letter written within square brackets (followed by a number if appropriate) following the locus designation. Example: *CHX1[C3]* (this would designate the wild-type *CHX1* locus derived from inbred strain C3).
2. For *Paramecium primaurelia* and *Paramecium tetraurelia*, no particular reference strain is used. The strain from which genes are derived should be referred to each time using the nomenclature indicated above for Tetrahymena.

Mutations

1. The allele designation for insertional mutants will be as for other altered alleles, but in addition will be followed by a double colon, which is followed by the name of the inserted element. Example: *btu2-1::neo2*.
2. All mutant alleles carrying known modifications have an appropriate designation following the hyphenated allele number. Example a: *btu2-2A251K* (this would indicate an allele of *BTU2* in which alanine at position 251 was replaced with lysine). Example b: *btu2-3Δ1-40* (this would designate an allele of *BTU2* in which the first 40 amino acids are deleted).

Randomly amplified polymorphic DNAs (RAPDs) (*Tetrahymena thermophila*)

RAPD polymorphisms are written in italics and conform to the following system:

a. A number indicating the laboratory of origin.

b. Two initials indicating the person who discovered or mapped the polymorphism.
c. A sequential number. Example: *1JB11*, *1JB12*.
d. If more than one polymorphic band is generated by the same primer combination, lowercase letters distinguish the bands. Example: *1JP18a* and *1JP18b*.
e. No further designators are used if the polymorphic band occurs in inbred strain B but not in inbred strain C3. Example: *1PM8*.
f. If polymorphisms are identified between inbred strain B and an inbred strain other than C3 (such as C2, or D, etc.), the designation of the second inbred strain would be appended after all the above designators in square brackets. Example: *2SA1[C2]* (for a band present in inbred strain B and absent in C2).
g. A capital R follows all the designations if the polymorphic band occurs in inbred strain C3, or in some other inbred strain, but not in inbred strain B. Example: *1EO3R* (for a band present in inbred strain C3, and not in B).
h. To distinguish the two alleles of a RAPD, "+" or "−" are added to all the previous designators to indicate the allele that determines the presence or absence of the polymorphic band, respectively. Example: *1EO3R*+ and *1EO3R*− designate the C3 and B alleles, respectively.

MACRONUCLEAR GENETICS

General

All genotypic and phenotypic designations are enclosed within a single pair of parentheses directly following the micronuclear genotype.

Genes

1. Genes conform to the conventions established above for micronuclear genes. Groups of somatically linked genes are separated by a slash. The simplest example of this is allelic variants of a single gene. Example: (*CHX1*/*chx1-1*).
2. If multiple genes are known to be somatically linked, they are listed together and separated by commas. Example: (*CHX1*, *PJB1*/*chx1-1*, *PJB1*).
3. Somatically unlinked genes are listed and separated by semicolons. Example: (*CHX1*, *PJB1*/*chx1-1*, *PJB1*; *MPR1*/*MPR1*).

Phenotypes

1. Phenotypes are lowercase and not italicized, generally using three letters, and separated by commas. If possible, drug phenotypes are indicated by two lowercase letters for the drug followed by a hyphenated *s* or *r* for sensitive or resistant. See Table 1 for current drug abbreviations. Example: (cy-s, pm-r).
2. Mating types
   a. Mating types of *T. thermophila* are written in roman numerals and listed last. Example: (*CHX1*, *PJB1*/*chx1-1*, *PJB1*; *MPR1*/*MPR1*; cy-r, mp-s, IV). The above example refers to a clone heterozygous in the MAC for wild-type CHX1 and homozygous for wild-type PJB1 and MPR1, expressing the phenotype cycloheximide-resistant, 6-methylpurine-sensitive and the mating type IV. (It should be noted that as a consequence of phenotypic assortment, phenotypes can change.)
   b. Mating types (mt) of both *P. primaurelia* and *P. tetraurelia* are indicated as "o" (odd) or "e" (even) and listed last. Mt "o" (odd) gathers all the previously odd-numbered mating types (mtI, mtIII, mtV . . . mtXXVII), and mt "e" (even) gathers the previously even-numbered mating types (mtII, mtIV, mtVI . . . mtXXVIII) from the exvarieties of *P. aurelia*, 1 to 14, which were later recognized as distinct species by Sonneborn (1975).

TABLE 1

Drug abbreviations

bs = blasticidin	noc = nocodazole
col = colchicine	or = oryzilin
cm = chloramphenicol	pm = paromomycin
cy = cycloheximide	tet = tetrazolium
dg = 2-deoxy-d-galactose	tx = paclitaxel (formerly taxol)
mp = 6-methylpurine	vb = vinblastine

Gene products

The protein product of a gene is indicated by the gene name followed by a lowercase "p." In this case the gene name is not italicized, and only the first letter is capitalized, for example, Btu2p for the wild-type protein. For mutant proteins, follow the conventions used in the literature for the most commonly engineered changes in proteins.

COMPREHENSIVE EXAMPLES

1. Strain CU427 is a heterokaryon with a micronucleus homozygous for a drug-resistant mutation of the strain B *CHX1* gene, with a macronucleus carrying the drug-sensitive wild-type allele of the *CHX1* gene, and expressing mating type VII, for example, *chx-1*/*chx-1* (*CHX1*, cy-s, VII).

2. Clone AAKO is a "knockout heterokaryon" that has a micronucleus homozygous for both an insertional mutation in the strain B *ATU1* gene as well as a drug-resistant mutation of the strain *MPR1* gene. Its macronucleus is wild type and undetermined for mating type. Example: *atu1-1::neo1*/*atu1-1::neo1*; *mpr1-1*/*mpr1-1* (*ATU1*; *MPR1*; mp-s).
3. AAKO-1 is a clone derived by somatic transformation of the insertional mutant *atu1-1* gene into the macronucleus of strain AAKO. Its macronucleus thus contains both wild-type and transformed alleles of *ATU1*. Example: *atu1-1::neo1*/*atu1-1::neo1*; *mpr1-1*/*mpr1-1* (*ATU1*/*atu1-1::neo1*; *MPR1;* pm-r, mp-s).
4. Strain SB3539 has a micronucleus homozygous for a mutation in the *CHX1* gene from strain C3 and a macronucleus that is wild type for the strain C3 *CHX1* gene and is mating type I. Example: *chx1 [C3]-1*/*chx1[C3]-1* (*CHX1[C3]*; cy-s, I).
5. In mutants of Paramecium restricted to mating type "o," the new macronucleus is able to differentiate normally towards "*O*" or toward "*E*" genotype, according to the genotype ("*O*" or "*E*") of the previous MAC before autogamy. However, the cell does not express the "e" mating type when it is macronuclearly determined to be "*E*," presumably because of the lack of a product, and expresses "o" by default. Thus, mutant *mtA-1* of *P. tetraurelia*, restricted to mating type "o," can be determined to be either "*O*" or "*E*" in its macronucleus, although it can only express "o." Example: *mtA-1*/*mtA-1* (*O*, o) OR *mtA-l*/*mtA-1* (*E*, o), where the first letter in parentheses refers to the macronuclear genotype and the second letter to the phenotype.

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