Condensins promote chromosome individualization and segregation during mitosis, meiosis, and amitosis in *Tetrahymena thermophila*

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ABSTRACT

Condensin is a protein complex with diverse functions in chromatin packaging and chromosome condensation and segregation. We studied condensin in the evolutionarily distant protist model *Tetrahymena*, which features noncanonical nuclear organization and divisions. In *Tetrahymena*, the germline and soma are partitioned into two different nuclei within a single cell. Consistent with their functional specializations in sexual reproduction and gene expression, condensins of the germline nucleus and the polyploid somatic nucleus are composed of different subunits. Mitosis and meiosis of the germline nucleus and amitotic division of the somatic nucleus are all dependent on condensins. In condensin-depleted cells, a chromosome condensation defect was most striking at meiotic metaphase, when *Tetrahymena* chromosomes are normally most densely packaged. Live imaging of meiotic divisions in condensin-depleted cells showed repeated nuclear stretching and contraction as the chromosomes failed to separate. Condensin depletion also fundamentally altered chromosome arrangement in the polyploid somatic nucleus: multiple copies of homologous chromosomes tended to cluster, consistent with a previous model of condensin suppressing default somatic pairing. We propose that failure to form discrete chromosome territories is the common cause of the defects observed in the absence of condensins.

INTRODUCTION

Condensin is a multi-subunit protein complex that was originally identified as a primary requirement for chromosome condensation in cell-free Xenopus oocyte extracts (Hirano and Mitchison, 1994). Subsequent studies in diverse organisms have shown it to be a highly conserved structural component of chromosomes that is important for proper chromosome segregation during both mitosis and meiosis (reviewed in Hirano, 2012, 2016). Condensin is proposed to act by encircling or extruding multiple DNA loops within the same chromatid, thus promoting or ensuring chromosome compaction (Nasmyth, 2001; Cuylen et al., 2011). In addition, condensin has been shown to function in dosage compensation (Caenorhabditis elegans), maintenance of rDNA repeats (*Saccharomyces cerevisiae*), tRNA gene clustering (*S. cerevisiae* and *Schizosaccharomyces pombe*), and DNA damage response and repair (Heale et al., 2006; Johzuka et al., 2006; Haeusler et al., 2008; Csankovszki et al., 2009; Iwaski et al., 2010; Sakamoto et al., 2011). These combined functions indicate that condensin is a crucial factor in genomic stability and is therefore implicated in cancer biology, genetic disorders, and reproductive health (Trimborn et al., 2006; Ham et al., 2007; Davalos et al., 2012; Je et al., 2014).

*Tetrahymena thermophila* is a free-living, freshwater ciliated protist. Like other ciliates, it exhibits nuclear dualism, that is, the germline and somatic genomes exist in separate nuclei within the same cell (Karrer, 2012). The germline nucleus has a diploid chromosome number of 10 and undergoes closed mitosis and meiosis (Figure 1). It is transcriptionally silent: its only role is to pass on genetic information during sexual reproduction. The somatic nucleus has a diploid chromosome number of 10 and undergoes closed mitosis and meiosis (Figure 1). It is transcriptionally silent: its only role is to pass on genetic information during sexual reproduction.
FIGURE 1: Vegetative and sexual cycles of Tetrahymena thermophila. During vegetative growth (left), the two nuclei of Tetrahymena divide asynchronously. First, the germline nucleus divides mitotically, then the somatic nucleus elongates and pinches off to form daughter nuclei, and, finally, the cleavage furrow closes to divide the cell. To induce meiosis, starved strains of two different mating types are mixed (right). The cells pair and their germline nuclei undergo synchronous meiosis, with pronounced nuclear elongation during prophase.

Materials and Methods. C-terminal tagging of Cph1 and Cph4 was unsuccessful, and therefore N-terminal HA tagging constructs were used to ectopically express these proteins. The ectopically expressed proteins could be visualized by immunofluorescence but caused aberrant nuclear divisions, indicating that either the proteins are not fully functional or that alteration of normal expression levels changed the dynamics of condensin function. Localization of tagged proteins is summarized in Table 1, and representative images are shown in Figure 3, B–H. In all cases, the condensin proteins were present throughout the cell cycle in both vegetative and mating cells. The localization of subunits indicates that the germline mitotic and meiotic functions of condensin are probably performed by complexes containing Smc2, Smc4, Cpd1, Cpg1, and Cph1 or Cph2. Condensin in the vegetative somatic nucleus is composed of Smc2, Smc4, Cpg1, Cpd1 or Cpd2, and Cph3 or Cph4, as shown in Figure 3A.

