Non-Mendelian Inheritance of Paralogs of 2 Cytoskeletal Genes in the Ciliate Chilodonella uncinata

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Recognition of the role of non-Mendelian inheritance is on the rise, particularly as epigenetic phenomena are shown to shape the transformation of genomes into phenotypes. Ciliates provide a model system in which to explore the role of epigenetics because ciliates have both a germ line (micronuclear) and somatic (macronuclear) genome within every cell. In the ciliate Chilodonella uncinata, the macronucleus is extensively fragmented such that many genes end up on their own chromosomes. Hence, it is possible to track the fate of unlinked genes within macronuclei of C. uncinata. Here we demonstrate that the pattern of inheritance in isolates of C. uncinata is complex and involves both Mendelian transmission between micronuclei and macronuclei and epigenetic phenomena. The macronuclei from 2 isolates of C. uncinata and their progeny share identical rDNA loci and 2 identical β-tubulin paralogs, yet have different actin paralogs and some β-tubulin paralogs that are not shared. We propose a model in which all the divergent paralogs are present in the ciliate macronuclei. Under this model, different paralogs are retained in developing macronuclei following conjugation. We further speculate that an epigenetic mechanism, such as RNA interference, is involved in selective retention of specific paralogs within lines. This system allows the exploration of epigenetic phenomena that shape somatic genomes and provides parallels to studies of the development of somatic nuclei within animals.

Introduction

Much like in animals, conventional models assume that loci present in ciliate “somatic” macronuclear genomes should follow Mendelian rules of inheritance, at least in the generations immediately following sexual conjugation (i.e., prior to any assortment of alleles). Here we specifically focus on the vertical transmission of loci, in this case duplicated (paralogous) genes, in somatic nuclei between generations. The null hypothesis is that paralogs present in the somatic genome of the parent should be found in offspring and that there is no intraspecific variation in the presence/absence of paralogs within somatic genomes.

Ciliates are defined by the occurrence of both a “germ line” micronucleus and somatic macronucleus in every cell. The micronucleus houses a typical eukaryotic genome with a small number of large chromosomes and divides by mitosis and meiosis. The macronucleus has a greater number of smaller chromosomes and is the site of virtually all vegetative transcription. The macronuclear genome is processed from a zygotic nucleus through chromosomal fragmentation, amplification, and the elimination of specific sequences within chromosomes (reviewed in Jahn and Klobutcher 2002; Yao et al. 2002).

In ciliates, sex is separated from reproduction and involves exchange of haploid nuclei during conjugation. Following conjugation, the resulting zygotic nucleus divides by mitosis and at least one resulting product is transformed into a macronucleus; the parental macronuclear degrades during this process. All ciliate division is asexual, with the germ line micronucleus dividing by mitosis and the processed somatic macronucleus dividing by amitosis, the latter being an imprecise mechanism that allows for differential assortment of alleles in at least some ciliates (reviewed in Jahn and Klobutcher 2002; Yao et al. 2002).

In 3 classes of ciliates—S militipharyng- ea (including Chilodonella uncinata, the focus of this study), and Armophorea—extensive processing generates macronuclear genomes with gene-sized chromosomes such that all genes are unlinked in the transcriptionally active macronuclei (Steinbrück et al. 1995; Katz 2001). This is in contrast to other ciliates where fragmentation of zygotic chromosomes generates longer macronuclear chromosomes (often >100 kb) that, in the case of the completely sequenced Tetrahymena thermophila genome, contain an average of approximately 100 genes per macronuclear chromosomes (Eisen et al. 2006). Phylogenetic analyses indicate that extensive fragmentation of macronuclear genomes has arisen at least twice, and possibly 3 times, within ciliates (Riley and Katz 2001).

There is increasing evidence of the role of epigenetics shaping patterns of inheritance in ciliate macronuclear genomes (reviewed in Meyer and Duhartcourt 1996; Yao et al. 2003; Mochizuki and Gorovsky 2004; McGrath et al. 2006). Data supporting epigenetics include experimental modifications of parental macronuclei (e.g., by inserting micronuclear-limited sequences) that are then inherited by newly developing macronuclei (Duhartcourt et al. 1995, 1998; Chalker and Yao 1996; Chalker 2005). One explanation of the non-Mendelian patterns of inheritance is that a RNA interference–based mechanism enables genome scanning between the zygotic and parental macronucleus to shape the newly developing macronucleus (Mochizuki et al. 2002; Mochizuki and Gorovsky 2004). Evidence for this model (reviewed in Chalker et al. 2005) includes the presence of small RNAs during conjugation of both T. thermophila (Howard-Till and Yao 2006; Lee and Collins 2006) and Paramecium tetraurelia (Garner et al. 2004) plus the presence of proteins such as Dicer (Malone et al. 2005; Mochizuki and Gorovsky 2005).

