

## 4 Summary

### I. DNA composition and organization of barley centromeres

The BAC clone 03J24 (named BAC 7) was selected from a genomic barley BAC library to study sequence composition and arrangement of barley centromeres since it yielded positive FISH signals exclusively at the centromeric regions of all barley chromosomes and a hybridization pattern similar to that of genomic DNA after digestion with *Dra* I and Southern hybridization with pGP7 (a barley homologue of the centromere-specific *Sau3A9* element of sorghum) and BCS2 (barley variant of the *cereal centromere sequence*1) (done by G. Presting).

The insert of BAC 7 (~23 kb), was found to harbour three copies of the *Ty3/gypsy*-like retroelement ‘*cereba*’ flanked by LTRs of ~1 kb and a sequence with the predominant motif AGGGAG. While the *cereba* element shows high similarity to *gypsy*-like elements within centromeres of other cereals, the G+C-rich satellite is barley-specific. Both sequences constitute the major DNA components of all barley centromeres. The CCS1 sequences (Aragón-Alcaide *et al.* 1996) proved to be parts of LTRs, as proposed by Presting *et al.* (1998).

About 200 *cereba* elements of ~7 kb each, are present per barley centromere (Presting *et al.* 1998), indicating a considerably higher density than calculated for wheat or sorghum centromeres. The completeness of the *cereba* elements is a novelty when compared to that within centromeric clones of other cereals (see Langdon *et al.* 2000).

The functional meaning of *gypsy*-like retroelements within cereal centromeres is not yet clear. Although their number may be reduced below the detectability by FISH within mitotically and meiotically stable barley telosomics (T. R. Endo, pers. communication), they are apparently involved in recruiting CENP-A like kinetochore proteins in maize

(Zhong *et al.* in press). These results leave open the question whether or not kinetochore assembly at cereal centromeres is epigenetically regulated.

## II. The upper chromosome size limit in barley

The observation of incomplete mitotic separation of sister chromatids of the recombinantly elongated arm 6<sup>1/7</sup>, the occurrence of micronuclei within meristematic cells and the reduced vigour of the recombinant cytotypes of barley correspond with the data reported for *Vicia faba* (Schubert and Oud 1997).

In barley, as well as in *V. faba*, chromosome arms which only slightly surpassed the length of half of the telophase spindle axis showed mitotic, but not meiotic disturbance based on non-separation of elongated arms since the spindle axis in meiocytes is significantly longer than in mitotic cells. Plants carrying the elongated chromosome in homozygous condition are slower growing and revealed reduced fertility, probably due to mitotic disturbances during early embryogenesis. In *Vicia*, the adverse effects on mitosis and plant development increase with extension of arm length (above half of the spindle axis). In barley, the long arm of chromosome 6<sup>1/7</sup> only slightly surpasses this limit. Further elongation of chromosome arm 6<sup>1/7</sup>L by recombination with a suitable translocation chromosome in future might yield even more severe effects as to mitotic non-separation of its sister chromatids, formation of micronuclei and disturbance of growth, development and fertility of carrier individuals.

The data obtained show that chromosome arms only slightly longer than half of the average spindle axis length may interfere with mitotic nuclear division and may cause cell death via chromatin deletion (Schubert *et al.* 1998a). Because dead cells arising from mis-division of meristematic cells may disturb tissue differentiation and thus affect the normal development of the organism concerned, half of the average spindle axis

extension defines the upper tolerance limit for chromosome arm length. This is apparently a rule, at least for higher plants.