Condensin subunit localization

Tetrahymena is likely to use condensin complexes with specific compositions to fulfill the different functions necessary for performing mitosis and meiosis in the germline nucleus and amitosis in the somatic nucleus. To determine which subunits are active in which nucleus, we epitope tagged the protein subunits identified in our bioinformatic searches. With the exception of Cph1 and Cph4, all subunits were C-terminally tagged by knock-in of the tag epitope (see Materials and Methods).
Next, RNAi-mediated depletion of germline condensin components was performed in meiotic cells. Germline chromosome behavior is more easily studied in meiosis than in mitosis because individual chromosomes are well separated, and meiotic divisions are more synchronous. RNAi-mediated \textit{SMC2} or \textit{CPDT1} knockdown in mating cells caused defects in meiotic DNA condensation and segregation (Figure 6, A and B). Chromosomes failed to condense into distinct bivalents, and in cells undergoing meiotic divisions, extensive DNA bridging was observed at anaphase. A time course of fixed \textit{smc2}i or \textit{cpdt1}i cells showed in all, 50% of \textit{smc2}i cells showed anaphase bridges, whereas all control cells showed normal mitotic anaphase (Figure 5B).

Single knockdowns of germline-specific \textit{CPH1} and \textit{CPH2} showed milder defects such as occasional DNA bridging at mitotic anaphase. In \textit{cph1}i strains, 3.4% of cells in anaphase had DNA bridges; in \textit{cph2}i strains, 14% of anaphase cells had DNA bridges (Figure 5B). To test for functional redundancy between these two subunits, double RNAi knockdown strains were produced. These showed DNA bridging at anaphase in 31% of cells, suggesting at least partial redundancy (Figure 5B).

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| Subunit   | Gene ID     | Localization | RNAi phenotype                                                                 |
|-----------|-------------|--------------|--------------------------------------------------------------------------------|
| Common condensins |             |              |                                                                               |
| Smc2      | TTHERM_00812950 | *            | Failure to segregate germline chromosomes in mitosis and meiosis, failure to divide somatic nucleus, somatic chromosomes cluster |
| Smc4      | TTHERM_00446400 | +            | Not done                                                                      |
| Cpg1      | TTHERM_00919690 | +            | Not done                                                                      |
| Cpdt1     | TTHERM_00486070 | +            | Failure to segregate germline chromosomes in mitosis and meiosis, failure to divide somatic nucleus, somatic chromosomes cluster |

Germline condensins

| Subunit   | Gene ID     | Localization | RNAi phenotype                                                                 |
|-----------|-------------|--------------|--------------------------------------------------------------------------------|
| Cph1      | TTHERM_00728870 | +            | Anaphase bridging in mitosis and meiosis, partially redundant to Cph2          |
| Cph2      | TTHERM_00540340 | +            | Anaphase bridging in mitosis and meiosis, partially redundant to Cph1          |

Somatic condensins

| Subunit   | Gene ID     | Localization | RNAi phenotype                                                                 |
|-----------|-------------|--------------|--------------------------------------------------------------------------------|
| Cph3      | TTHERM_00554600 | *            | Failure to divide somatic nucleus, somatic chromosomes cluster                 |
| Cph4      | TTHERM_01299730 | +            | None detected                                                                  |
| Cpdt2     | TTHERM_00392760 | +            | Failure to complete sexual reproduction                                         |

*Localization was inferred from the RNAi phenotype.

