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Evolution of the telomere-associated protein POT1a in *Arabidopsis thaliana* is characterized by positive selection to reinforce protein-protein interaction

Beilstein M., Renfrew K., Song X., Shakirov E., Zanis M., Shippen D.
Kazan Federal University, 420008, Kremlevskaya 18, Kazan, Russia

Abstract

© The Author 2015. Published by Oxford University Press on behalf of the Society for Molecular Biology and Evolution. Gene duplication is a major driving force in genome evolution. Here, we explore the nature and origin of the POT1 gene duplication in *Arabidopsis thaliana*. Protection of Telomeres (POT1) is a conserved multifunctional protein that modulates telomerase activity and its engagement with telomeres. *Arabidopsis thaliana* encodes two divergent POT1 paralogs termed AtPOT1a and AtPOT1b. AtPOT1a positively regulates telomerase activity, whereas AtPOT1b is proposed to negatively regulate telomerase and promote chromosome end protection. Phylogenetic analysis uncovered two independent POT1 duplication events in the plant kingdom, including one at the base of Brassicaceae. Tests for positive selection implemented in PAML revealed that the Brassicaceae POT1a lineage experienced positive selection postduplication and identified three amino acid residues with signatures of positive selection. A sensitive and quantitative genetic complementation assay was developed to assess POT1a function in *A. thaliana*. The assay showed that AtPOT1a is functionally distinct from single-copy POT1 genes in other plants. Moreover, for two of the sites with a strong signature of positive selection, substitutions that swap the amino acids in AtPOT1a for residues found in AtPOT1b dramatically compromised AtPOT1a function *in vivo*. *In vitro*-binding studies demonstrated that all three sites under positive selection specifically enhance the AtPOT1a interaction with CTC1, a core component of the highly conserved CST (CTC1/STN1/TEN1) telomere protein complex. Our results reveal a molecular mechanism for the role of these positively selected sites in AtPOT1a. The data also provide an important empirical example to refine theories of duplicate gene retention, as the outcome of positive selection here appears to be reinforcement of an ancestral function, rather than neofunctionalization. We propose that this outcome may not be unusual when the duplicated protein is a component of a multisubunit complex whose function is in part specified by other members.

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Keywords

Arabidopsis, Brassicaceae, CTC1, positive selection, POT1, telomere