Stable barley chromosomes without centromeric repeats

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The satellite sequences $(AGGGAG)_n$ and Ty*3*/*gypsy*-like retrotransposons are known to localize at the barley centromeres. Using a gametocidal system, which induces chromosomal mutations in barley chromosomes added to common wheat, we obtained an isochromosome for the short arm of barley chromosome 7H (7HS) that lacked the barley-specific satellite sequence $(AGGGAG)_n$. Two telocentric derivatives of the isochromosome arose in the progeny: 7HS* with and 7HS without the pericentromeric C-band. FISH analysis demonstrated that both telosomes lacked not only the barley-specific centromeric $(AGGGAG)_n$ repeats and retroelements but also any of the known wheat centromeric tandem repeats, including the 192-bp, 250-bp, and Tail sequences. Although they lacked these centromeric repeats, 7HS* and 7HS** both showed normal mitotic and meiotic transmission. Translocation of barley centromeric repeats to a wheat chromosome 4A did not generate a dicentric chromosome. Indirect immunostaining revealed that all tested centromere-specific proteins (rice CENH3, maize CENP-C, and putative barley homologues of the yeast kinetochore proteins CBF5 and SKP1) and histone H3 phosphorylated at serines 10 and 28 localized at the centromeric region of 7HS*. We conclude that the barley centromeric repeats are neither sufficient nor obligatory to assemble kinetochores, and we discuss the possible formation of a novel centromere in a barley chromosome.**

centromere | chromosome aberration

Centromeric DNA sequences are not highly conserved, and their functional importance in many higher eukaryotes is still a matter of debate (for reviews, see refs. 1 and 2). Nevertheless, clusters of tandem repeats interspersed with retroelements are typical features of regular centromeres in several plant species (2). Two repetitive sequences, CCS1 (3) and Sau3A9 (4), are conserved within centromeres of cereal species and represent parts of the LTR and of the integrase region of a Ty*3*/*gypsy*-like retroelement (e.g., *cereba* or centromeric retrotransposon of barley), respectively (5). Complete or truncated copies of this retroelement family were found in centromeres of barley, maize, sorghum, wheat, rice, rye, oats, *Aegilops* (5–10), and even in dicotyledonous *Beta* species (11) interspersed irregularly between species-specific tandem repeats (12–15). Preferential coprecipitation of both centromeric sequence types with the CENP-A-like centromeric histone H3 variant CENH3 indicates their functional importance for centromere assembly (16), although not all centromeric repeats were associated with CENH3 on extended chromatin fiber preparations (17).

If regular centromeres are lost, a few regions without the typical centromeric repeats of human chromosomes (18) or regions of *Drosophila* chromosomes adjacent to the centromere (19) may assemble kinetochores and constitute a *de novo*-formed centromere. Neocentromeres of plants are different because they are observed in meiotic cells as chromatin extensions directed to the spindle poles and are present together with a normal centromere. Consequently, the corresponding chromosome behaves as a dicentric at meiosis, whereas in mitosis, only the normal centromere is functional. Plant neocentromeres, best known from rye (20) and maize, are represented mostly by heterochromatic terminal knobs and are composed of tandem repeats different from those of regular centromeres (21, 22). Kinetochore proteins such as CENP-C (23) and CENH3 are absent from these neocentromeres (17). The neocentric activity in maize is part of a complex meiotic drive system regulated by several genes of an "aberrant chromosome 10." Neocentromere movement along spindle fibers differs from that of normal centromeres (22). Neocentromeres that fully substitute for regular centromeres have not yet been described for plants.

To elucidate the functional significance of centromeric repetitive sequences, we applied a gametocidal system to induce deletions in barley chromosomes. A gametocidal chromosome derived from a related species, *Aegilops cylindrica*, when introduced into common wheat (*Triticum aestivum*) in monosomic condition, induces structural chromosome aberrations such as deletions in the gametes not carrying the gametocidal chromosome (24, 25). This gametocidal system can induce chromosomal mutations in barley and rye chromosomes added to common wheat (26, 27).