**TABLE 1:** Localization and depletion phenotypes of \textit{Tetrahymena} condensin subunits.

FIGURE 2: Phylogenetic tree reconstruction indicates that \textit{Tetrahymena} condensins are more closely related to condensin I than condensin II. To determine relationships of (A) Cap-D and (B) Cap-H proteins, sequences were aligned with Clustal Omega, and, subsequently, maximum-likelihood trees were generated using PhyML v3.1 with the LG substitution model (Guindon et al., 2010; Sievers et al., 2011). Colored boxes highlight the condensin I (yellow) and condensin II (green) genes. Branch support is indicated by bootstrap values in percentages shown near each branch. The branch-length scale bar represents the estimated number of substitutions per amino acid site. Both \textit{Tetrahymena} Cap-D homologues group within the Cap-D2 subfamily of HEAT repeat proteins and all four \textit{Tetrahymena} Cap-H homologues group within the Cap-H kleisin subfamily with high bootstrap support, confirming that all \textit{Tetrahymena} condensin complexes were derived from condensin I.
FIGURE 3: Composition and localization of condensin proteins in Tetrahymena. (A) Condensin is a multi-subunit protein complex composed of two "structural maintenance of chromosomes" proteins, Smc2 and Smc4, each consisting of a hinge domain joined to an ATPase head domain by a long coiled coil. The two Smcs associate via the hinge domains, and the head domains interact with a kleisin subunit, Cph1, 2, 3, or 4. The Cph subunit interacts with two additional subunits, Cpg1 and the Cpdt1 or Cpdt2 HEAT repeat protein. In Tetrahymena, germline and somatic nuclei contain condensin complexes with distinct subunit composition, as shown by epitope tagging experiments (B–H). Scale bars equal 5 μm. Merged images show DAPI staining in magenta and tagged proteins in green. (B) Smc4 localizes to both the somatic and germline nuclei, a mitotic cell is shown. Smc4 appears to be highly abundant and distributed throughout the somatic nucleus. The protein appears less abundant in the germline and forms some brighter foci on the dividing chromosomes. (C, D) Cpg1 and Cpdt1 localize to both somatic and germline nuclei in mitotic cells (top panels) and meiotic cells (bottom panels). Cpg1 (C) is shown in anaphase of meiosis I, and Cpdt1 (D) is shown at metaphase/diakinesis. (E, F) Cph1 and Cph2 are found only in the germline nucleus. Top panels show mitotic cells, bottom panels show cells in meiosis II. Cph1 is shown at telophase II (E), Cph2 at early anaphase II. Again, bright foci can be seen within the germline nuclei. (G, H) Cpdt2 and Cph4 localize only to somatic nuclei. Vegetative cells are shown.
FIGURE 4: Colocalization of condensin and centromeres. Cells expressing GFP-tagged Ndc80 were mated with cells expressing Cpdtp1-HA. One cell of each pair is shown; scale bar represents 5 μm. Higher intensity Cpdtp1-HA foci colocalizing with Ndc80-GFP are visible on chromosomes from metaphase onward but are most obvious at anaphase of meiosis I. Top panels, early anaphase; bottom panels, late anaphase.

FIGURE 5: RNAi-mediated knockdown of germline condensin genes during vegetative growth causes segregation defects in germline nuclei. (A) In wild-type cells, the germline nucleus divides prior to somatic amitosis and formation of the cleavage furrow. RNAi of germline condensin genes resulted in anaphase bridges during mitosis. Top panel, normal mitotic divisions; bottom panel, examples of bridged mitosis in cph1i/cph2i cells. Scale bar equals 5 μm. (B) Mitotic chromosome segregation was assayed by counting anaphase cells in which the two new nuclei were separated by at least the diameter of the somatic nucleus. If the two chromatin masses of the dividing germline were not completely separated, anaphase was classified as “bridged.” Quantitation of RNAi-treated cells versus controls shows that anaphase bridging is more frequent in cph1i/cph2i double RNAi cells than in single RNAi cells. Four strains each of control, cph1i, cph2i, and double RNAi cells were evaluated, with 50 anaphases counted under each condition. Two strains of smc2i cells were evaluated, with 100 anaphases counted for each.
At 4.5 h after induction of meiosis, 77% of cells in meiosis I or II showed anaphase bridges, compared with 0% in wild-type (WT) cells (average of three matings, 100 cells counted). Delayed progression of meiosis. At 5.5 h after induction of meiosis, only 20% of smc2i or 12% of cpdt1i cells had reached anaphase II, compared with 56% of SMC2 control or 28% of CPDT1 control matings. Meiotic divisions were also abnormal in cph1i, cph2i double-depleted cells: at 4.5 h after induction of meiosis, 77% of cells in meiosis I or II showed anaphase bridges, compared with 0% in wild-type (WT) cells (average of three matings, 100 cells counted).

