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Construction of a barley (*Hordeum vulgare* L.) YAC library and isolation of a *Hor1*-specific clone

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Abstract. We have constructed an *Eco*RI-based YAC (*y*east *a*rtificial *c*hromosome) library from barley (*Hordeum vulgare* L. cv. Franka) using the vector pYAC4. The library consists of approximately 18000 recombinant YACs with insert sizes ranging between 100 and 1000 kb (average of 160 kb) corresponding to 50% of the barley genome. Size fractionation after ligation resulted in an increased average insert size (av. 370 kb) but also in a substantial decrease in cloning efficiency. Less than 1% of the colonies showed homology to a plastome-specific probe; approximately 50% of the colonies displayed a signal with a dispersed, highly repetitive barley-specific probe. Using a primer combination deduced from the sequence of a member of the small *Hor1* gene family coding for the C-hordein storage proteins, the library was screened by polymerase chain reaction and subsequently by the colony hybridization technique. A single YAC, designated Y66C11, with a 120 kb insert was isolated. This DNA fragment represents a coherent stretch from the terminal part of the *Hor1* gene region as judged from the correspondence of the restriction patterns between Y66C11 DNA and barley DNA after hybridization with the *Hor1*-specific probe. Restriction with the isoschizomeric enzymes *Hpa*II/*Msp*I suggests a high degree of methylation of the *Hor1* region in mesophyll cells but not in YAC-derived (yeast) DNA.

Key words: Barley – *Hordeum vulgare* – C-hordeins – Yeast artificial chromosomes (YAC)

Introduction

Modern strategies for analysing complex genomes rest on the study of high molecular weight (> 100 kb) DNA molecules. Since many genes of interest are not accessible to conventional cloning techniques, the use of *y*east *a*rtificial *c*hromosome (YAC) vectors (Burke et al. 1987) in combination with high-density restriction fragment length polymorphism (RFLP) maps, pulsed field gel electrophoresis (PFGE) and chromosome walking techniques provides access to genes via the concept of "reverse genetics". The assembly of contigs consisting of overlapping YAC clones permits the molecular analysis of long, coherent stretches of DNA. This in turn offers the possibility to bridge the gap between genetically and physically amenable distances.

Representative YAC genomic libraries have been constructed from various organisms such as man (Brownstein et al. 1989; Anand et al. 1990; Albertsen et al. 1990), *Caenorhabditis elegans* (Coulson et al. 1988), *Drosophila melanogaster* (Garza et al. 1989), *Arabidopsis thaliana* (Ward and Jen 1990; Grill and Somerville 1991), carrot (Guzmán and Ecker 1988), maize (Edwards et al. 1992), or tomato (Martin et al. 1992). The quality of a YAC library depends on the average insert size and the percentage coverage of a given genome. Average insert sizes between 100 kb (Traver et al. 1989) and 1000 kb (Chumakov et al. 1992a) have been reported for human YAC libraries and their application has been impressively demonstrated by long-range contiguous coverage of DNA segments, e.g., by the contig across chromosome 21q (Chumakov et al. 1992b), the characterization of YACs containing the entire class II region of the major histocompatability complex (Ragoussis et al. 1991), or the β-globin gene region (Gaensler et al. 1991). YAC libraries from crops have been screened with RFLP markers for individual clones. Martin et al. (1992) reported the isolation of clones hybridizing with RFLP probes that cosegregate with the *Tm-2a* or the *Pto* loci of tomato conferring resistance to tobacco mosaic virus and *Pseudomonas syringae* pv. tomato, respectively, and a clone bearing the maize *Adh1* gene has been selected from a maize YAC library (Edwards et al. 1992).

RFLP maps have been developed recently for various crops including barley (*Hordeum vulgare* L.; Graner et al. 1991; Heun et al. 1991). Such maps of sufficient resolution are a prerequisite for the molecular charac-

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terization of complex loci such as the *Mla* region, which confers resistance to powdery mildew and consists of at least 24 alleles (Jahoor and Fischbeck 1993), or the *Hor1* locus carrying 5–7 genes encoding the C-hordein storage proteins, both located on the short arm of chromosome 1H. Although sequence data for some C-hordein cDNAs are available (Rasmussen and Brandt 1986), information on the organization and the chromosomal context of the *Hor1* locus is still limited. No intra-allelic recombination has been observed to date, but a long-range restriction map indicated a maximal size of 135 kb for this locus and a GC-rich island in the vicinity of the locus (Siedler and Graner 1991). Further to investigate the number and chromosomal organization of the C-hordein genes as well as the *Mla* locus we have constructed a YAC library from barley DNA and describe the selection of a recombinant yeast chromosome encoding part of the *Hor1* gene region.

Materials and methods

Plant material and DNA preparation. High molecular weight DNA was isolated from the barley cultivar Franka, which is resistant to barley yellow mosaic virus (BYMV) and carries the *Mla6* locus (Schwarzbach and Fischbeck 1981). Isolation of leaf mesophyll protoplasts, embedding and subsequent lysis in agarose plugs were performed essentially as described in Siedler and Graner (1991). The DNA concentration used was 0.1–0.2 µg per µl agarose.

Host strain and vector preparation. The vector pYAC4 (Burke et al. 1987) and the *Saccharomyces cerevisiae* host strain AB 1380 (*MAT***a**, psi$^+$, *ura3, trp1, ade2-1, can1-100, lys2-1, his5*) were kindly provided by Dr. M. Olson (St Louis, Mo., USA). Vector DNA was prepared chromatographically using Quiagen columns according to the manufacturer's protocol (Diagen GmbH, Düsseldorf, FRG). The DNA was digested with *Eco*RI, monitored for complete digestion by inability to transform *Escherichia coli,* and then cut with *Bam*HI. Before use, the restricted vector DNA was treated with alkaline phosphatase (Boehringer-Mannheim, 0.1 units per µg DNA), extracted twice with phenol, concentrated by ethanol precipitation, and dissolved in double distilled water (1 µg/µl).