Applying the gametocidal system to the formation of aberrations in a barley chromosome 7H added to common wheat, we obtained an isochromosome of the short arm of 7H that was lacking the *gypsy*-like retroelement *cereba* and the G+C-rich satellite sequence $(AGGGAG)_n$, both representing the major components of centromeric DNA in barley (12). Telocentric chromosomes (or telosomes) were derived from this isochromosome in the next generation. In this study, we used centromere-specific DNA probes and antibodies to elucidate the molecular organization of the centromeres of the telosomes. We also investigated the transmission of the truncated telosomes to understand the effect of centromeric repetitive sequences on chromosome segregation in mitosis and meiosis, and we discuss the possibility of neocentromere formation in barley.

Materials and Methods

Plant Material. Aberrant 7H chromosomes examined in this study were found in a common wheat (*T. aestivum* cv. Chinese Spring, $2n = 6x = 42$) line carrying a pair of barley (*Hordeum vulgare* cv. Betzes) 7H chromosomes and one gametocidal chromosome 2C of *Ae. cylindrica*. This line was developed for selecting 7H aberrations (28, 29). Barley cultivars Betzes and Shinebis as well as Chinese Spring lines carrying the whole, the short arm, or the long arm of barley chromosome 7H (30, 31) were used as controls.

PCR Analysis. To test in wheat background for the typical sequence organization of barley centromeric repeats as observed in the representative clone BAC7 (12), primers for PCR were designed according to the LTR sequences of *cereba* and the G+C-rich satellite sequence $(AGGGAG)_n$. The primers were *cereba*LTR-F (5′-GTTGATCGTGCTCCGGTGTGATCA-3′),

Abbreviations: *cereba*, centromeric retrotransposon of barley; GISH, genomic *in situ* hybridization.

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*cereba*LTR-R (5′-TGATCACACCGGAGCACGATCAAC-3′), $(AGGGAG)_3$-F (5′-AGAGGGAGAGGGAGAGGGAG-3′), and $(AGGGAG)_3$-RG (5′-CTCCCTCTCCCTCTCCCTCTG-3′). PCR was performed in 20 μl containing ≈100 ng of template DNA, 0.25 mM each dNTP, 4 pmol of forward and reverse primers in 1× PCR buffer with 0.15 mM $MgCl_2$, and 2.5 units of AccuPrime Taq DNA polymerase (Invitrogen) by using an iCycler (Bio-Rad). The PCR conditions were as follows: initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturion (96°C, 10 sec), annealing (67°C, 30 sec), and extension (68°C, 1 min). The reaction was completed by additional extension at 68°C for 7 min. Amplification products were electrophoresed on a 1% agarose gel and were visualized by ethidium bromide staining.

Giemsa N- and C-Banding and FISH. N- and C-banding was performed according to Gill *et al.* (32). Three sequences of the two major components of the barley centromere contained within the barley centromere-specific clone BAC7 were used as FISH probes to detect centromeric repeats on chromosomes: the G+C-rich satellite sequence $(AGGGAG)_n$, the plasmid clone pGP7 containing the RNase H and the integrase region of the Ty*3*/*gypsy*-like retroelement *cereba*, and the LTRs of *cereba* (12). To verify that the aberrant barley chromosomes did not acquire centromeric or other sequences from wheat during their formation, FISH was conducted with the 192-bp repeat (33), the 250-bp repeat (34), and the TaiI tandem repeat (35), which hybridize to centromeric regions of wheat chromosomes. Additionally, genomic *in situ* hybridization (GISH) was conducted with genomic DNA of common wheat as a probe and with barley genomic DNA in 300-fold excess for blocking. To identify the arms of the 7H chromosome, C-banding, FISH with the barley-specific subtelomeric repeat HvT01 (36), and GISH with total barley genomic DNA were combined. The DNA probes were labeled with digoxigenin-11-dUTP or with biotin-11-dUTP according to the manufacturer's instructions (Roche Applied Science, Indianapolis) and detected with either fluorescein-conjugated anti-digoxigenin antibody (Roche Applied Science) or streptavidin-Cy3 (Amersham Pharmacia Biosciences). Chromosomes were counterstained with DAPI or propidium iodide. FISH, GISH, and sequential C-banding/FISH/GISH were performed as described in refs. 37 and 38. Chromosome images were recorded with a fluorescence microscope equipped with a charge-coupled device camera (SenSys, Photometrics, Tucson, AZ).

