The Evolution of Telomeres in Algae

Abstract

Telomeres, protective nucleoprotein structures of linear chromosome termini, are a ubiquitous and conserved structure of eukaryotic genomes. Telomeric DNA formed by a long arm of mononucleotide motifs may vary between eukaryotic groups. Algae are of interest for telomere biology, because they represent a substantial portion of the eukaryotic phylogenetic diversity. We employed an experimental approach to probe telomeric minisatellites. Our results show that there are a few variants of the telomeric motifs that are conserved in particular groups of algae. Telomeric motif TTAGGG, conserved in most green algal lineages, is ancestral for Chloroplastida. However, an interesting diversity of telomeres was described in the order Chlamydomonadales, where at least two independent changes to the ancestral TTAGGG telomeric repeat occurred. The TTTAGGG telomeric sequence is also present in the photosynthetic alga Chromera velia and in Xanthophyceae, in contrast to the presence of the TTAGGG motif in other ochrophytes studied. Glaucochrysins and haptophytes exhibit TTAGGG telomeric type. To expand knowledge about the distribution of different telomere types in various groups of eukaryotic phylogeny, we performed in silico analyses of genomic data from major eukaryotic lineages. These analyses confirm the TTAGGG telomeric repeat as the most common and possibly ancestral in eukaryotes, but alternative motifs replaced it along the phylogeny of diverse eukaryotic lineages.

Telomere Repeat Amplification Protocol (TRAP)

The first step of TRAP assay is the extension of a non-telomeric oligonucleotide substrate by the action of telomerase, a special reverse transcriptase, which uses a short region inside its own RNA subunit as a template determining directly the sequence of telomeric repeats. The products of the telomerase-mediated extension of the substrate are amplified in the second step (PCR) using the substrate primer and the reverse primer that is complementary to the G-telomeric strand synthesized by telomerase. The PCR products are then separated on non-denaturating polyacrylamide gel. The pattern of TRAP reaction products is a ladder of bands with a periodicity corresponding to the size of the telomeric repeat.

Results

We used the sequence analysis of cloned TRAP products, dot-blot hybridization, restriction digestion, and Southern hybridization with various radiolabeled telomeric probes to study the diversity of telomeric repeats in a phylogenetically wide array of algal species. Our results support the view that the Arabidopsis-type telomeric repeat TTTAGGG is ancestral for Chloroplastida and has been conserved in most lineages, including Mamiellophyceae, Chlorodendrophyceae, Trebouxiophyceae, Chlorophyceae and Zygmenatales. An alternative model of the phylogenetic distribution of telomeric repeats in each of the phylogenetically distinct eukaryotic lineages was previously described. To determine the sequence of the repeats added by telomerase, the PCR products of each reaction are given above lane. The negative product was used in the TRAP reaction. The sequence of the repeats in the TRAP reaction was confirmed by sequencing. The negative product was used in the TRAP reaction.