Partial digestion, ligation in agarose and size fractionation. The high molecular weight DNA within the agarose plug was treated twice with TE (10 mM TRIS, 1 mM EDTA, pH 8.0) for 30 min at 50° C, twice with TE/phenylmethyl sulphonyl fluoride (PMSF, 1 mM; Boehringer-Mannheim) for 30 min at 50° C, and then with TE for 1 h at room temperature. It was equilibrated with 1.5 × *Eco*RI restriction buffer (Boehringer-Mannheim) for 30 min at room temperature. Aliquots (40 µl) of the agarose plugs were incubated with various *Eco*RI (Boehringer-Mannheim) concentrations ranging between 0.1 and 15 units per µg DNA. The reaction assay was kept on ice for 15 min followed by 1 h at 37° C. The digestion was stopped by adding proteinase K (Merck, Darmstadt, FRG) and EDTA (final concentrations 1 mg/ml and 50 mM, respectively). After incubation for 30 min at 37° C the extent of digestion was checked by pulsed field gel electrophoresis using the CHEF DR II system (Biorad, Richmond, CA, USA; ramped switching time from 60 to 90 s at 6 V/cm for 20 h). Between 2 and 4 units *Eco*RI per µg DNA were used for large-scale digestions. Finally the DNA was washed with TE/PMSF as described above.

For ligation, the agarose blocks were equilibrated with 66 mM TRIS-HCl, 5 mM $MgCl_2$, 30 mM NaCl, 0.75 mM spermidine, 0.30 mM spermine pH 7.5, three times for 30 min at room temperature. The buffer was discarded and vector DNA added to the agarose block in a weight ratio of 1 : 1 to ensure a 50- to 100-fold molar excess of vector DNA. The blocks were melted at 68° C for 10 min and cooled to 37° C for 10 min. Ligation buffer and T4 DNA ligase (Boehringer-Mannheim; 1 unit per µg DNA) were then added, mixed by gentle stirring with a pipet tip, and the solution was kept at room temperature overnight. The agarose in the assay containing the ligated DNA was melted, digested with agarase (1 unit/100 µl), and used directly for transformation. Alternatively, it was loaded onto a pulsed field gel for size fractionation.

For PFGE, the agarose was melted and carefully loaded onto 1% low melting point agarose (Sigma, St Louis, Mo., USA) gels with a blunted pipet tip. The gels were run for 16 h with a switching time of 30 s at 5 V/cm allowing the separation of DNA shorter than 350 kb; larger molecules gathered in the limiting mobility band. Gel slices containing a size marker and an aliquot of the ligation reaction were excised and stained with ethidium bromide. The limiting mobility band was visualized under UV and its position marked by a notch on each side. The stained gel slice was repositioned to the rest of the gel and the limiting mobility band was excised from the unstained part of the gel (Anand et al. 1989). The agarose slice was liquefied at 68° C, cooled down to 40° C and digested for 1 h with 1 unit of Gelase (Biozym, Hameln, FRG) per 100 µl agarose solution. The liquefied fractionation assay was used directly for transformation.

Transformation, selection and storage of recombinant YAC clones. Yeast cells were grown in YPD medium (Rose et al. 1990) to an OD_{600} of 1.5. Spheroplasts were generated with 4000 units Lyticase (Sigma) for usually 10 min for a 200 ml overnight culture and used for transformation as described by Burgers and Percival (1987). Spheroplasting was monitored under phase contrast microscopy. Spheroplasts (150 µl; $3–5 \times 10^8$ cells/ml) were transformed either with 10 µl of the unfractionated, agarase-treated ligation assay or with 50 µl of the size-selected DNA. The centromeric yeast plasmid pRS316 served as a control (Sikorski and Hieter 1989). The transformed cells were plated onto solidified synthetic dextrose (SD) minimal medium that lacked uracil but was supplemented with amino acids (Rose et al. 1990). Transformants were grown for 4–5 days at 30° C, then transferred to a plate containing the same medium lacking uracil and tryptophan. Yeast colonies were scraped off

after 3 days and transferred into microtiter plates containing liquid YPD with 20% glycerol (v/v). The microtiter plates were shock-frozen in liquid nitrogen and stored at −70° C.

Polymerase chain reaction (PCR)-based screening of the library. Yeast colonies from four microtiter plates were grown for 2–3 days on solidified YPD, pooled in batches of 384 individual YAC clones and DNA was isolated from each pool as described in Green and Olson (1990) except that Zymolyase was replaced by Lyticase. PCR reactions were carried out in a total volume of 25 µl with 500 ng of template DNA, 1 µM of each primer in 10 mM TRIS-HCl, pH 8.3, 1.5 mM $MgCl_2$, 50 mM KCl, 0.1 mg/ml gelatin, 0.2 mM each dATP, dCTP, dGTP and dTTP, and 0.5 units *Taq* DNA polymerase (Boehringer-Mannheim) in 30 cycles (denaturation at 95° C for 1 min, annealing at 60° C for 1 min, extension at 72° C for 2 min) using a programmable thermal cycler (Perkin-Elmer/Cetus). Initial denaturation was for 4 min at 94° C. The amplification products were separated on a 1.5% agarose slab gel (Seakem, FMC, Rockland, Md.). The DNA was then transferred onto Biodyne B membrane (Pall, Dreieich, FRG) for hybridization. Individual clones were finally identified by colony hybridization. For colony lifts, autoclaved nylon membranes were placed onto solidified YPD plates containing 15 µg/ml tetracycline (USB, Cleveland, Ohio, USA) and the yeast cells were transferred from the microtiter plate onto the membrane using a 96-pin device. After incubation at 30° C for 2–3 days, the membranes were removed and treated as described in Larin et al. (1991) again using Lyticase instead of Zymolyase for cell lysis.

