Both subtelomeric regions are required and sufficient for specific DNA fragmentation during macronuclear development in Stylonychia lemnae

ABSTRACT

Our results indicate that an inverted repeat with the core sequence 5'-TGAA present in both subtelomeric regions acts as a Cbs in Stylonychia. The results allow us to propose a mechanistic model for DNA processing in this ciliate.

INTRODUCTION

Background Programmed DNA-reorganization and DNA-elimination processes are frequently observed in differentiating eukaryotic cells. Examples include the mating type switch in yeast, the antigen variation in trypanosomes and other parasitic flagellates, the specific DNA elimination observed during embryogensis of nematodes, Cyclops and Sciara, and the processing of mammalian immunoglobulin and T-cell receptor genes. However, these processes are most extreme in hypotrichous ciliates, which therefore serve as model systems to study the molecular basis of programmed DNA reorganization, DNA elimination and specific DNA fragmentation during cellular differentiation. One characteristic of ciliated protozoa is the occurrence of two morphologically and functionally different nuclei in one cell: the generative micronucleus and the somatic macronucleus, which is responsible for transcription during vegetative growth. After sexual reproduction, a new macronucleus is formed as a derivative of the micronucleus while the old macronucleus degenerates. The stages of macronuclear development in hypotrichous ciliates, such as Oxytricha, Euplotes or Stylonychia, are summarized in Figure 1. First, a DNA-synthesis phase leads to the formation of polytene chromosomes. During this developmental stage, transposon-like elements, as well as short internal eliminated sequences (IES), are excised in the form of DNA circles. The polytene chromosomes then disintegrate and the DNA to be eliminated is enclosed into vesicles where DNA degradation takes place; depending on the organism up to 95% of the DNA is eliminated. The remaining DNA is specifically fragmented into small DNA molecules and telomeric sequences are added de novo. In a second DNA-synthesis phase, each molecule is amplified to a specific copy number, resulting in the somatic macronucleus containing millions of individual DNA molecules, each carrying one gene and all control sequences required for replication and expression (for reviews see). The molecular mechanisms of these processes are still not well understood, but in the holotrichous ciliate Tetrahymena, and lately in the hypotrichous ciliate Euplotes, conserved cis-acting sequences have been identified that are involved in directing the process of specific fragmentation. In Tetrahymena thermophila, a 15 base pair (bp) sequence, the chromosome breakage sequence (Cbs), is located in the eliminated sequences flanking the macronuclear precursor. Sequence comparisons in Euplotes crassus have identified a 5 bp sequence, the proposed E-Cbs, that either resides inside the macronuclear precursor at position 18, or is located in the flanking micronuclear-specific DNA 12 bp from the macronuclear precursor. A model for chromosome fragmentation in E. crassus was proposed on the basis of the positions of the E-Cbs and the finding of overlapping sequences, involving a 6 bp staggered cut on both sides of the macronuclear precursor. To date, no consensus sequence has been found at defined positions near the fragmentation sites in Stylonychia lemnae. We therefore decided to identify cis-acting sequences involved in DNA fragmentation and telomere addition by injecting modified macronuclear precursor sequences into the

developing macronuclei. Recently, we demonstrated that injection of such a construct (pCE5) into the developing macronucleus resulted in correct fragmentation and de novo telomere addition. This construct contained two macronuclear precursor sequences homologous to a 1.1 kb and a 1.3 kb macronuclear DNA molecule (; GenBank accession numbers X72955 and X72956). They are separated by an 11 bp spacer and are flanked by micronuclear-specific sequences. To distinguish between the injected precursor sequence and the endogenous macronuclear DNA molecule, the 1.3 kb precursor sequence was modified by inserting a 500 bp polylinker sequence. Moreover, we showed that neither sequences of the neighboring 1.1 kb macronuclear precursor sequence nor flanking micronuclear-specific sequences are required for specific fragmentation and telomere addition. Deletion of 70 bp of the 3' end of the 1.3 kb precursor sequence resulted in no detectable processing, however, indicating that a sequence located in this subtelomeric region is indeed required for fragmentation and/or telomere addition. In contrast, deletion of 69 bp of the 5' end still led to a processed product. Surprisingly, this processed product contained the subtelomeric sequences that were deleted in the construct, suggesting the presence of a so far uncharacterized proofreading mechanism during macronuclear development. Only after deletion of 520 bp of the 5' end was a processed product no longer observed. Here, we show that both subtelomeric regions are required for correct DNA fragmentation but, at least in the case of the 1.3 kb precursor sequence, the distance of cis-acting sequences from the fragmentation sites are different in the 3' and 5' region. In addition, we show that the subtelomeric regions of the α1-tubulin gene are sufficient for correct DNA processing. Sequence analysis of all these regions revealed the presence of an inverted repeat with a sequence almost identical to the core E-Cbs described in Euplotes crassus.

CONCLUSION

Conclusions Our data indicate that no micronuclear specific DNA sequences are required for specific DNA fragmentation during macronuclear development of the hypotrichous ciliate Stylonychia. Instead they are found in both subtelomeric regions of macronuclear precursor sequences, although they do not have to be localized at identical positions with respect to the breakage site. We show that these sequences are not only required but also sufficient for DNA fragmentation. Moreover, a functional analysis of an inverted repeat found in this region revealed that it functions as a St-Cbs.