Indirect Immunostaining. Polyclonal rabbit antibodies against histone H3 phosphorylated at serine-10 (Upstate Biotechnology, Lake Placid, NY) and a rat monoclonal antibody against H3 phosphorylated at serine-28 (39) were diluted 1:400 in PBS/3% BSA. After a 12-h incubation of slides containing squashed root tip meristems in primary antibody solution at 4°C and washing for 15 min in PBS, the slides were incubated in rhodamine-conjugated anti-rabbit IgG and FITC-conjugated anti-rat IgG, both from Dianova (Hamburg, Germany), in PBS/3% BSA for 1 h at 37°C. After final washes in PBS, the preparations were mounted in antifade containing DAPI as a counterstain (40).

For immunostaining of kinetochore proteins, suspensions of nuclei and chromosomes were prepared as described in refs. 41 and 42 from synchronized root tip meristems of seedlings of the wheat/barley telosomic addition line containing the telocentric chromosome without the centromeric repeats (7HS*, see *Results*). Suspended nuclei and chromosomes were spun down onto microscopic slides (at 18 × *g* for 5 min) by using a Cytospin3 (Shandon, Pittsburgh) cytological centrifuge and stored in glycerol at 4°C until use.

Polyclonal rabbit antisera raised against synthetic peptides derived from sequences encoding putative barley homologues of the yeast kinetochore proteins CBF5 and SKP1 (43) and rabbit antisera against the centromeric CENH3 (the histone H3 variant corresponding to the human CENP-A) protein of rice (44) and against maize ZmCENP-C (23) were used as primary antibodies. Slides with isolated nuclei/chromosomes were washed three times for 20 min each in PBS, postfixed in 4% formaldehyde (vol/vol) in PBS for 10 min, blocked (3% BSA/10% horse serum, in PBS) for 1 h at 37°C, and then incubated for 1 h at room temperature in primary antiserum in AK buffer (1% BSA/10% horse serum/0.1% Tween 20, in PBS). After three 5-min washes in PBS, the slides were incubated for 1 h at room temperature in secondary antiserum (Alexa Fluor 488-conjugated goat anti-rabbit IgG, Molecular Probes, or FITC-conjugated goat anti-rabbit IgG, Sigma) in AK buffer and washed in PBS. DNA was counterstained with DAPI in Vectashield (Vector Laboratories). For subsequent FISH, coverslips were removed by using liquid nitrogen. After washing in PBS and postfixation in 4% paraformaldehyde/3.4% sucrose for 10 min at room temperature, FISH with the rhodamine-labeled subtelomeric repeat HvT01 was carried out as described in ref. 37.

Results

Aberrant Derivatives of Chromosome 7H Without Centromeric Satellite Sequences and *cereba* Retroelements Are Stably Transmitted. The primary constrictions of wild-type barley chromosomes appeared after N-banding as thin stretched chromatin threads that were oriented to the opposite spindle poles and thus formed a diamond shape (Fig. 1*A*). This chromatin structure colocalizes with the *cereba* sequence pGP7 (Fig. 1*A*) and the $(AGGGAG)_n$ repeat located between the dark pericentromeric C bands of the short arm (7HS) and the long arm (7HL) of chromosome 7H (Fig. 1*B*) and at the centric ends of the telocentrics 7HS (Fig. 1*C*) and 7HL (Fig. 1*D*). These data indicate that the 7H centromeric region contains many of these repeats.

A wheat–barley Robertsonian translocation involving 7HS and an unidentified wheat chromosome arm with a centromere containing the barley-specific centromeric $(AGGGAG)_n$ repeats (Fig. 1*E*) but no detectable wheat-specific centromeric 192-bp repetitive sequence (33) was found. From this translocation chromosome, a 7HS isochromosome was derived that no longer contained $(AGGGAG)_n$ sequences detectable by FISH within its primary constriction (Fig. 1*F*) but at least a part of the pericentromeric C-band was retained (Fig. 1*G*). Thus, the $(AGGGAG)_n$ repeats were demonstrated to be located proximally to the pericentromeric C-band of 7HS and were absent at a cytological level from the 7HS isochromosome. Minor $(AGGGAG)_n$ signals were detected on wheat chromosomes 3B and 5B when images were exposed longer than usually required for detecting the corresponding signals on normal barley chromosome 7H (Fig. 1 *F* and *R*).