YAC clone analysis. Individual YAC clones were grown for 3 days at 30° C in 5 ml liquid SD medium lacking uracil and tryptophan and supplemented with 15 µg/ml tetracycline to prevent bacterial contamination. High molecular weight yeast DNA was prepared in low melting point agarose as described in Larin and Lehrach (1990) but using Lyticase (see above). The chromosomal DNA was separated for 24 h by electrophoresis in agarose gels with a ramped switching time of 30–70 s at 6 V/cm. The DNA in the gel was then nicked for 3 min with UV light (302 nm), denatured (0.5 M NaOH, 1.5 M NaCl) for 30 min, and transferred onto Biodyne B membrane using the alkaline technique described by the manufacturer. Hybridization was performed at 60° C (Jung et al. 1990) followed by two subsequent washes in 0.5 × SSC (1 × SSC is 0.15 M NaCl, 15 mM sodium citrate) and 0.2% SDS at 60° C for 30 min. YAC DNA was restricted with 6 units enzyme (*Eco*RV, *Xba*I, *Hpa*II and *Msp*I from Boehringer-Mannheim; *Bst*NI from New England Biolabs, Bad Schwalbach, FRG) per µg DNA at 37° C for 2 h as described above except that the agarose blocks were liquefied by agarase digestion. The DNA was separated on a 0.75% agarose gel and prepared for hybridization as outlined above.

Plasmid probes and PCR primers. The *Apa2* probe carries a 580 bp insert cloned from a highly repetitive satellite DNA, which is dispersed throughout the barley genome (Lehfer et al. 1991). Probe pPSII/51 coding for the 51 kDa chlorophyll *a* binding protein of the reaction centre of photosystem II of spinach (*psbB* gene) was chosen as a plastome-specific probe. The 210 bp *Pst*I fragment of pBSC5 (Siedler and Graner 1991) was used as a *Hor1*-specific probe. The DNA fragments were labelled by the random priming method in the presence of [α-^{32}P]dATP and [α-^{32}P]dCTP (Feinberg and Vogelstein 1983). *Hor1*-specific primers for PCR were designed from the partial cDNA clone pchor 1-3 (Rasmussen and Brandt 1986). The following sequences were used: HORC-1, 5′-CTCATTTGAACTAGTATAGGCCGGG-3′; HORC-2, 5′-CAGCAACCACAACAACCATTCCCAC-3′. The sequences selected for the *Apa2* primers were: *APA2*-A, 5′-GGCCCACTCGGTAATACAAC-3′; *APA2*-B, 5′-GGGGTAACCAAATGAAGTCA-3′.

Results

Construction and characterization of the library

The currently available YAC library of the barley genome consists of ca. 18 000 clones with insert sizes ranging between 100 and 1000 kb and an average of 160 kb (Fig. 1). It represents approximately 52% of the barley genome. The transformation efficiency was monitored by transforming the centromeric yeast plasmid pRS316, which gave 5×10^5 cfu of the Ura$^+$ phenotype per µg plasmid DNA. Recombinant YACs containing non size-selected DNA transformed with a frequency of 150 to 200 cfu of the Ura$^+$ phenotype per µg DNA.

The DNA of 120 individual Ura$^+$/Trp$^+$ yeast colonies was separated by PFGE to estimate the frequency, size distribution and origin of the inserts. Non size-selected YACs ranged between 100 and 500 kb with an average of 140 kb (Fig. 1). Size selection after ligation increased the average size of the YACs to 370 kb with individual YAC clones obtained of sizes up to 1000 kb, but transformation efficiency dropped conspicuously, between 10- and 50-fold. All 120 yeast clones checked contained an

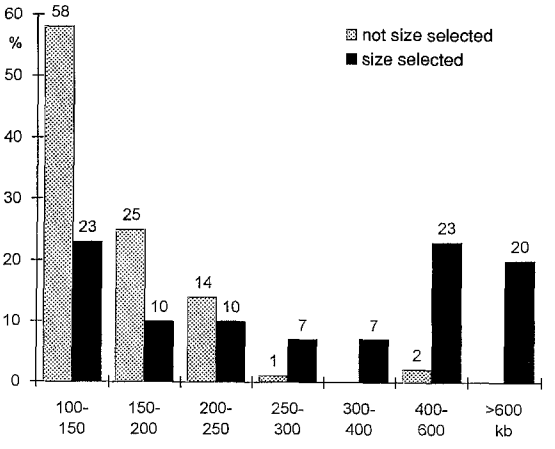

Fig. 1. Size distribution of 90 non size-selected (average insert size 140 kb) and 30 size-selected (average insert size 370 kb) yeast artificial chromosome (YAC) clones