We set out to test the hypothesis that patterns of inheritance of gene-sized chromosomes in the macronuclei of C. uncinata follow the principles of Mendelian genetics—specifically that paralogous loci will be inherited in somatic macronuclei vertically between generations. Alternatively, patterns of inheritance might be nonvertical...
such that the somatic genome varies substantially following conjugation events. We propose this alternative hypothesis in light of the extensively processed nature of the *C. uncinata* macronuclear genome, resulting in each gene being unlinked within the somatic macronucleus, coupled with the possibility of widespread epigenetic phenomena in ciliates.

Our preliminary observations from 2 geographically isolated strains of *C. uncinata*, one from Poland and the other from the United States, indicated that macronuclei from these ciliates shared identical rDNA sequences but some highly divergent protein genes. To distinguish between geographic differences versus noncanonical patterns of inheritance, we generated clonal lines from the Poland and US isolates and then assessed the diversity of rDNA sequences and actin and β-tubulin paralogs between clonal lines and in lineages generated from crossing strains from the 2 locations.

**Materials and Methods**

**Ciliate Culture and DNA Isolation**

Two strains of *C. uncinata* were acquired from 1) American Type Culture Collection (designated here as USA; ATCC 50194, Manassas, VA) and 2) from a colleague, Stefan Radzikowski (designated here as Poland or POL; University of Warsaw, Warsaw, Poland). Morphospecies identity of the strain from Stefan Radzikowski was confirmed to be *C. uncinata* by ciliate taxonomist Wilhem Foissner, University of Salzburg. All strains were cultured at room temperature in the dark. Clonal lines of both cultures were generated by passing single cells through 3 rounds of isolation. Isolation of DNA from strains was performed with a phenol/chloroform protocol previously described in Riley and Katz (2001).

Conjugation between the 2 cultures was accomplished by placing a single cell from each strain into a droplet of pond water with a small amount of the bacterium *Klebsiella pneumonia* provided as food. Cells were placed into single droplets and checked every hour for conjugation. While waiting for the cells to undergo conjugation, some cells went through asexual division; hence, there is some uncertainty (discussed below) as to whether conjugation occurred between lines or within lines. Once conjugation was observed, the mating pair (designated as “F1” to reflect generation of novel macronucleus following conjugation) was pipetted into a 96-well plate and allowed to undergo asexual divisions. During this time, additional conjugation and multiple asexual divisions occurred. Once conjugation was observed within the F1s, a new conjugating pair (designated “F2”) was isolated as described above.

**Characterization of Protein-Coding Genes and rDNA Sequences**

The coding domains of actin and β-tubulin were amplified using eukaryotic-specific primers (Tekle et al. 2007), whereas the internal transcribed spacer (ITS) region was amplified with ciliate-specific primers (Snoeyenbos-West et al. 2002). The polymerase Phusion (Finnzymes, New England Biolabs, Ipswich, MA) and TaqGold were used for polymerase chain reactions (PCRs) (20 μl reactions). Resulting PCR products were cleaned using either a gel-isolating technique or MicroClean. With gel isolation, the PCR product was run on a 1.0% SeaKem agarose gel, extracted, and cleaned in a Millipore column. Cleaned PCR products were then cloned using Novagen’s cloning kit with pSTBlue-1 vector and competent cells or Zero Blunt TOPO Cloning Kit (Invitrogen, Carlsbad, CA).

Clones were miniprepped using one of 2 methods. The Qiaprep Spin MiniPrep kit (Qiagen, Valencia, CA) was used to miniprep individual clones. For large-scale analyses, Invitrogen’s 96-well format harvested-cell method, protocol B, was used to miniprep clones on a larger scale. Sequences were produced from all clones in both directions using the BigDye terminator RR mix from PE Applied Biosystems (Wellesley, MA, 4303152). Sequencing reactions were cleaned using gel filtration columns from Edge Biosystems (Gaithersburg, MD) and analyzed on a Perkin-Elmer ABI-3100 automated sequencer.