Of 26 plants examined in the selfed progeny of the wheat line with the 7HS isochromosome, 5 plants contained the same isochromosome as a monosomic addition and another 5 had single telocentric chromosomes derived from this isochromosome. We analyzed the 7HS telosomes by sequential C-banding, FISH, and GISH, and identified two aberrant derivatives of 7HS: one with the pericentromeric C-band (7HS*, Figs. 1*H* and 2) and the other without the pericentromeric C-band (7HS**, Figs. 1*I* and 2).

Both types of truncated telosomes were stably transmitted to subsequent generations at rates similar to that of a regular telosome 7HS. In the monosomic addition, 7HS was transmitted to 24.1% (7 of 29 plants) of the selfed progeny, whereas 7HS* and 7HS** were transmitted to 30.2% (119 of 394 plants; 10 plants were disomic for 7HS*) and to 25.8% (17 of 66 plants; two plants were disomic for 7HS**) of the selfed progeny, respectively. We established a stable disomic 7HS* addition line of Chinese Spring wheat that showed the regular bivalent formation

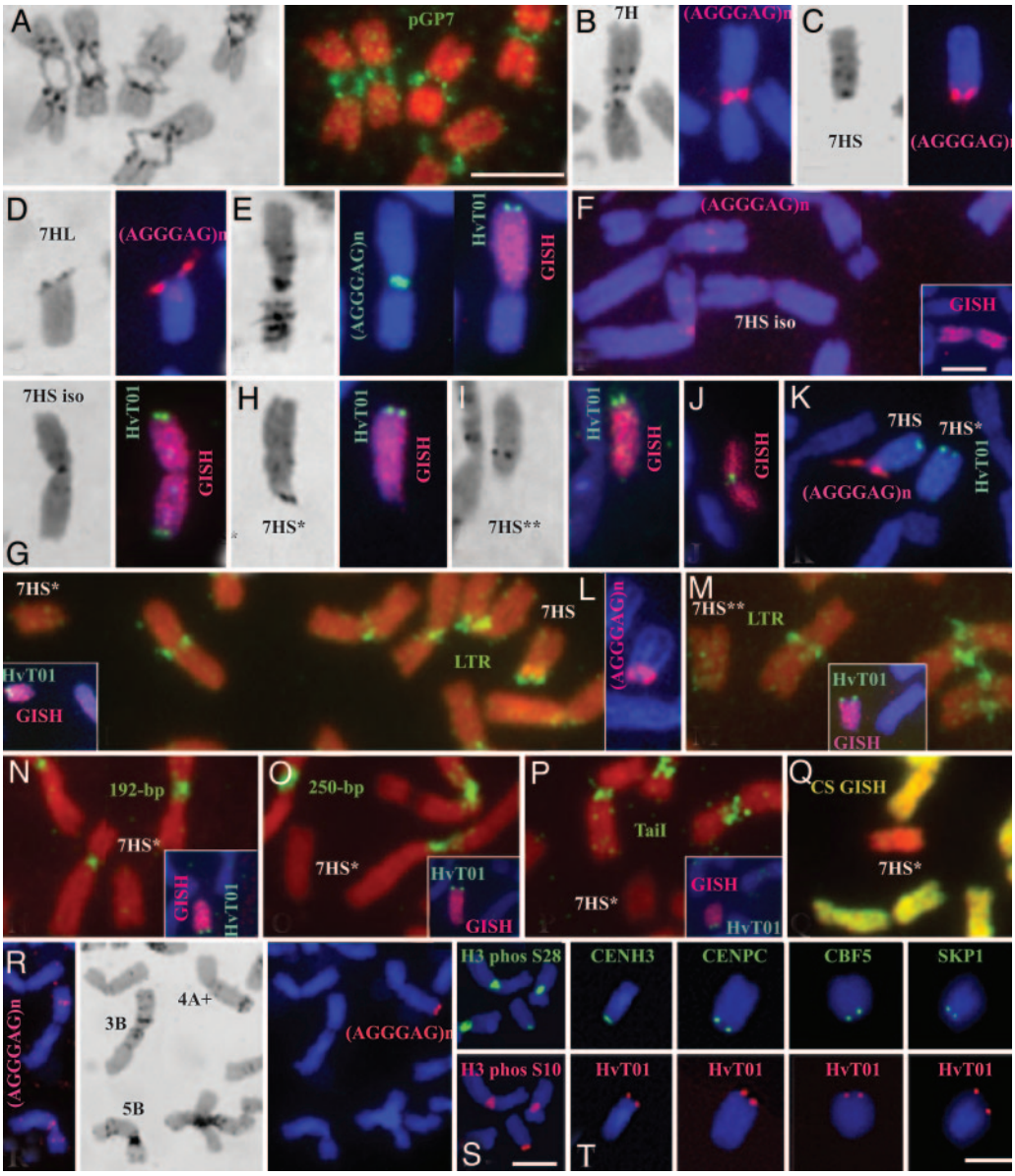

Fig. 1. Characterization of barley centromeres in regular and altered chromosomal positions. (*A*) Partial mitotic metaphase cell of barley cv. Shinebis after N-banding (*Left*) and FISH using the pGP7 probe (green signal) counterstained with propidium iodide (*Right*). (*B–D*) Chromosomes 7H (*B*), 7HS (*C*), and 7HL (*D*) after C-banding (*Left*) and FISH using the $(AGGGAG)_n$ probe (*Right*). (*E*) Robertsonian translocation between 7HS and a wheat chromosome segment after C-banding (*Left*), FISH using the $(AGGGAG)_n$ probe (green, *Center*), and FISH/GISH (*Right*) using the HvT01 (green) and genomic barley DNA (red, *Right*). (*F*) The 7HS isochromosome after FISH with $(AGGGAG)_n$ and GISH with genomic barley DNA (red, *Inset*). (*G*) The 7HS isochromosome after C-banding (*Left*) and subsequent FISH/GISH with HvT01 (green) and barley genomic DNA (red, *Right*). (*H*) 7HS* after C-banding (*Left*) and subsequent FISH/GISH with HvT01 (green) and barley genomic DNA (red, *Right*). (*I*) 7HS** after C-banding (*Left*) and subsequent