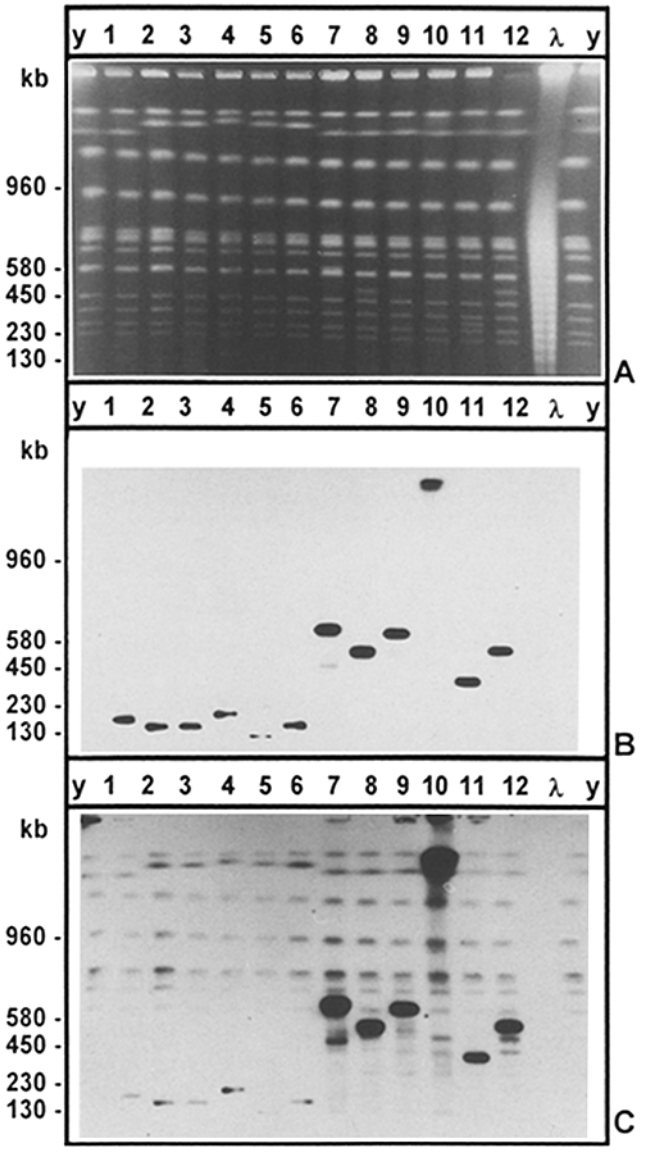

Fig. 2A–C. Pulsed field gel electrophoresis (PFGE) analysis and Southern analysis of 12 randomly chosen YAC clones (lanes 1–6, not size-selected; lanes 7–12, size selected; Y, yeast chromosomal DNA marker AB1380-, λ, λ-ladder). **A** Ethidium bromide-stained pulsed field gel, **B** Autoradiograph after hybridization with pBR322 DNA; exposure, 1 day. **C** Autoradiograph after hybridization with genomic barley DNA; exposure, 3 days

extrachromosomal DNA fragment that hybridized with vector-specific sequences (pBR322) as well as with genomic barley DNA though with different signal intensity, probably because of varying portions of repetitive DNA (Fig. 2). The plastome-specific probe pPSII/51, hybridized to a subset of 384 YAC clones on four colony filters, resulted in three signals. This indicates that less than 1% of the YAC clones contained plastid-specific inserts (see the Discussion). For the identification of *Hor1*-specific clones, the DNA of approximately 12 000 colonies was extracted in pools, each of 384 individual recombinants, and colony filters were prepared from all these individual clones.

Screening with the repetitive Apa2 *probe*

The origin of the YAC inserts was verified by hybridization with the *Apa2* sequence, a highly repetitive, dispersed DNA element of the barley genome. This probe showed no homology to yeast DNA and was used in combination with the *Apa2*-derived PCR primers to calibrate the colony and PCR screening technique as well as to estimate the distribution and average spacing of the *Apa2* elements. Ten YAC DNA pools representing 3840 clones were monitored by PCR using these primer sequences. All pools generated a 550 bp amplification product which was also visible with barley DNA. As was expected, no amplification products were detectable with yeast DNA (Fig. 3). In a second experiment, 384 randomly selected YAC clones were screened with the *Apa2* probe by colony hybridization. Approximately 200 colonies gave signals of varying intensity. Ten of these were further investigated by separating restricted DNA in conventional agarose gels and hybridization with the *Apa2* probe. The varying signal intensities obtained were similar to those of the colony hybridization (data not shown).

Isolation and characterization of a Hor1*-specific YAC clone*

The YAC library was subsequently screened for *Hor1*-homologous sequences using the YAC-PCR technique in

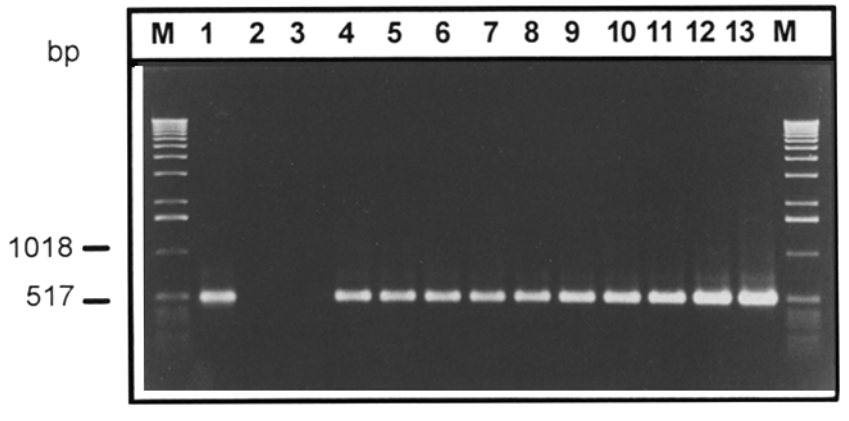

Fig 3. Polymerase chain reaction (PCR) amplification with the *Apa2* primers of DNA from 10 YAC pools (lanes 4–13), barley DNA (lane 1), yeast strain AB1380 (lane 2) and vector pYAC4 (lane 3). A 1 kb ladder (BRL, Gaithersburg) was used as size standard (lane M)