We use a working definition of paralogs as sequences within a single species that are more than 2% divergent at the nucleotide level. This percentage is often used as the cutoff for defining separate species and therefore represents a substantial level of divergence. Based on this definition, we assume that sequences that differ by less than 2% are due to recent gene duplication, allelic variation, and experimental error. Hence, in the present study, we are focusing only on relatively old paralogs or divergent alleles. Using this approach, we have chosen not to describe patterns of allelic variation—although we did observe low levels of allelic variation for some loci (e.g., 3 segregating sites within 1.2 kb of one β-tubulin paralog), our sampling strategy was not sufficient to confidently track the fate of alleles at each locus.

**Data Analysis**

Sequences were manually edited, contigs assembled, and putative paralogs identified using Seqman software (DNAStar, Inc., Madison, WI). Representatives of each paralog were then transferred to MegaAlign (DNAStar, Inc.) where open reading frames were examined. Sequence alignments were produced using the ClustalW algorithm (Thompson et al. 1994) as employed by Megalign (DNAStar, Inc.) with a gap penalty of 10 and a gap length penalty of 0.2 for multiple alignment parameters. Alignments were adjusted by hand and codon positions calculated in the program MacClade. The best-fit models of protein evolution for the actin and β-tubulin alignments are WAG + G and Blosum62, respectively (ProTest; Abascal et al. 2005). Genealogies and posterior probabilities were generated using these best-fit models with our amino acid alignments in MrBayes. Four simultaneous Metropolis-Coupled Markov Chain Monte Carlo chains were run for 4,000,000 generations sampling every 100 generations. Stationarity in likelihood scores was determined by plotting the −lnL against the generation. All trees below the observed stationarity level were discarded, resulting in a “burn-in” of 100,000 generations. The 50% majority-rule consensus tree was determined to calculate the posterior probabilities for each node. Bootstrap values were calculated under maximum
Table 1
Occurrence of Paralogs in Clone Libraries

<table>
<thead>
<tr>
<th>Strains</th>
<th>β-Tubulin</th>
<th>Actin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Shared ITS</td>
<td>Shared POL</td>
</tr>
<tr>
<td>Poland Pop†</td>
<td>1</td>
<td>8</td>
</tr>
<tr>
<td>Blue</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Green</td>
<td>22</td>
<td>2</td>
</tr>
<tr>
<td>F1GO</td>
<td>28</td>
<td>4</td>
</tr>
<tr>
<td>F2GO</td>
<td>1</td>
<td>21</td>
</tr>
<tr>
<td>USA-original line</td>
<td>21</td>
<td></td>
</tr>
<tr>
<td>Orange</td>
<td>4</td>
<td>15</td>
</tr>
<tr>
<td>Red</td>
<td>22</td>
<td>7</td>
</tr>
<tr>
<td>F1RB</td>
<td>41</td>
<td>6</td>
</tr>
<tr>
<td>F2RB</td>
<td>5</td>
<td>6</td>
</tr>
</tbody>
</table>

Note.—ITS = internally transcribed spacer regions of rDNA locus plus 5.8S rDNA.
† Five paralogs were identified from the original Poland population that were never found in any subsequent clonal line; these 5 paralogs are included in figure 1. Blue and green lines represent 2 clonal lines isolated from the Poland population, whereas red and orange lines are 2 clonal lines from the USA (ATCC) accession. The GO and RB lines are crosses between green and orange cells and red and blue lines, respectively. F1 and F2 designations refer to order of isolation of clonal lines resulting from crosses.