FIGURE 6: Condensin is required for DNA segregation in meiosis. (A) RNAi against SMC2 has no effect on nuclear stretching at meiotic prophase but results in reduced condensation of metaphase chromosomes and extensive DNA bridging at anaphase I and II compared with WT cells. Scale bar equals 5 μm. (B) Enlarged images of metaphase and telophase chromosomes show that chromosomes are less condensed in smc2i cells than in WT cells. Scale bar equals 2 μm. (C) Matings between smc2i and spo11i cells show DNA bridging at anaphase I, indicating that bridge formation is independent of double-strand breaks. Scale bar equals 5 μm. For each sample, 100 meiotic cells were counted and scored as showing either normal meiotic divisions (meiosis I and II categories) or bridged anaphase. Values plotted are the average of two experiments.
Live cell imaging of meiosis in smc2i cells was performed to observe germline chromosome segregation defects in real time (Figure 7 and Supplemental Movies S1 and S2). In vivo observation of the meiotic divisions revealed spectacular failures in segregating the chromosomes. One such example is shown in Figure 7B (Supplemental Movie S2). As cells attempted the first meiotic division, two masses of chromatin separated, with some DNA remaining between. This DNA bridge most likely represents entangled chromosomes that act like an elastic band, subsequently pulling the two masses of chromatin back toward each other after the failed attempt at division. However, the meiotic program progressed, and another attempt at division was made. The second attempt resulted in two masses of chromatin that were mostly separate but appeared to remain connected by chromatin links. In some cells, a large amount of
DNA was unable to segregate to the poles before the spindle collapsed; this later appeared as a separate chromatin body in the cytoplasm. Therefore, at the end of meiosis, cells often contained three small bodies of germline chromatin (i.e., aberrant nuclei), which were eventually degraded, leaving only the somatic nuclei.

**Segregation defects are not dependent on meiotic DNA double-strand breaks**

Clearly, some type of DNA entanglement prevents segregation of mitotic and meiotic chromosomes in the absence of condensin. These DNA linkages could merely represent a failure of sister chromatid decatenation after replication; alternatively, in meiosis, they could be caused by incomplete repair of meiotic double-strand breaks (DSBs). In meiosis in budding yeast condensin mutants, anaphase bridges were shown to be recombination dependent; they were alleviated in the spo11 mutant background, in which DSBs are not formed (Yu and Koshland, 2003). To investigate whether anaphase bridges during meiosis in Tetrahymena smc2i cells are recombination dependent, we mated smc2i cells with spo11i cells (Figure 6C). As RNAi expressed in one cell can pass into the partner cell during mating, in this way we could achieve the double knockdown of SMC2 and SPO11. In control matings, 65.5% of cells completed meiosis II by 5.5 h after induction of meiosis. In matings between smc2i and control cells, 75.5% of cells had bridged anaphase I nuclei and only 10.5% of cells completed meiosis II by 5.5 h post-induction. In smc2i × spo11i matings, the number of bridged anaphase I cells increased to 88.5%, and only 4.5% of cells reached anaphase II. This result indicates that in the absence of Spo11, the delay in meiosis and the defects in segregation were slightly increased rather than reduced. Therefore, the linkages that prevent segregation are not dependent on persistent homologous connections but rather on nonhomologous linkages or entanglements that occur in the absence of proper condensation.

**Depletion of condensin from the somatic nucleus prevents chromosome segregation and alters nuclear organization**

As described previously, after partial SMC4 knockdown (Cervantes et al., 2006), chromatin is unevenly distributed in the dividing somatic nucleus. Here, we similarly found that when the somatic nucleus divides in smc2i, cpdt1i, or cph3i strains, one half usually receives most of the DNA, while the other half receives almost none. In other cells, the cleavage furrow ends up cutting through the mass of chromatin, and much of the DNA in the middle is lost (Figure 8A). Interestingly, the size of the nearly DNA-free nucleus is almost normal; this was particularly evident when the nuclear membrane was stained using an antibody against the nuclear pore protein Nup93 (Figure 8B).

