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LTL1 Protein Regulates DNA Rearrangement Boundaries in *Tetrahymena thermophila*

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The somatic genome of ciliated protozoan, *Tetrahymena thermophila* undergoes extensive reorganization during development to allow for efficient gene expression. *Tetrahymena* possess two functionally different nuclei, a micronucleus and a macronucleus. The transcriptionally silent micronucleus, or germline nucleus, contains five pairs of condensed chromosomes. The macronucleus, or somatic nucleus, is transcriptionally active, providing for all gene expression. During sexual development, new somatic and germline nuclei differentiate, which for the somatic genome requires thousands of site-specific deletions of DNA segments called internal eliminated sequences (IESs). Removal of IESs eliminates approximately 30% of the genome from over 10,000 individual loci to create a streamlined somatic nucleus that drives gene transcription. We identified a protein, encoded by *LTL1*, required for precise excision of a specific subset of IESs. Wild-type cells exhibit reproducibly accurate excision of IESs; however, in Δ*LTL1* cells, this subset of IESs, which includes IES D, is excised with aberrant and heterogeneous boundaries. Thus, *LTL1* appears to determine the position of deletion boundaries for specific IESs. To establish that loss of *LTL1* alone is responsible for the observed defects in IES excision, I introduced a wild type copy of *LTL1* into Δ*LTL1* cells and found that excision accuracy of IES D was restored. I attempted to pinpoint the specific cis-acting sequences required for accurate IES excision of IES D by introducing site-specific mutations in the region flanking this IES and assessed the impact on excision boundaries. Mutagenesis of sequences upstream of -75bp resulted in aberrant excision boundaries, indicating that *LTL1* requires these to precisely excise IES D. This work provides clear evidence that *LTL1* regulates the excision boundaries of specific IESs through recognition of sequences within the flanking region. Investigating the role *LTL1* plays in reorganizing the *Tetrahymena* genome sheds light onto the complexity by which chromatin domains are established in eukaryotes.