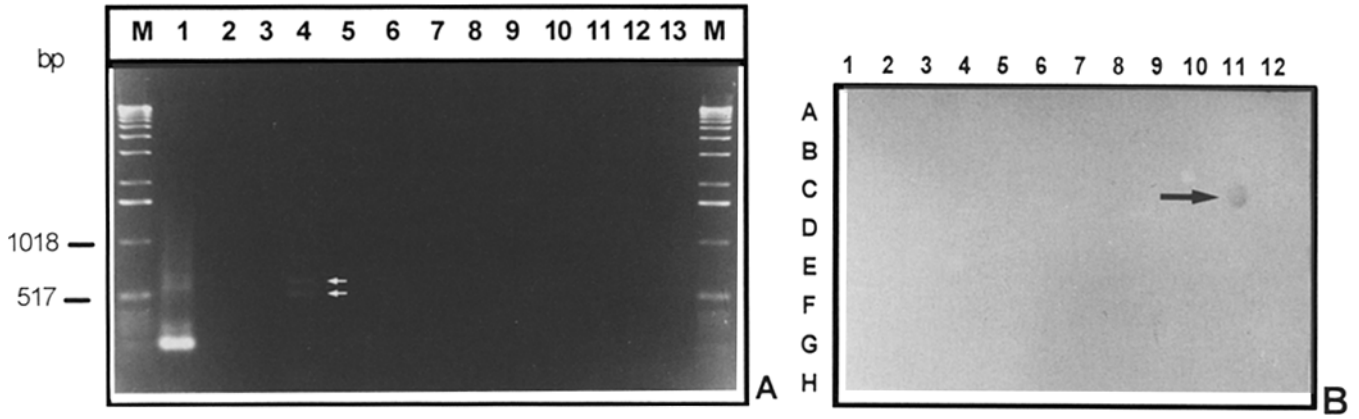

Fig. 4A, B. Isolation of *Hor1*-specific YAC Y66C11. **A** PCR amplification with the *Hor1*-specific primer pair of DNA from 10 YAC pools (lanes 4–13; *arrows*), barley DNA (lane 1), yeast strain AB1380 (lane 2) and vector pYAC4 (lane 3). The 1 kb ladder was used as size standard (lane M). **B** Autoradiograph of the colony lift after hybridization with the *Hor1*-specific probe *(arrow)*. Exposure, 5 days. The coordinates indicate the positions of the 96 colonies on the filter

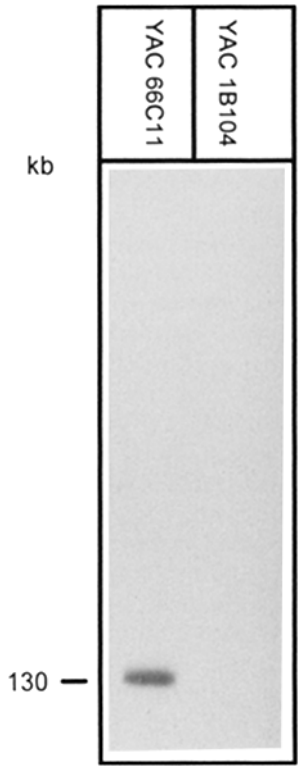

Fig. 5. Characterization of YAC clone Y66C11. Non-restricted Y66C11 DNA and DNA of an anonymous barley YAC clone were separated by PFGE and hybridized with the *Hor1*-specific probe. Exposure, 3 h

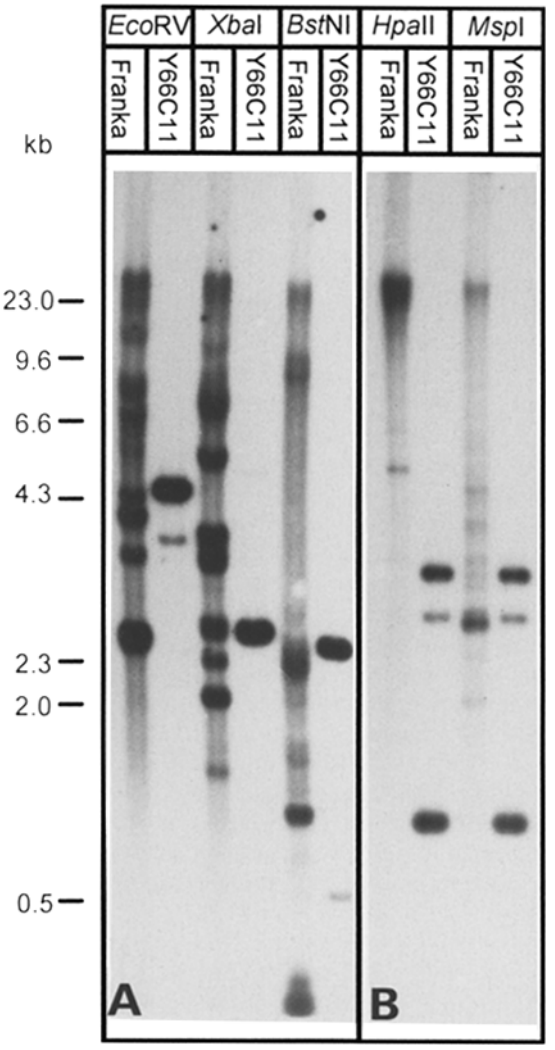

Fig. 6A, B. Restriction analysis of Y66C11 DNA. **A** Ten nanograms YAC DNA and 10 µg barley DNA were restricted with *Eco*RV, *Xba*I or *Bst*NI and hybridized with the *Hor1*-specific probe. **B** Methylation at the *Hor1* locus; 10 ng YAC DNA and 10 µg barley DNA were restricted separately with the isoschizomers *Hpa*II and *Msp*I, and hybridized with the *Hor1* probe. Exposure, 4 days

combination with the *Hor1*-specific primer pair. Barley and yeast DNA were used as controls. The following results were obtained. PCR with genomic barley DNA generated multiple bands of amplified DNA fragments (Fig. 4A). No bands were observed with yeast DNA. DNA amplification was only apparent with DNA of one YAC pool. Of the two fragments produced (500 bp and 550 bp), the larger one comigrated with fragments amplified from genomic DNA (Fig. 4A, lane 4). When the DNA of the PCR gel was blotted and hybridized with the pBSC5 probe, barley DNA and the two amplified DNA fragments of the YAC pool displayed strong signals (data not shown). Colony hybridization with the four corresponding filters and probe pBSC5 resulted in a