Results

Strains and Crosses

Initial studies were performed on a clonal line generated from an accession of *C. uncinata* (designated here USA-original line) and a “population” of cells received from Dr Radzikowski (Poland Pop). Once we found unusual patterns of sequences, we created additional lines for intensive study. We successfully generated 2 clonal lines from the USA sample (designated “red” and “orange”) and 2 clonal lines from the Poland population (designated “blue” and “green”). We then set up 2 crosses between these lines: red × blue (designated F1RB and F2RB) and green × orange (designated F1GO and F2GO). For all strains, DNA was prepped once cell number reached 20,000-40,000 cells (roughly 15 generations of asexual division); given the high amplification of macronuclear genomes relative to micronuclear genomes (estimated at 100- to 1,000-fold for ciliates with extensively processed genomes [Jahn and Klobutcher 2002]), this relatively small number of generations is insufficient for random assortment alone to remove alleles or paralogs. We also followed the fate of paralogs into subsequent generations, and after 9 months later, we characterized β-tubulin paralogs from single cells of 2 of the clonal lines (blue [Poland] and orange [USA]).

rDNA Locus

The Polish and USA lines of *C. uncinata* appear to contain identical rDNA loci in their macronuclei. We sequenced a limited number of small subunit rDNA (ssu-rDNA) genes from the Poland and USA lines and 127 clones of the “ITS region” that consists of the ITS 1, 5.8S rDNA, and ITS 2 (table 1). Previous work has shown that the ITS region has equivalent or slightly greater diversity within ciliates than the full-length ssu-rDNA gene (Snoeyenbos-West et al. 2002), and this short region is easy to amplify. Only a single ITS sequence was found in original lines and all crosses (table 1; GB#EU047813).

β-Tubulin

In contrast to the morphology and rDNA data, analyses of β-tubulin sequences reveal a complex picture of inheritance within *C. uncinata*. We first characterized β-tubulin from the clonal lines and crosses described above and found 2 putative paralogs that were shared between virtually all lines (shared P1 = GB#EU047814 and shared P2 = GB#EU047815). The 2 exceptions were the lack of shared paralog P1 among 34 clones analyzed for the F2GO line and 40 clones from the original USA line (table 1).

We also identified 6 additional paralogs that were unique to one line or the other (POL P1 = GB#EU047816, POL P2 = GB#EU047818, POL P3 = GB#EU047820, USA P1 = GB#EU047817, USA P2 = GB#EU047819, and USA P3 = GB#EU047821; table 1 and fig. 1). These 6 paralogs are found at varying frequencies among the isolates (table 1). The cells resulting from the green × orange cross (F1GO and F2GO) retain the shared paralogs and only the Poland-specific paralogs (POL P1–P3; table 1). In contrast, the cells resulting from the red × blue cross (F1RB and F2RB) retain the shared paralogs and only the USA-specific paralogs (USA P1–P3; table 1). These data suggest that both crosses may have resulted from conjugation between 2 cells of the same lines (e.g., red × red for the F1RB and F2RB crosses). However, not every paralog appears to be retained in all lines; for example, the shared paralog P1 and the POL P1 paralog were not found in the F2GO line (table 1). The absence of these paralogs may be due to the vagaries of PCR and cloning. Yet, as discussed below, data from actin at least for the red × blue cross indicate that this cross is not simply the result of conjugation between 2 cells from the same clonal line.

Genealogical analyses of inferred β-tubulin amino acid sequences encoded by these paralogs reveal that they
fall into 4 major groups: one clade containing the 2 shared paralogs and 3 other clades each pairing one Poland and one USA paralog (POL P1 + USA P1, POL P2 + USA P2, and POL P3 + USA P3; fig. 1A). The 2 shared paralogs are identical at the amino acid level, though they vary by 4.6% (uncorrected nucleotide differences) in nucleotide sequence. The average uncorrected nucleotide divergence among the remaining pairs of paralogs ranges from 5.7% for POL P1 and USA P1 to 13.5% for POL P3 and USA P3. The similarity in amino acid sequences between paralogs that are unique to the 2 isolates (e.g., POL P1 and USA P1) suggests that such paralogs are functionally related.

Of the 2 crosses we analyzed, β-tubulin paralogs in one cross, F1GO and F2GO, are more similar to the Poland parent as it contained the shared paralogs plus the unique Poland paralogs (POL P1–P3). The other cross, F1RB and F2RB, is more like the USA parent (table 1) and contains the shared paralog plus USA P1–P3. Not every paralog was retained in the lines; for example, the shared paralog P1 was not found in the F2GO line (table 1). The absence of this paralog may be due to the vagaries of PCR and cloning or may reflect loss from the macronucleus.