FISH/GISH with HvT01 (green) and barley genomic DNA (red, *Right*). (*J*) The 7HS* bivalent at meiotic metaphase I after FISH with HvT01 (green) and GISH (red). (*K*) Mitotic chromosomes 7HS* and normal 7HS in the same cell after FISH with HvT01 (green) and $(AGGGAG)_n$ (red). (*L*) 7HS* and normal 7HS after FISH with LTR sequences of *cereba* (green), 7HS* after subsequent FISH with HvT01 (green) and GISH (red) (*Inset Left*), and 7HS after subsequent FISH with $(AGGGAG)_n$ (red, *Right*). (*M*) 7HS** after FISH with LTR (green) and after subsequent FISH/GISH with HvT01 (green) and genomic barley DNA (red, *Inset*). (*N*) 7HS* after FISH with the 192-bp centromeric repeat of wheat (green) and after subsequent FISH/GISH with HvT01 (green) and genomic barley DNA (red, *Inset*). (*O*) 7HS* after FISH with 250-bp centromeric repeat of wheat (green) and after subsequent FISH/GISH with HvT01 (green) and genomic barley DNA (red, *Inset*). (*P*) 7HS* after FISH with TaiI (green) and after subsequent FISH/GISH with HvT01 (green) and genomic barley DNA (red, *Inset*). (*Q*) 7HS* (counterstained red) after GISH with wheat labeled genomic DNA (green) and an excess of unlabeled barley DNA. (*R*) C-banding (*Center*) and FISH (*Left* and *Right*) with $(AGGGAG)_n$ (red) on chromosomes of the 4A+ line; note that much longer exposure was required to detect the minor signals on chromosomes 3B and 5B (*Left*) than to detect those of 4A+ (*Right*). (*S*) Partial metaphase of wheat including chromosome 7HS* after immunostaining with antibodies against histone H3 phosphorylated at serines 10 (red, *Lower*) and 28 (green, *Upper*). (*T*) Isolated telosomes 7HS* after immunostaining with antibodies (green) against rice CENH3 (*Upper Left*), ZmCENP-C (*Upper Second*), CBF5 (*Upper Third*), and SKP1 (*Upper Right*). Red signals of subsequent FISH with HvT01 are shown in *Lower*, demonstrating that the telosomes were 7HS*. (Scale bars, 5 μm. The magnification is the same for *A–P*, except for *Insets*.)

of 7HS* during meiotic metaphase I (Fig. 1*J*), normal fertility, and regular transmission of 7HS* to the subsequent generations.