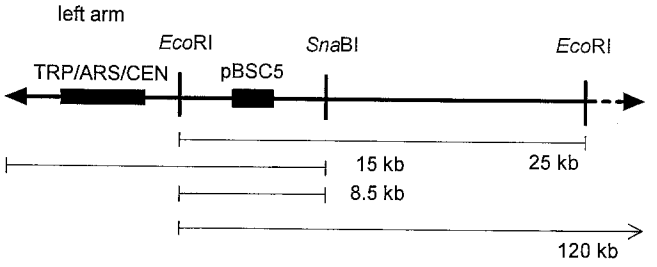

Fig. 7. *Eco*RI/*Sna*BI restriction map of the left end of the 135 kb Y66C11 DNA. The *Hor1*-specific probe pBSC5 resides within the terminal 8.5 kb *Eco*RI/*Sna*BI restriction fragment

single signal (Fig. 4B). The positive yeast clone was designated Y66C11 and contained a single YAC with an insert of approximately 120 kb in size, which hybridized strongly with the *Hor1* probe (Fig. 5). Randomly chosen recombinant YAC clones that served as a control in this experiment did not give any signal.

To verify colinearity between the Y66C11 insert and genomic barley DNA, 10 ng YAC DNA and 10 µg barley DNA were each digested with *Eco*RV, *Xba*I or *Bst*NI and hybridized with the *Hor1*-specific probe (Fig. 6A). For each restriction digest, fragments of identical size were labelled with barley and YAC DNA. In one case (*Eco*RV) an additional fragment was visible with YAC DNA which was not detectable with barley DNA under the conditions chosen (10 µg DNA, 4 days exposure). The location of the *Hor1* gene within the YAC insert was determined by digestion of Y66C11 DNA with the rare cutter enzyme *Sna*BI and subsequent hybridization with the *Hor1* probe pBSC5 and a vector-specific probe (pBR322). A 15 kb fragment was detected with both probes (data not shown), indicating that the *Hor1*-specific region is located terminally. After double-digestion with *Eco*RI and *Sna*BI a fragment of 8.5 kb was labelled with the *Hor1* probe (Fig. 7).

To check the degree of CpG methylation around the *Hor1* region, YAC and barley mesophyll DNA were both restricted with the isoschizomers *Msp*I and *Hpa*II. *Hpa*II is inhibited by methylation at the two C residues whereas *Msp*I is only sensitive to methylation of the first C in its recognition sequence (Nelson and McClelland 1987). After Southern analysis with the *Hor1* probe, the YAC DNA digestions displayed identical banding patterns (Fig. 6B; cf Profitt et al. 1984), but different restriction fragments between the two enzymes were observed with barley DNA indicative of methylation at the *Hor1* region. As expected, the *Msp*I restriction patterns in barley and YAC DNA resembled each other except for a 1.2 kb fragment visible only with YAC DNA.

Discussion

The applicability of a YAC library for the selection of individual clones using single-copy sequences as probes depends on three principal criteria: (i) the number of clones, (ii) the average insert size, and (iii) the accessibility of the library for screening with a given probe. The YAC library presented here is obviously suitable for selecting recombinant single-copy clones for three reasons. Firstly, a remarkably high proportion of clones (99%) contained inserts; only a relatively small fraction (1%) contained plastid DNA inserts, which is in accordance with results previously described for a tomato YAC library (Martin et al. 1992). This figure is a maximal estimate since it is known that plastid DNA sequences may react with genomic DNA (e.g. Timmis and Scott 1985).

Secondly the average insert size is in the range reported for other plant YAC libraries: 150 kb for *Arabidopsis* (Ward and Jen 1990), 145 kb for maize (Edwards et al. 1992) and 140 kb for tomato (Martin et al. 1992). Attempts to raise the average insert size by size fractionation resulted in a dramatic decrease of the transformation efficiency, which is consistent with findings reported from maize (Edwards et al. 1992) and human YAC libraries (Albertsen et al. 1990). Work is in progress to increase the number of clones to 1.5 genome equivalents with both size-selected and non size-selected DNA. Thirdly, a two-step procedure based on PCR screening of pooled YAC DNA (Green and Olson 1990) followed by colony hybridization resulted in the identification of a discrete, individual clone.

In this study, we have used two different barley sequences for screening the library. The highly repetitive *Apa2* sequence constitutes approximately 2.4% of the barley genome as judged from Southern analysis (data not shown). Approximately 50% of the recombinant YACs hybridized with this probe, thus corroborating and extending results from in situ hybridization studies on barley metaphase chromosomes (Lehfer et al. 1991) that these repeats are interspersed repetitive elements. The different signal intensities of individual YAC DNAs most probably reflect varying numbers of this element. Analysis of these clones can provide further insight into the genomic organization of this repeat.