We first chose to label one of the smaller chromosomes (79 kb). In wild-type cells, FISH signals formed distinct spots that were more clustered distribution (Figure 9). In cells in which Smc2, Cph3, or Cpd1 had been depleted for 24 h, the signals remained distinct spots and did not appear substantially larger or more diffuse than those of WT nuclei, suggesting that condensin may have a more important role in organizing the

![FIGURE 8: RNAi-mediated knockdown of somatic condensin genes disrupts amitotic division.](image)

(A) In WT cells, the somatic nucleus elongates and splits into two new nuclei prior to cell division. In contrast, smc2i or cph3i cells divide with unequal distribution of DNA in somatic nuclei, or DNA remains in the cleavage furrow and is lost when the cells separate. Interphase cells often contain somatic nuclei with very little DNA. Arrowheads indicate areas of nuclear membranes lacking DAPI-stained chromatin. (B) cpdt1i cells show a similar phenotype to smc2i and cph3i cells, that is, loss of somatic DNA. Control cells stained with an anti-Nup93 antibody (red) show the nuclear membrane closely surrounding the DNA. cpdt1i nuclei have large, DAPI-free areas within the nuclear membrane, indicating lower DNA content. Scale bar equals 5 μm in A and B. (C) Western blotting of protein extracts from Cpdt1HA + cpdt1i cells before induction or 4 and 24 h after induction of RNAi. After 24 h, protein levels are comparable to 1/10 that of uninduced cells (lane 2). Total protein (Bio-Rad stain free visualization) is shown as a loading control.
homologous chromosomes have a tendency to interact unless actively prevented from doing so (Joyce et al., 2016) and suggest that condensin may act as an anti-pairing factor. This action is also consistent with the concept of chromosome territories, in which each chromosome maintains its own distinct space and is not comingled or intertwined with other chromosomes (Cremer and Cremer, 2010).

**DISCUSSION**

**The evolutionary diversification of condensins**

In multicellular eukaryotes, two forms of condensin (condensin I and II) have evolved from a common ancestor by the diversification of kleisins and HEAT repeat subunits, with the Smc subunits remaining unchanged. These two forms have different functions in axial versus lateral chromosome compaction and centromere organization (Hirano, 2012).

**Caenorhabditis elegans** employs a third condensin that has a modified Smc4 subunit but is otherwise identical to condensin I, with a function in sex chromosome dosage compensation (Csankovszki et al., 2009). This degree of functional radiation is surpassed by the diversification of condensin components in somatic chromosomes in three-dimensional space than in condensing them.

Chromosome clustering became progressively more severe over time after induction of SMC2 RNAi (Figure 9A). To determine whether clustering was due to general clumping of chromatin within one area of the nucleus, we labeled a second chromosome (131 kb) in a different color (Figure 9B). Double FISH showed that the two chromosome clusters did not colocalize; hence, FISH signal clustering was not due to general chromatin clumping. The 131-kb chromosome showed only moderate clustering compared with the 79-kb or 367-kb chromosomes, indicating that clustering is independent of chromosome size (Figure 9B).

Notably, chromosome clustering is not due to a failure to separate DNA copies after replication. In most cells at 4 h after RNAi induction, somatic chromosomes are not dividing; therefore, if replication continued without separation of newly replicated copies, we would expect the foci to become brighter but would not expect the overall number or distribution to change. Instead, our results support the somewhat controversial theory that daughter or homologous chromosomes have a tendency to interact unless actively prevented from doing so (Joyce et al., 2016) and suggest that condensin may act as an anti-pairing factor. This action is also consistent with the concept of chromosome territories, in which each chromosome maintains its own distinct space and is not comingled or intertwined with other chromosomes (Cremer and Cremer, 2010).

**FIGURE 9:** Condensin depletion causes chromosome clustering in somatic nuclei. (A) Representative images from FISH of a 79-kb chromosome in the somatic nucleus show examples of evenly distributed, unevenly distributed, or clustered chromosomes. Quantitation of chromosome clustering in smc2i, cph3i, and cpdt1i cells shows the various effects of depleting different condensin subunits. Temporal analysis after induction of RNAi in smc2i cells shows a gradual increase in chromosome clustering. RNAi was induced in starved cells, which were then refed to induce cell division and fixed at the indicated time points. (B) FISH of two different chromosomes shows nonoverlapping clusters, with various degrees of clustering for different probes. Scale bar equals 5 μm in A and B.
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Condensin's contribution to mitosis and meiosis