Actin

Similar to the β-tubulin data, analyses of actin from the same lines also reveal complex patterns of inheritance. In contrast to β-tubulin, each line that we examined contained only one actin paralog (table 1), with the exception of the 2 nearly identical actins in the F1RB strains as described below. The USA lines (orange and red) shared a sequence (Actin-USA1, GB#EU047828), whereas one of the Poland lines (green) and one set of crosses (F1GO and F2GO) contained a second sequence (Actin-POL1). This suggests that the green × orange cross may actually have been autogamous. The Actin-USA1 and Actin-POL1 sequences are very similar, differing at 2.5% uncorrected nucleotide divergence. The actin sequence from the blue line (GB#EU047826) differs from the green line at one silent site.

Surprisingly, divergent sequences containing one intron were obtained from the offspring of the red and blue cross (Actin-F1RB = GB#EU047822 and Actin-F1RB-2 = GB#EU047823). The Actin-F1RB and Actin-F1RB-2 sequences diverge from each other at only 2 silent sites within the coding domains, though there are numerous differences within the introns; only Actin-F1RB is included in genealogical analyses. A further divergent sequence emerged from the line resulting from conjugation between 2 F1RB cells (Actin-F2RB, GB#EU047834; fig. 1B). This last paralog contains 3 canonical eukaryotic introns, and hence, this larger fragment is expected to amplify less efficiently if other paralogs were present within F2RB macro- nuclei. Moreover, the predicted coding domains of the Actin-F1RB and Actin-F2RB paralogs are highly divergent from both the Actin-USA1 and Actin-POL1 sequences (uncorrected amino acid distances ~33% for each comparison). Finally, as part of our preliminary work for this study, we also isolated 5 actin paralogs from the original Poland population (GB#EU047829–GB#EU047833) that were never found in any subsequent experiment (P1–P5, fig. 1).

Experimental Controls

We ran 2 important controls to demonstrate that the presence of divergent paralogs among lines were not simply an artifact of PCR bias. First, 2 of the amplifications
were repeated using a different polymerase (TaqGold instead of Phusion) and PCR conditions. These repeated amplifications yielded similar results in terms of presence and frequencies of putative paralogs. Second, quantitative PCR analysis of one gene from the USA strains revealed that the frequency of paralogs in our clone libraries corresponded to the frequencies of sequences in the ciliate genomes relative to micronuclear genomes. PCR from total ciliate DNA is most likely amplification of macronuclear DNA. Moreover, with only one exception, no micronuclear-limited internally excised sequences (IESs) were found among the approximately 900 clones sampled for this study, even though we know that at least one \( \beta \)-tubulin paralog (USA P3) has IESs (Zufall and Katz 2007). The one exception was 2 small IESs found in 5 of 41 actin clones characterized from the 2 Poland clonal lines; these sequences differ by a single silent substitution within the micronuclear-destined sequences and 4 sites within the IESs (blue = GB#EU047826 and green = GB#EU047824). The IES-containing sequences represent either failed processing during macronuclear development or contamination by micronuclear DNA.

### Discussion

#### Non-Mendelian Inheritance

We observed nonvertical inheritance of both \( \beta \)-tubulin and actin paralogs within somatic macronuclei of the morphospecies *C. uncinata*. For \( \beta \)-tubulin, our original lines shared 2 paralogs plus we found 3 paralogs unique to each population (Poland P1–P3 and USA P1–P3). However, the frequencies and sometimes presence/absence of these paralogs varied among lines and crosses (table 1). After 9 months in culture, paralogs were generally retained within lines, though we once found a USA-specific paralog in a recent conjugation between 2 Poland cells (blue exconjugant 1; table 2). Moreover, one of the shared \( \beta \)-tubulin paralogs (shared P1) was only found in one clone from approximately 300 analyzed from 2 lines following 9 months in
culture. For actin, each line only had a single paralog, but for one cross (red × blue), the F1 and F2 isolates had unique paralogs (Actin-F1RB and Actin-F2RB; table 2) that were not identified in the parents. Intriguingly, these 2 red × blue–specific actins are both intron containing and therefore less likely to be amplified if in the same macronucleus as the intron-less Actin-USA1 or Actin-POL1 paralogs. Together, the data from β-tubulin and actin indicate that paralogs are not inherited in a simple Mendelian fashion in *C. uncinata*.