The second probe, pBSC5, originating from one of the barley *Hor1* genes has been chosen to select a recombinant YAC representing a low copy sequence. Additionally, we were interested in the organization of the 18.4 cM chromosome segment between the *Hor1* and *Hor2* loci on barley chromosome 1HS (Schüller et al. 1992). The *Hor1* locus has recently been physically mapped and its maximum extension estimated to be approximately 135 kb (Siedler and Graner 1991). RFLP analysis has indicated 5–7 *Hor1* genes within this genomic region (Rasmussen and Brandt 1986). Our data suggest that we have cloned a region of some 120 kb from the terminal part of the *Hor1* gene region.

(1). The insert strongly hybridizes with the *Hor1*-specific probe. Hybridization with a *Hor2*-specific probe, encoding a B-hordein and which displays low homology to the *Hor1* sequences, resulted in >10 times weaker signals (data not shown). (2). Identical restriction patterns have been observed in donor (barley) and YAC DNA (disregarding methylation-sensitive restriction enzymes). The additional band at 1.2 kb that appeared in the YAC lanes (Fig. 6B) can be explained by CNG-methylation that inhibits *Hpa*II as well as *Msp*I restriction activity and is known to occur in plants (Gruenbaum

et al. 1981). (3). The detection of three fragments in Y66C11 DNA digested with *Hpa*II and *Msp*I (Fig. 6B), the limitation of the signal to a terminal 8.5 kb *Eco*RI-*Sna*BI fragment and the appearance of two amplification products generated with the *Hor1*-specific primer pair (Fig. 4A) suggest that at least two *Hor1* sequences are located terminally on the YAC insert near the left arm of the vector (Fig. 7). This implies that the cloned sequence represents a terminal part of the 135 kb *Hor1* gene region and offers the possibility of directed chromosome walking. During the past 18 months no evidence for chimeric YACs, as reported for YAC libraries with large inserts (Chumakov et al. 1992b), has been noted in the barley library. The *Hor1* locus is methylated in leaf cells (Fig. 6B) in accordance with previous findings (von Wettstein 1992). Since DNA methylation may be involved in silencing genes in discrete plant tissues (Renckens et al. 1992) it is tempting to assume that this mechanism contributes to the developmental and spatial regulation of C-hordein genes which are only active in the endosperm.

The cloning of long coherent stretches of DNA provides the opportunity to probe the genomic organization, the regulation of a given locus, and, provided high density RFLP maps are available, to isolate genes of economic interest such as the *Mla* locus conferring powdery mildew resistance and which is located in the *Hor1*/*Hor2* genomic region. This locus has been found to be closely linked (0.7 cM) to RFLP marker 1H036 (Schüller et al. 1992), and additional RFLP probes have been selected from a recently established plasmid library constructed with microdissected 1HS metaphase chromosomes (Schondelmaier et al. 1993).

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References

Albertsen HM, Abderrahim H, Cann HM, Dausset J, Le Paslier D, Cohen D (1990) Construction and characterization of a yeast artificial chromosome library containing seven haploid human genome equivalents. Proc Natl Acad Sci USA 87:4256–4260

Anand R, Villasante A, Tyler-Smith C (1989) Construction of yeast artificial chromosome libraries with large inserts using fractionation by pulsed-field gel electrophoresis. Nucleic Acids Res 17:3425–3433

Anand R, Riley JH, Butler R, Smith JC, Markham AF (1990) A 3.5 genome equivalent multi access YAC library: construction, characterisation, screening and storage. Nucleic Acids Res 18:1951–1956

Brownstein BH, Silverman GA, Little RD, Burke DT, Korsmeyer SJ, Schlessinger D, Olson MV (1989) Isolation of single-copy human genes from a library of yeast artificial chromosome clones. Science 244:1348–1351

Burgers PMJ, Percival KJ (1987) Transformation of yeast spheroplasts without cell fusion. Anal Biochem 63:391–397

Burke DT, Carle GF, Olson MV (1987) Cloning of large segments of exogenous DNA into yeast by means of artificial chromosome vectors. Science 236:806–812

Chumakov IM, LeGall I, Billault A, Ougen P, Soularue P, Guillou S, Rigault P, Bui H, Detand MF, Barillot E, Abderrahim H, Cherif D, Berger R, Lepaslier D, Cohen D (1992a) Isolation of chromosome-21-specific yeast artificial chromosomes from a total human genome library. Nature Genet 1:222–225

Chumakov I, Rigault P, Guillou S, Ougen P, Billaut A, Guasconi G, Gervy P, LeGall I, Soularue P, Grinas L, Bougueleret L, Bellanné-Chantelot C, Lacroix B, Barillot E, Gesnouin P, Pook S, Vaysseix G, Frelat G, Schmitz A, Sambucy J-L, Bosch A, Estivill X, Weissenbach J, Vignal A, Riethman H, Cox D, Patterson D, Gardiner K, Hattori M, Sakaki Y, Ichikawa H, Ohki M, Le Paslier D, Heilig R, Antonarakis S, Cohen D (1992b) Continuum of overlapping clones spanning the entire human chromosome-21q. Nature 359:380–387

Coulson A, Waterston R, Kiff J, Sulston J, Kohara Y (1988) Genome linking with yeast artificial chromosomes. Nature 335:184–186

Edwards KJ, Thompson H, Edwards D, de Saizieu A, Sparks C, Thompson JA, Greenland AJ, Eyers M, Schuch W (1992) Construction and characterization of a yeast artificial chromosome library containing three haploid maize genome equivalents. Plant Mol Biol 19:299–308

Feinberg AP, Vogelstein B (1983) A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Anal Biochem 132:6–13