We confirmed the absence of the centromeric repeats from the centromere of 7HS* in hybrid plants that carry one 7HS and one 7HS*. As expected, no $(AGGGAG)_n$-specific signal was detected on 7HS*, whereas a strong signal occurred at the centric

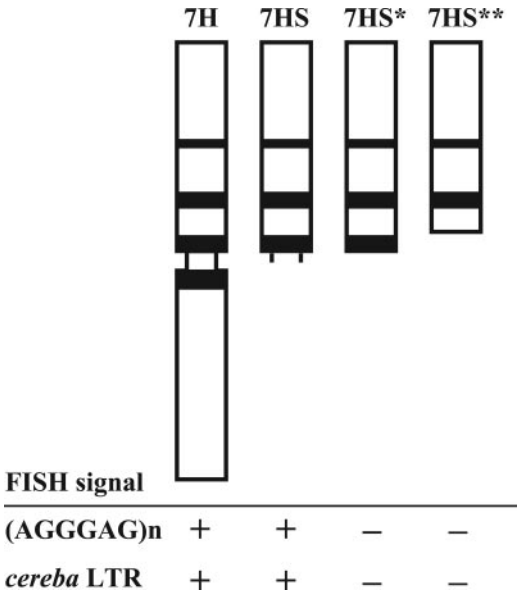

Fig. 2. C-bands (solid bars) and the presence (+) or absence (–) of FISH signals in chromosomes 7H, 7HS, 7HS*, and 7HS**.

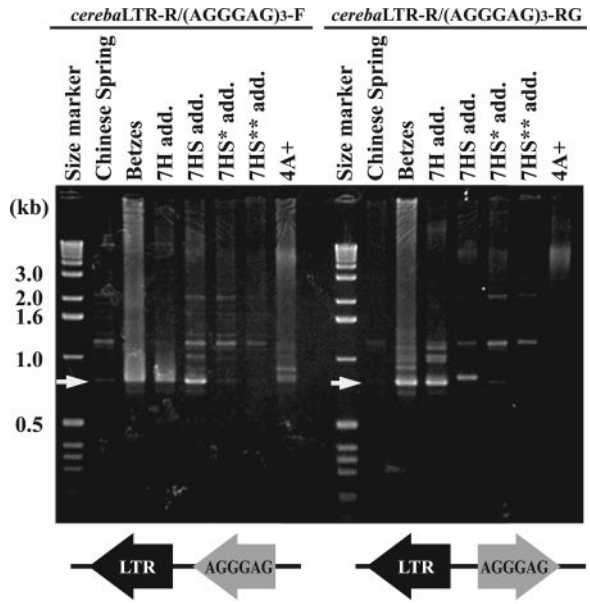

Fig. 3. PCR amplification of the regions between *cereba* LTR and adjacent $(AGGGAG)_n$ sequences. Primer combinations used were *cereba*LTR-R/$(AGGGAG)_3$-F (*Left*) and *cereba*LTR-R/$(AGGGAG)_3$-RG (*Right*). The barley-specific amplification products are indicated with arrows. Opposite orientations of *cereba* LTR and $(AGGGAG)_n$ repeats inferred from the different amplification products are shown below the gel.

end of 7HS (Figs. 1*K* and 2). After FISH with the LTR probe of the centromere-specific Ty*3*/*gypsy*-like retroelement *cereba*, no signals were found in the centromeric regions of 7HS* (Figs. 1*L* and 2) and 7HS** (Figs. 1*M* and 2), whereas an intense hybridization signal was present on 7HS that was comparable with that observed on wheat chromosomes (Figs. 1*L* and 2). FISH on the same 7HS, re-probed with $(AGGGAG)_n$ repeats, revealed that these sequences were present in a region extending beyond the region containing the centromeric *cereba* sequences (Fig. 1*L Right*). No FISH signals of the three wheat centromeric repeats, 192-bp, 250-bp, and TaiI, were detected on 7HS* (Fig. 1 *N–P*), and no GISH signals of wheat genomic DNA were found on 7HS* (Fig. 1*Q*).

Translocation of the Barley Centromeric $(AGGGAG)_n$ Repeat onto a Wheat Chromosome Does Not Generate a Dicentric Chromosome. In the siblings of the Robertsonian wheat–barley translocation line from which plants with the 7HS isochromosome were derived, we found a wheat chromosome 4A carrying many $(AGGGAG)_n$ repeats at the tip of the long arm (4A+ in Fig. 1*R*, *Center* and *Right*). The 4A+ chromosome was regularly transmitted through male and female gametes. Disomic 4A+ plants behaved normally throughout their life cycle. Thus, no dicentric was formed by translocation of this barley centromere repeat onto a wheat chromosome.