One important caveat for these experiments is that we cannot definitively demonstrate that our crosses were successful as autogamy is also a possibility. In fact, inheritance at both the β-tubulin and actin loci for the USA orange by Poland green cross (F1GO and F2GO) are consistent with this line being the result of autogamy between 2 Poland blue cells. However, the patterns of inheritance in the USA red × Poland blue cross are inconsistent with autogamy as novel actin paralogs emerge in the subsequent F1RB and F2RB lines. Further, the combination of shared identical paralogs and unique paralogs (e.g., Poland-specific and USA-specific β-tubulin paralogs, novel actin paralogs in F1RB and F2RB lines) indicated that inheritance of paralogs in processed macronuclei is not strictly vertical. This observation challenges an essential assumption of Mendelian inheritance—that loci (i.e., paralogs) present in somatic nuclei will be conserved across generations and within species.

### Functional Conservation of β-Tubulin Paralogs

A further striking pattern for the β-tubulin data is in the functional redundancy of the paralogs. The shared paralogs found in both the USA and Poland lines are identical at the amino acid level for the region examined, suggesting that they are functionally equivalent or at least very similar. Consistent with this argument, all *C. uncinata* cells have one or both of these 2 shared paralogs (tables 1 and 2). The most frequent Poland-specific paralog, as measured by presence in cells, is the highly divergent POL P3 (tables 1 and 2). Similarly, the most common USA-specific paralog, USA P3, is present in virtually all USA lines and cells samples (tables 1 and 2). These data suggest that 1) at least one of the shared paralogs is required for survival, 2) the USA and Poland strains then each have a second most common paralog that is highly divergent in amino acid sequence, and 3) the remaining paralogs are not essential for the ciliates.

### A Model

There are at least 2 possible mechanisms to explain the nonvertical transmission of paralogs in the macronuclei of *C. uncinata*. The first is population differentiation due to geographic isolation. Under such a model, the divergent β-tubulin paralogs (POL P1–P3 vs. USA P1–P3; table 1 and fig. 1) would have diverged over a period of reduced gene flow. However, this explanation is not consistent with the single, identical rDNA haplotype shared between populations or the presence of some shared β-tubulin paralogs that are found in macronuclei in both populations (shared P1 and P2; table 1 and fig. 1). Similarly, the occurrence of novel actin paralogs in sequences following crosses is inconsistent with geographic isolation as an explanation for divergent sequences.

An alternative hypothesis is that there is considerable intraspecific variation during the generation of macronuclei within *C. uncinata*, particularly when crosses are performed between strains from different locations. Under this hypothesis, all the paralogs we found (8 for β-tubulin and 4 for actin) exist within the macronuclear genome of members of both Poland and USA populations, and only a subset of these paralogs are retained in the macronucleus following conjugation. The presence of unlinked gene-sized chromosomes in *C. uncinata* may allow this ciliate species to “pick and choose” from among paralogs in generating macronuclei following conjugation.

In a simplified depiction of this second hypothesis (fig. 2), 4 paralogs of a gene are present in the micronucleus of *C. uncinata*, but only a few paralogs are included in the macronucleus following conjugation and subsequent macronuclear genome processing. There are 2 ways in which differential macronuclear processing could occur. Either paralogs could be “chosen” at random following each conjugation event (fig. 2A) or epigenetics influences the “choice” of paralogs such that macronuclei contain paralogs similar to those of the previous generation (fig. 2B). The first option seems unlikely as random inheritance of paralogs might result in a ciliate that lacks an essential sequence. Moreover, a model of random paralog choice is inconsistent with the retention of both shared (shared P1 or P2) and divergent (POL P3 or USA P3) β-tubulin paralogs within virtually every ciliate, as discussed above.

We speculate that epigenetic factors, such as RNA pools in cytoplasm, underlie patterns of inheritance in ciliate macronuclei. The nonrandom distribution of β-tubulin paralogs supports such factors, as does the resemblance between parent and offspring in clonal lines maintained over 9 months (table 2). This model is consistent with the appearance of novel actin paralogs in the F1RB and F2RB crosses as resulting from the epigenetic effects emerging from crosses with divergent cytoplasm (i.e., divergent RNA pools in the Poland and USA lines). An epigenetic mechanism involved in regulating paralogs in the macronucleus can ensure that essential sequences are retained.