Gaensler KML, Burmeister M, Brownstein BH, Taillon-Miller P, Myers RM (1991) Physical mapping of yeast artificial chromosomes containing sequences from the human β-globin gene region. Genomics 10:976–984

Garza D, Ajioka JW, Burke DT, Hartl DL (1989) Mapping the *Drosophila* genome with yeast artificial chromosomes. Science 246:641–646

Graner A, Jahoor A, Schondelmaier J, Siedler H, Pillen K, Fischbeck G, Wenzel G, Hermann RG (1991) Construction of an RFLP map of barley. Theor Appl Genet 83:250–256

Green ED, Olson MV (1990) Systematic screening of yeast artificial-chromosome libraries by use of the polymerase chain reaction. Proc Natl Acad Sci USA 87:1213–1217

Grill E, Somerville C (1991) Construction and characterization of a yeast artificial chromosome library of *Arabidopsis* which is suitable for chromosome walking. Mol Gen Genet 226:484–490

Gruenbaum Y, Naveh-Many T, Cedar H, Razin A (1981) Sequence specificity of methylation in higher plant DNA. Nature 292:860–862

Guzmán P, Ecker J (1988) Development of large DNA methods for plants: Molecular cloning of large segments of *Arabidopsis* and carrot DNA into yeast. Nucleic Acids Res 16:11091–11105

Heun M, Kennedy AE, Anderson JA, Lapitan NLV, Sorrels ME, Tanksley SD (1991) Construction of a restriction fragment polymorphism map for barley *(Hordeum vulgare)*. Genome 34:437–447

Jahoor A, Fischbeck G (1993) Identification of new genes for mildew resistance of barley at the *Mla* locus in lines derived from *Hordeum spontaneum*. Plant Breeding 110:116–122

Jung C, Kleine M, Fischer F, Herrmann RG (1990) Analysis of DNA from a *Beta procumbens* chromosome fragment in sugar beet carrying a gene for nematode resistance. Theor Appl Genet 79:663–672

Larin Z, Lehrach H (1990) Yeast artificial chromosomes: an alternative approach to the molecular analysis of mouse developmental mutations. Genet Res 56:203–208

Larin Z, Monaco AP, Lehrach H (1991) Yeast artificial chromosome libraries containing large inserts from mouse and human DNA. Proc Natl Acad Sci USA 88:4123–4127

Lehfer H, Wanner G, Herrmann RG (1991) Physical mapping of DNA sequences on plant chromosomes by light microscopy and high resolution scanning electron microscopy. In: Herrmann RG, Larkins BA (eds) Proceedings of the Sixth NATO Advanced Study Institute on Plant Molecular Biology, Elmau, Bavaria, pp 277–284

Martin G, Ganal MW, Tanksley SD (1992) Construction of a yeast artificial chromosome library of tomato and identification of cloned segments linked to two disease resistance loci. Mol Gen Genet 233:25–32

Nelson M, McClelland M (1987) The effect of site-specific methylation on restriction-modification enzymes. Nucleic Acids Res 15 (Suppl): r219–r230

Proffitt JH, Davie JR, Swinton D, Hattman S (1984) 5-Methylcytosine is not detectable in *Saccharomyces cerevisiae* DNA. Mol Cell Biol 4:985–988

Ragoussis J, Monaco A, Mockridge I, Kendall E, Campbell RD, Trowsdale J (1991) Cloning of the HLA class II region in yeast artificial chromosomes. Genetics 88:3753–3757

Rasmussen SK, Brandt A (1986) Nucleotide sequences of cDNA clones for C-hordein polypeptides. Carlsberg Res Commun 51:371–379

Renckens S, De Greve H, Van Montagu M, Hernalsteens J (1992) Petunia plants escape from negative selection against a transgene by silencing the foreign DNA via methylation. Mol Gen Genet 233:53–64

Rose MD, Winston F, Hieter P (1990) Methods in yeast genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York

Schondelmaier J, Martin R, Jahoor A, Houben A, Graner A, Koop U, Herrmann RG, Jung C (1993) Microdissection and microcloning of the barley (*Hordeum vulgare* L.) chromosome 1HS. Theor Appl Genet, in press

Schüller C, Backes G, Fischbeck G, Jahoor A (1992) RFLP markers to identify the alleles on the *Mla* locus conferring powdery mildew resistance in barley. Theor Appl Genet 84:330–338

Schwarzbach E, Fischbeck G (1981) Mehltau-Resistenzfaktoren von Sommer- und Wintergerstensorten in der Bundesrepublik Deutschland. Z Pflanzenzücht 87:309–318

Siedler H, Graner A (1991) A construction of physical maps at the *Hor1* locus of two barley cultivars by pulsed field gel electrophoresis. Mol Gen Genet 226:177–181

Sikorski RS, Hieter P (1989) A system of shuttle vectors and yeast host strains designed for efficient manipulation of DNA in *Saccharomyces cerevisiae*. Genetics 122:19–27

Timmis JN, Scott NS (1985) Movement of genetic information between the chloroplast and nucleus. In: Hohn B, Dennis ES (eds) Genetic flux in plants. Springer, Wien New York, pp 61–78

Traver CN, Klapholz S, Hyman RW, Davis RW (1989) Rapid screening of a human genomic library in yeast artificial chromosomes for single-copy sequences. Proc Natl Acad Sci USA 86:5898–5902

von Wettstein D (1992) The future of barley as an experimental organism. In: Munck L (ed) Proceedings of the Sixth International Barley Genetics Symposium, Helsingborg, Sweden, II, pp 1087–1097

Ward ER, Jen GC (1990) Isolation of single-copy-sequence clones from a yeast artificial chromosome library of randomly-sheared *Arabidopsis thaliana* DNA. Plant Mol Biol 14:561–568