Characterization of relic DNA from barley genome

D. A. Belostotsky* and E. V. Ananiev

Plant Molecular Genetics and Genetic Engineering Laboratory, N.I. Vavilov Institute of General Genetics, Gubkin st. 3, B333 Moscow, USSR

Received July 5, 1989; Accepted April 11, 1990
Communicated by Yu. Gleba

Summary. High-molecular-weight “relic” DNA fraction can be electrophoretically separated from the bulk of barley DNA digested with different restriction enzymes. We have cloned and analyzed a population of relic DNA fragments. The majority of AluI-relic DNA clones contained barley simple sequence satellite DNA and other families of repetitive DNA. One of these families, designated HvRT, has been analyzed in detail. This family is composed of tandemly arranged 118-bp monomers and is present in 7 x 10^5 copies in the barley genome. Clones representing the HvRT family were sequenced. HvRT repeats were found to contain high levels of methylated cytosine. The HvRT family was found in the genomes of H. vulgare, H. leporinum, H. marinum, H. jubatum, and wheat. Different barley species and cultivars show restriction fragment length polymorphism with the HvRT probe. Chromosome-specific subfamilies of HvRT were found to be present on different barley chromosomes, providing the possibility of using the HvRT probe as a chromosome-specific marker. HvRT fragments up to 810 kbp in length were resolved by pulsed field gel electrophoresis.

Key words: Chromosome marker – Hordeum vulgare – Relic DNA – Tandemly repeated sequences

Introduction

Repetitive DNA sequences constitute up to 80% of higher plant DNA (Flavell 1982). Some are scattered in the genome, whereas others are organized as clusters of tandemly repeated units. Sequences of both types were found in different higher plant species including barley (Ananiev et al. 1988). Tandemly repeated DNA sequences (TRS) were characterized in detail at the molecular level in rye (Bedbrook et al. 1980), maize (Dennis and Peacock 1984), rice (Wu and Wu 1987), melon (Lederc and Siegel 1987), onion (Barnes et al. 1985), broad bean (Kato et al. 1984), Arabidopsis (Martinez-Zapater et al. 1986), and some other Cruciferae (Benslimane et al. 1986; Grellet et al. 1986). They share some features in common: (1) tandem organization in the genome, (2) high copy number, (3) incomplete identity of monomeric units, (4) similar length of these units (usually 170–360 bp), (5) high level of cytosine methylation, (6) absence of transcriptional activity, and (7) predominant localization of clusters in the centromeric or telomeric regions of most, if not all, chromosomes. Some TRS families may not have all these features simultaneously. As was shown earlier (Bedbrook et al. 1980), plant genomic DNA fraction enriched with TRS can be obtained in the form of relic DNA resistant to the action of restriction enzymes. This relic DNA may be composed of different either related or unrelated sequence families and represents a good source for species-specific probes (Metzlaff et al. 1986; Junghans and Metzlaff 1988), which are used as molecular genetic markers. Moreover, such probes can be applied as cytogenetic markers in combination with in situ hybridization techniques.

Materials and methods

Seeds of different barley cultivars were obtained from Dr. A. Pomortzev (Institute of General Genetics, Moscow). Barley-wheat addition lines as well as parental forms were kindly provided by Dr. A.K.M.R. Islam (CSIRO, Australia). Total DNA was isolated from 5-day-old barley seedlings according to Del-
porta et al. (1983). Relic DNA for cloning was purified by centrifugation of the restriction enzyme-digested total barley DNA in NaCl gradient (Grosveld et al. 1982). Partial fragmentation of barley relic DNA was done using DNaseI in the presence of Mn$^{2+}$ ions (Anderson 1981). Phagemid vector pBS(+) and E. coli recipient strain XL-1 Blue (Stratagene) were used for cloning. Plasmid DNA was isolated using the rapid alkaline extraction method (Kieser 1984). The alkaline procedure was used for DNA blotting (Reed and Mann 1985). DNA was sequenced by the dideoxy-method (Sanger et al. 1977) either after recloning the inserts into M13tg131 (Kieny et al. 1983) or using the single-stranded form of the recombinant phagemid as a template, produced in the presence of the M13K07 helper phage. All other DNA manipulations were performed following standard protocols (Maniatis et al. 1982). Pulsed field gel electrophoresis (Schwartz and Cantor 1984) was performed using an LKB Pulsaphor apparatus. DNA sequences were analyzed using the SEQBUS software for sequence analysis (Institute for Molecular Genetics, Moscow) and the repetitive DNA sequences data base constructed by Dr. N.V. Milshina in our laboratory.

Results

Characterization of barley AluI-relic DNA using restriction endonucleases

High-molecular-weight “relic” DNA fraction can be observed after electrophoretic separation of total barley DNA digested with different restriction enzymes. This fraction consists of the mixture of extremely long restriction fragments, which most probably are built up from tandemly arranged monomeric units lacking particular restriction enzyme sites, or having these sites methylated and thus inaccessible to cleavage. AluI-generated relic DNA was chosen for further analysis because this particular fraction can be efficiently separated from the bulk of hydrolyzed DNA (Fig. 1). All restriction enzyme digests were carried out in the presence of λ phage DNA as internal control to confirm the completeness of digestion.

We found that AluI-relic DNA cannot be completely converted into low-molecular-weight fragments by digestion of either total barley DNA or isolated AluI-relic DNA with restriction enzymes EcoRI, BamHI, BglIII, HindIII, HinfI, AluI, Sau3A,MspI, BspRI, TaqI, and all of their possible pairwise combinations. However, when different restriction enzyme digests of barley DNA were blot-hybridized with AluI-relic DNA as a probe, a set of fragments of low molecular weight as well as the band of relic DNA were observed (Fig. 2A). At least two different ladders consisting of regularly spaced bands were discerned (e.g., in EcoRI and Sau3A digests) against the background of a rather complex hybridization spectrum. This pattern is typical of tandemly organized repetitive

Fig. 1. Electrophoretic pattern of total barley DNA digested with AluI restriction endonuclease (lane 1). λ DNA digested with PstI was used for size markers (lane 2). Relic DNA is indicated on the left (R)

Fig. 2. Hybridizations of A $^{32}$P-labelled AluI-relic DNA; B $^{32}$P-labelled insert of HvR 11; C $^{32}$P-labelled HvRT probe to Southern blots of total barley DNA digested with various restriction endonucleases and separated by electrophoresis on 2% agarose gel. T-TaqI; Br-BspRI; S-Sau3A; A-AluI; Hf-HinfI; Hd-HindIII; Bl-BglII; Bm-BamHI; E-EcoRI. The size of 118-bp oligomers is indicated in kb (C, left margin)