Tetrahymena has small chromosomes and performs closed mitosis and meiosis. Mitotic chromosomes are difficult to study because of their small size, and the only visible effect of condensin depletion in mitosis was difficulty in separating daughter nuclei (Figure 5). In contrast, meiosis is more amenable to cytological inspection, and the observed meiotic anomalies in condensin mutants may also pertain to mitosis. Tetrahymena has a somewhat unusual meiotic program. When starved cells of different mating types are mixed, they pair and initiate sexual reproduction (Figure 1) (reviewed in Cole and Sugai, 2012). The prophase germine nucleus elongates to about twice the length of the cell (Figure 6A). During this stage, the chromosomes are roughly aligned within the tubular nucleus with the centromeres at one end and the telomeres at the other (Loidl and Scherthan, 2004; Loidl et al., 2012). As the nucleus shortens, DSBs are repaired and chromatin condenses until bivalents are formed into distinct bivalents (Yu and Koshland, 2003; Hartl et al., 2008b; Lee et al., 2011). In budding yeast, there is evidence that meiotic bridging is caused by physical linkage of chromosomes, because condensin is required in meiosis for cohesin removal and resolution of recombination-dependent linkages (Yu and Koshland, 2003). In Tetrahymena, however, inhibition of meiotic recombination by SPO11 depletion increased rather than abrogated the severity of DNA bridging in condensin-depleted cells. Without recombination, homologues do not become linked and should separate freely in meiosis I. Therefore, the linkages causing persistent bridging in anaphase I must occur between either homologous or heterologous chromosomes, as reported during male meiosis in Drosophila cap-H2 mutants (Hartl et al., 2008b). Without condensin, it is likely that proper chromosome territories are not formed, resulting in intertwines between heterologous chromosomes.

Condensin in the organization of the polyplloid nucleus

In diploid nuclei, cohesion of sister chromatids prior to cell division is critical for achieving equal segregation during mitosis. However, amitosis of Tetrahymena's polyplloid somatic nucleus requires a different strategy to ensure nearly equal distribution of the ~50 copies of each chromosome. Experiments demonstrating pheno-typic assortment imply that chromosome copies are distributed randomly to daughter nuclei (Allen and Nanney, 1958; Orias and Flacks, 1975). If correct, then this would require a mechanism for maintaining the separation, rather than cohesion, of chromosome copies (Figure 10). Condensin appears to be performing such a function in the somatic nucleus of Tetrahymena. We found dramatic clustering of somatic chromosome copies in cells depleted of the core condensin gene SMc2, the HEAT repeat gene CPD1T, or the soma-specific kleisin CPH3. In addition to a role for condensin in promoting territory formation (Hartl et al., 2008b; Bauer et al., 2012; Ito and Narita, 2015; Iwasaki et al., 2016) and decatenation (Leonard et al., 2015; Sen et al., 2016), there are several reports that the complex has a specific function in separating homologous sequences. In mouse neuronal stem cells, condensin II was found to prevent hyperclustering of pericentromeric regions known as chromocenters (Nishide and Hirano, 2014). In Drosophila, condensin II was reported to promote dissolution of polyteny chromosomes and antagonize transvection, a process in which homologous loci influence each other's transcription through their physical interaction (Hartl et al., 2008a; Nguyen et al., 2015). The action of condensin in the Tetrahymena somatic nucleus seems to be yet another example of such anti-interaction functions of condensin in interphase nuclei.

The complete failure to segregate somatic chromatin in the absence of condensin cannot be entirely explained by clustering of chromosome copies. Massive interlinkage of all chromosomes would have to occur to prevent segregation; however, this is improbable due to the number and small size of somatic chromosomes. Therefore, it seems likely that condensin plays an additional, more active, role in somatic nuclear division. A previous study showed that partial knockdown of SMc4 disrupted microtubule formation within the dividing somatic nucleus (Cervantes et al., 2006). Exploration of the molecular or genetic interactions between condensin and microtubules in the dividing somatic nucleus may therefore lead to a better understanding of the unconventional amitotic segregation mechanism.
A universal model for condensin action