Truncated 7HS Telosomes Lack the Barley-Specific Sequence Organization of Centromeric Repeats. To verify the *in situ* hybridization results that the barley centromeric $(AGGGAG)_n$ repeats and retroelements had been lost in the truncated 7HS telosomes, we investigated the Chinese Spring lines having 7H, 7HS, 7HS*, 7HS**, and 4A+, together with Betzes barley and Chinese Spring wheat, by PCR using four primer-pair combinations: *cereba*LTR-F/$(AGGGAG)_3$-F, *cereba*LTR-F/$(AGGGAG)_3$-RG, *cereba*LTR-R/$(AGGGAG)_3$-F, and *cereba*LTR-R/$(AGGGAG)_3$-RG. The former two primer combinations yielded no distinct PCR product (data not shown). With the latter two primer combinations, two fragments of similar size (≈800 bp) were amplified in Betzes but not in Chinese Spring (Fig. 3). The primer pair *cereba*LTR-R/$(AGGGAG)_3$-F amplified the barley-specific fragments from the 7H and 7HS additions and from the 4A+ line but not from the 7HS* and 7HS** additions (Fig. 3). The primer pair *cereba*LTR-R/$(AGGGAG)_3$-RG amplified both fragments from the 7H addition, only one of the fragments from the 7HS line, and no fragment from the 7HS* and 7HS** additions and from the 4A+ line (Fig. 3). These results suggested that (*i*) 7H and 7HS both contained the *cereba* LTR and $(AGGGAG)_n$ sequences in adjacent position and in different orientations, (*ii*) 4A+ contained both sequences adjacently in one orientation, and (*iii*) 7HS* and 7HS** had lost the centromeric region where both sequences were in close vicinity.

Derivatives of Chromosome 7H Without Centromeric Repeats Possess Centromere-Specific Proteins. During mitosis, histone H3 phosphorylation levels at serine positions 10 and 28 are high in plant pericentromeric regions (40, 45, 46). We explored whether H3 undergoes the same posttranslational modification at the centromeric end of the telosome 7HS* that normal pericentromeres do. The histone H3 phosphorylation revealed no difference between regular wheat chromosomes and the aberrant telosome 7HS* (Fig. 1*S*). Our observation agrees with previous findings that only regions around functional centromeres contain H3 phosphorylated at serine-10 from prophase until telophase (40).

Immunostaining with antibodies against rice CENH3, maize ZmCENP-C, and CBF5 and SKP1, barley homologues of yeast kinetochore proteins, revealed the presence of all these centromere-specific proteins at the centric end of isolated 7HS* telosomes (Fig. 1*T Upper*). The opposite terminus of the same telosomes showed signals after FISH with the barley subtelomeric repeat HvT01 (Fig. 1*T Lower*).

Discussion

Are Centromeric Repeats Necessary for Assembling Functional Kinetochores? It is generally assumed that the centromere is located in the primary constriction of monocentric chromosomes where chromatin is weakly stained after chromosome banding. The primary constriction of regular barley chromosomes contains extended repetitive sequences as shown by FISH and, as re-

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ported by Singh and Tsuchiya (47), forms a diamond-shaped structure after N-banding (Fig. 1*A*). The kinetochore, where specific proteins accumulate and mediate attachment to spindle fibers during nuclear divisions, is positioned at the centromere, hence at the primary constriction.