### Evaluating the Model

In a preliminary test of the model, we carried out PCR experiments to assess whether the various line-specific paralogs could be amplified from other lines (e.g., primers specific to β-tubulin paralog USA P1 were tried on DNAs from the Poland lines). Here, we continued to use total DNA, which is predominantly macronuclear given the high level of genome amplification in ciliates with extensively processed genomes; extensively fragmenting ciliates can amplify macronuclear chromosomes as many as 1,000× compared with micronuclear copy number (Ammermann 1987; Jahn and Klobutcher 2002). Our hypothesis was that we would be able to amplify specific paralogs from lines in
which they appeared to be absent in initial screens with
degenerate primers if either 1) they were present at low copy
number within the macronucleus or 2) the paralogs were
present in the micronucleus and could be readily amplified
(i.e., contained small IESs and could be amplified from
a mixture of macronuclear and micronuclear DNA).

We designed specific primers for line-specific
\( \beta \)-tubulin paralogs, demonstrated that they worked on their
source lines, and then tried them on lines that lacked a
specific paralog. For all but one primer pair, PCRs on non-
source lines either failed or produced nontarget sequences
(i.e., PCR artifacts). The one exception was with primers spe-
cific for the POL P3 \( \beta \)-tubulin paralog (TTAggAACCTTg
CTgATTAaTTgC and ggTCCTAATTTTgCTTCTATCATg
re amplified with internal primers TTCgATAACgAAgCT-
CTgATCCTgCTA and GCTTCTATCATGGTTGGAGc-
cagt), which successfully amplified a 190-bp piece from
the USA red and orange lines. However, this nested set of
amplifications went 40 cycles each (80 total), whereas the pri-
mers amplified their source line after 35 cycles. We interpret
these data as indicating that the POL P3 \( \beta \)-tubulin paralog is
present in the USA lines but that it only exists within the mi-
cronucleus. Additional experimental tests of the model are
underway and require isolation of large quantities of micro-
nuclear-limited DNA.

Additional evidence for the proposed model includes
micro- and macronuclear \( \alpha \)-tubulin data from \textit{C. uncinata}
(Katz et al. 2003). Of 2 micronuclear forms discovered, one
was not amplified by PCR from total genomic DNA, sug-
gest that it was not present (or rare) in the macronucleus
(Katz et al. 2003). This is consistent with our hypothesis
that there are more paralogs in the micronucleus than will
be seen in any given macronucleus.

There is also a substantial and growing literature on
non-Mendelian patterns of inheritance in ciliates, with ex-
amples ranging from morphology (Grimes et al. 1980)
to molecules (e.g., Mochizuki and Gorovsky 2004;
Duham et al. 1995, 1998; Chalker and Yao 1996;
Chalker 2005). For example, non-Mendelian inheritance
of expression patterns have been reported for surface anti-
gens in \textit{P. tetraurelia} (Epstein and Forney 1984) and im-
mobilization antigens in \textit{T. thermophila} (Doerder and
Berkowitz 1987). Further, the epigenetic control of paral-
logs may favor, or at least allow, the maintenance of large
and diverse gene families as detected from both individual
gene studies from diverse ciliates (e.g., Israel et al. 2002;
Snoeyenbos-West et al. 2002; Katz et al. 2004; Zufall
et al. 2006) and analyses of 2 completed ciliate genomes
(Aury et al. 2006; Eisen et al. 2006). In sum, our results
elucidate patterns of inheritance within a single ciliate

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure2}
\caption{Model of non-Mendelian inheritance of paralogs in macronuclei of \textit{Chilodonella uncinata}: all paralogs are found in micronucleus (upper
oval within each cell), but only a subset is retained in the macronucleus (lower oval within each cell) following the chromosomal rearrangements
that occur after conjugation. Two possible mechanisms underlying this non-Mendelian pattern including: (A) a subset of paralogs present in the
micronucleus are retained at random following conjugation or (B) epigenetics plays a role in the retention of paralogs between subsequent conjugation
events such that there is similarity among macronuclei within lines. As depicted in this simplified drawing, 4 paralogs are present in all micronuclei
(indicated by 4 different shadings) but only a subset is found in macronucleus and at varying numbers. Excon refers to exconjugants—cells generated
following conjugation and subsequent macronuclear development.}
\end{figure}
species and, at broader level, inform our understanding of the transformations that take place in genomes during development of somatic nuclei in eukaryotes in general.

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