Telomere-mediated truncation of barley chromosomes

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Abstract Engineered minichromosomes offer an enormous opportunity to plant biotechnology as they have the potential to simultaneously transfer and stably express multiple genes. Following a top-down approach, we truncated endogenous chromosomes in barley (Hordeum vulgare) by Agrobacterium-mediated transfer of T-DNA constructs containing telomere sequences. Blocks of Arabidopsis-like telomeric repeats were inserted into a binary vector suitable for stable transformation. After transfer of these constructs into immature embryos of diploid and tetraploid barley, chromosome truncation by T-DNA-induced de novo formation of telomers could be confirmed by fluorescent in situ hybridisation, primer extension telomere repeat amplification and DNA gel blot analysis in regenerated plants. Telomere seeding connected to chromosome truncation was found in tetraploid plants only, indicating that genetic redundancy facilitates recovery of shortened chromosomes. Truncated chromosomes were transmissible in sexual reproduction, but were inherited at rates lower than expected according to Mendelian rules.

Introduction

Considerable progress has been made towards developing chromosome-based vector systems either by artificial composition of cloned chromosomal constituents into functional chromosomes ('bottom-up' approach) or by engineering endogenous chromosomes ('top-down' approach) in mammalian cells. Such engineered minichromosomes have been used for the development of chromosome-based vector systems (Grimes and Monaco 2005) and to address questions concerning the function of specific chromosomal domains (e.g. centromeric regions) (Nakano et al. 2008).

The 'bottom-up' strategy relies on cell-mediated chromosome assembly after transfection of a cell line with recombinant constructs comprising cloned centromeric sequences and a selectable marker gene, with or without telomeric and other genomic DNA. This is an established method in yeast (Clarke and Carbon 1980; Murray and Szostak 1983) and even mammalian cells (Harrington et al. 1997; Ikino et al. 1998). However, the process of de novo chromosome assembly within cells is hard to control and has been achieved only in a limited number of mammalian cell lines (Irvine et al. 2005). For plants, the 'bottom-up' strategy has not yet yielded sufficiently robust solutions (Houben et al. 2008).

Our limited understanding of centromere function and maintenance is one of the obstacles on the way to generate artificial chromosomes in higher eukaryotes. To circumvent the necessity of de novo centromere formation, modification of existing chromosomes of the host cell to generate a chromosome-based vector can be alternatively achieved by chromosome truncation. In this 'top-down' approach as shown first by Farr et al. (1991) for mammals, transformation of cells with cloned telomeric repeats may truncate the distal portion of a chromosome by the formation of a new telomere at the integration site. A comparable telomere