The retroelement *cereba* and $(AGGGAG)_n$ sequences represent all centromeric repeats reported so far for barley (12). The $(AGGGAG)_n$ sequence in 7HS extends from the centric end beyond the *cereba* LTR sequences (Fig. 1*L*). As summarized in Fig. 2, both repetitive sequences are absent from 7HS* and 7HS**, and 7HS** also lacks the centromere-adjacent C-band (Fig. 1*I*). PCR analyses using the primer pairs designed to amplify the region between the *cereba* LTR and $(AGGGAG)_n$ revealed neither for 7HS* nor for 7HS** the centromere-specific organization of both sequences that has been described for the BAC7 insert. This finding indicates that 7HS* and 7HS** have lost most of these repeats. GISH with barley genomic DNA and unlabeled excess BAC7 DNA yielded only very weak signals at the barley centromeres, indicating the absence of considerable amounts of repeats other than *cereba* and $(AGGGAG)_n$ (12). Therefore, it is unlikely that *cereba* LTR and $(AGGGAG)_n$ sequences were eliminated preferentially to leave only other unknown centromeric repeats in 7HS* and 7HS**. The absence of any wheat chromatin (see Fig. 1 *N–Q*) does not support the idea that the truncated 7HS telosomes gained wheat-specific centromeric sequences during their formation from the 7HS isochromosome. The stable mitotic and meiotic transmission of 7HS* and 7HS** suggests that both telosomes retain functional centromeres and that presently known centromeric repeats in such amounts detectable by FISH are not essential for the normal centromere function. The conserved epitopes with high similarity to the yeast kinetochore proteins SKP1 and CBF5, to ZmCENP-C, and to rice CENH3, as well as the signals for histone H3 phosphorylated at serines 10 and 28, were all detected at one terminus of chromosome 7HS*, confirming the presence of a functional centromere. The 4A+ chromosome with a cluster of the $(AGGGAG)_n$ sequences, together with the *cereba* LTR, at the terminus of the long arm showed regular mitotic and meiotic transmission, suggesting that these barley centromeric repeats are not sufficient to organize a functional centromere on the wheat chromosome.

Where Is the Position of the Kinetochore? The kinetochore of 7HS might be located on the distal side of the pericentromeric C-band (see Fig. 1*H*), not in the primary constriction, because 7HS** had a normal centromere function despite the loss of the pericentromeric C-band. If this is the case, the normal 7H chromosome should have at least two functional centromeres, one on 7HS and the other on 7HL or in the primary constriction. Alternatively, if the kinetochore of 7HS is organized at the centromeric repeats within the primary constriction, as generally assumed, 7HS* and 7HS** should have centromeres that might have been established close to the position of original centromeric sequences in a similar way as *Drosophila* neocentromeres were formed (19). Three arguments support the view that the telosomes 7HS* and 7HS** contain fully functional centromeres different from the neocentromere-type of maize that also lacks repeats of regular centromeres: (*i*) Centromeric satellite and retroelement sequences of maize associate preferentially with the centromeric isoform CENH3 of histone H3 (17), therefore it seems reasonable to assume that the same basic step of kinetochore assembly takes place also at the corresponding repeats of barley. Then, these regions represent the position of regular barley centromeres and are deleted from the truncated telosomes. (*ii*) Functional human neocentromeres and the centric ends of 7HS* but not the transient meiotically neocentric knobs of maize (48) are associated with all tested kinetochoric proteins. (*iii*) The centric end of 7HS* is enriched in histone H3 phosphorylated at serine-10, which is typical for fully functional barley centromeres but not for inactive ones such as found on a semidicentric chromosome (40).

About 60 positions within the human chromosome complement have been reported to develop centromeric activity without containing detectable amounts of centromeric repeats (18). This results in an average of 1.3 potential neocentromeres per chromosome. However, frequent acentric chromosome fragments, which appear during the first nuclear division after genotoxin exposure, form micronuclei and eventually disappear rather than establishing a neocentromere spontaneously. According to the hypothesis of Mellone and Allshire (49), stretching centromeric regions by attachment to spindle fibers during nuclear divisions is required to exchange nucleosomal histone H3 by CENP-A/CENH3. If this happened somewhere on an acentric fragment, full kinetochores might become established and perpetuated during subsequent nuclear divisions. The observation of *Drosophila* neocentromeres at chromosomal positions close to that of the original centromeres could be due to remnants of the original centromere/kinetochore, which allow attachment of a few spindle fibers to generate tension during nuclear division. The consequence might be an evolutionary ''migration'' of the centromeric position in case of (partial) deletion of the original centromere. On the one hand, the presence of CENH3 as well as of other kinetochore proteins and of pericentromere-specific histone modifications at the centric end of chromosome 7HS* suggests that a fully functional neocentromere might have originated after deletion of the original centromere at stretched chromatin-containing sequences other than centromere-specific repeats. On the other hand, the centromeric repeats translocated to the terminus of the wheat chromosome 4A+ do not generate an additional centromere at this chromosomal position, possibly because the translocated regions did not become exposed to stretching forces exerted by spindle microtubules.

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