In this study, we show that condensin is required to segregate chromosomes in the two very different nuclei of *Tetrahymena*. Condensin mediates the condensation and resolution of germline chromosomes and promotes the spatial distribution and segregation of the smaller chromosomes of the polyploid somatic nucleus (Figure 10). One proposed mechanism for chromosome condensation is “loop extrusion” (Nasmyth, 2001; Goloborodko et al., 2016a,b). In this scenario, cohesin or condensin complexes bind a linear DNA molecule and, through the extrusion of loops, create a compact “noodle” closely resembling a condensed eukaryotic chromosome. By taking into account specific parameters for condensin occupancy, loop size, and the presence of a Topo II-like activity, loop extrusion can remarkably recapitulate the process of chromosome territory formation, sister chromatid resolution, and condensation (Goloborodko et al., 2016a). Condensin has been shown to mediate Topo II recruitment and resolution of DNA intertwiners on chromosome arms (Leonard et al., 2015). A recent study showed that Topo II can both create and resolve DNA intertwiners and that close physical proximity of sister chromatids promotes the catenation reaction in the absence of condensin-dependent supercoiling (Sen et al., 2016). Therefore, it is likely that the combined actions of cohesin removal and condensin supercoiling promote decatenation of sister chromatids (Figure 10). This model fits well with the action of condensin on germline *Tetrahymena* chromosomes and can be extended to account for condensin’s functions in the somatic nucleus. Chromosomes of *Tetrahymena’s* polyploid somatic nucleus are much smaller than most eukaryotic chromosomes, but their spacing nevertheless implies that they form distinct territories. Therefore, if a loop extrusion mechanism can produce chromosome territories within large chromosomes, then it should also be sufficient to separate replicated copies of small chromosomes, as well as untangle any unwanted interactions that occur between homologous sequences. In many organisms, it is now assumed that cohesin complexes are involved in loop extrusion (Schwarzer et al., 2017). However, cohesin is not present in *Tetrahymena’s* somatic nucleus, and therefore condensin may have been harnessed for this function.

Duplication and divergence of condensin subunits in *Tetrahymena* may explain the evolution of condensin complexes with various DNA-binding positions, on-off rates, or loop processivity, as well as the ability to interact with additional chromatin-bound proteins. Altering these properties, but not the basic action (i.e., DNA looping) of each condensin complex, may be sufficient to produce the range of condensin functions in both the germline and somatic nuclei.

**MATERIALS AND METHODS**

**Strains and growth conditions**

*Tetrahymena* strains B2086 and Cu428 obtained from the *Tetrahymena* stock center (https://tetrahymena.vet.cornell.edu/) were used as wild-type strains for transformations and as genomic DNA sources for amplification of regions used in tagging and RNAi constructs. Both strains were grown in modified Neff medium using standard methods (Orias et al., 2000).

**Protein tagging and localization**

Endogenous C-terminal protein tagging was performed by a knock-in strategy, as previously described (Howard-Till et al., 2013). In short, 500 base pairs of the C terminus of the gene of interest and 500 base pairs downstream of the gene were amplified by PCR and combined with the tagging epitope and Neo4 cassette by Gibson assembly using the NEBuilder HiFi DNA assembly master mix (New England Biolabs, Frankfurt, Germany). Primers used for amplification and assembly are listed in Supplemental Table S1. Plasmids pHNeo4, pGFP-Neo4, and pmCherry-Neo4 were used as the sources of tagging cassettes (gifts of K. Mochizuki). Transformation of strains by biolistic particle bombardment was performed as previously described (Cassidy-Hanley et al., 1997; Bruns and Cassidy-Hanley, 2000). Smc4-GFP, Cpg1-HA, Cpd1-mCherry, Cpd2-HA, and Cph2-mCherry strains were all constructed in this way, and protein localization was visualized in fixed cells by either direct detection or immunofluorescence (IF), as previously described (Loidl and Scherthan, 2004; Howard-Till et al., 2013). Primary antibodies used for IF were rabbit polyclonal anti-HA (1:100; Sigma, St. Louis, MO) and Living Colors mouse monoclonal JL-8 anti-GFP (1:50) and rabbit...
polyclonal anti-dsRed (1:100; Clontech Laboratories, Mountainview, CA). Endogenous C-terminal tagging of the other Cph subunits was unsuccessful; therefore, we ectopically expressed N-terminal HA-tagged proteins from the Mti1 promoter. HA-Cph1 and HA-Cph4 expression constructs were created by amplifying the entire coding region of each gene and inserting it into pBNM22-HA and the HHT2-mCherry construct. Ece Sahi performed the live imaging experiments, and Emine Ali (Max F. Perutz Laboratories, Vienna, Austria) assisted with epitope tagging. Thanks also to Kazufumi Mochizuki (National Center for Scientific Research, Montpellier, France) for tagging and expression constructs. Finally, we thank Maria Novatchkova (Research Institute of Molecular Pathology, Vienna, Austria) for bioinformatics advice. This work was supported by grant P 28336-B28 from the Austrian Science Fund (FWF).

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Liver cell imaging

For live imaging, a Commodore Compressor device was used to flatten and immobilize the cells (Yan et al., 2014). Mating cells were concentrated by centrifugation and resuspended in 3% polyethylene oxide (Sigma, St. Louis, MO) to increase the viscosity of the medium, and 1 µl of the cell suspension was placed into the compressor. Cells were transformed with an HHT2-mCherry construct to visualize DNA (gift of K. Kataoka). Imaging was performed with a Zeiss Axioskop 2 wide-field fluorescence microscope, and images were aligned and processed using Adobe Photoshop and ImageJ software.

Fluorescence in situ hybridization

For live imaging, a Commodore Compressor device was used to flatten and immobilize the cells (Yan et al., 2014). Mating cells were concentrated by centrifugation and resuspended in 3% polyethylene oxide (Sigma, St. Louis, MO) to increase the viscosity of the medium, and 1 µl of the cell suspension was placed into the compressor. Cells were transformed with an HHT2-mCherry construct to visualize DNA (gift of K. Kataoka). Imaging was performed with a Zeiss Axioskop 2 wide-field fluorescence microscope, and images were aligned and processed using Adobe Photoshop and ImageJ software.

**Fluorescence in situ hybridization**

FISH was performed as previously described (Loidl and Scherthan, 2004), using probes generated by PCR amplification of 8–10 different regions (each ~10 kb long) spanning the entire length of the somatic chromosome of interest. The total amount of DNA labeled was similar for each chromosome. Primers are listed in Supplemental Table S1. Somatic chromosome scaffolds of probed chromosomes were as follows: scf_8253887 (367,094 base pairs), scf_8254632 (79,368 base pairs), and scf_8254505 (131,308 base pairs). Sequences were retrieved using the genome browser of the Tetrahymena Genome Database (www.ciliate.org).

**RNAi**

RNAi is performed by expressing a hairpin RNA molecule from an inducible promoter and can reduce RNA levels by up to 90% (Howard-Till and Yao, 2006; Howard-Till et al., 2013). RNAi constructs were created and introduced into cells as previously described for pREC8hpCYH (Howard-Till et al., 2013). Primers used to amplify gene fragments are listed in Supplemental Table S1. RNAi was induced by adding 0.3 µg/ml CdCl2 to cells growing in Neff medium or 0.05 µg/ml CdCl2 to cells in starvation medium. Starved cells were centrifuged and resuspended in starvation medium lacking cadmium prior to mixing to induce mating. Additional RNAi constructs for CPH1 and CPH2 were constructed to perform double RNAi experiments. These constructs were integrated into the BTU1 locus using the NEO5 selection cassette and expressed the RNAi hairpin from the MTT2 copper inducible promoter (Boldrin et al., 2008). RNAi from these constructs was induced by adding 100 µM CuSO4 to growing cells or 10 µM CuSO4 to starving or mating cells. Wild-type controls carried the relevant unexpressed RNAi construct. Confirmation of CPDT1 RNAi was performed by Western blotting analysis of tagged protein levels for a Cpd1HA strain transformed with the CPDT1 hairpin construct (Figure 8C). Protein extracts were run on a Mini-PROTEAN TGX Stain-Free 4–20% gradient gel (Bio-Rad, Hercules, CA). The gel was blotted, and the membrane was probed with rabbit polyclonal anti-HA (1:1000; Sigma, St. Louis, MO). For the remaining constructs, RT-qPCR was used to assay RNA levels for the appropriate gene (Supplemental Figure S1). RNA was extracted from cells using TriFAST reagent (VWR, Radnor, PA), and RNA levels for the appropriate gene (Supplemental Figure S1). RNA was extracted from cells using TriFAST reagent (VWR, Radnor, PA), and RT-qPCR was performed using the LUNA Universal One-Step RT-qPCR kit (New England BioLabs, Frankfurt, Germany).

Live cell imaging

For live imaging, a Commodore Compressor device was used to flatten and immobilize the cells (Yan et al., 2014). Mating cells were concentrated by centrifugation and resuspended in 3% polyethylene oxide (Sigma, St. Louis, MO) to increase the viscosity of the medium, and 1 µl of the cell suspension was placed into the compressor. Cells were transformed with an HHT2-mCherry construct to visualize DNA (gift of K. Kataoka). Imaging was performed with a Zeiss Axioskop 2 wide-field fluorescence microscope, and images were aligned and processed using Adobe Photoshop and ImageJ software.

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