The promises of a new era in chromosome technology

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The introduction of DNA:DNA *in situ* hybridisations on chromosomal targets dates back to the late sixties and can be considered as one of the major milestones in chromosome research. However, the technology first needed the developments of advanced epi-fluorescence microscopic systems with superb optics and versatile multi-spectral filter sets and molecular biological innovations to make it as versatile and powerful as we experience it nowadays (de Jong 2003). One of its important applications is Fluorescence *in situ* Hybridizations (FISH) and is widely applied to establish positions of single copy and repeat sequences on mitotic and meiotic chromosomes, directly on DNA molecules (de Jong *et al.* 1999). FISH was also adapted to discriminate parental genomes in interspecific hybrids using total genomic DNA of one of the parental species as probe and the other for blocking. With this method the parental species in the hybrid and its backcross derivatives could be identified and evolutionary relations of nuclear DNAs between related taxa established (review in Raina & Rani 2001).

A second important application of FISH technology is chromosome painting, based on chromosomal *in situ* suppression (CISS) in which pooled DNA sequences from flow sorted or microdissected chromosomes were used as probe for hybridisation in the presence of highly repetitive Cot1 as blocking DNA. Chromosome painting has advanced clinical cytogenetics enormously in studying chromosome aberrations in specific heritable and cancer diseases, and revealed chromosomal rearrangements in the evolution of mammalian karyotypes by what is now known as ZOO-FISH. However, a comparable technique for plant chromosomes could not be developed as plant genomes have most of their repeats homogenised over all chromosomes preventing painting individual chromosomes by CISS (Schubert et al. 2001). Alternative strategies were developed including the genome painting of monosomic and disomic additions showing the alien chromosome (pair) in the background of the related species (Chang and de Jong, 2004). This type of chromosome painting is especially favourable in studying the effect of *Ph1* controlling chromosome pairing and pre-meiotic association of wheat additions (Mikhailova *et al.* 1998; Maestra *et al.*, 2002) and elucidating molecular organisation of repeats of a singe chromosomes (de Jong *et al.* 2000). A major breakthrough in true chromosome painting in plants was claimed by Lysak et al. (2001) who selected pools of repeat-poor BACs and used them for painting on pachytene chromosomes in Arabidopsis. So far the technique has been tested only for Arabidopsis and related Brassica's (Lysak et al. 2003) and seems more appropriate for small size genome species.

FISH technologies were further improved by increasing spatial resolution of neighbouring targets and sensitivity detection of minor signals. One way to do so is using pachytene chromosomes in stead of mitotic metaphase complements as targets. Chromosomes at this meiotic stage are 10-50 times longer than mitotic chromosomes and resolution in Arabidopsis of 2 MB at metaphase can be improved up to 50 kb in euchromatin segment in pachytene chromosomes. Even more importantly, pachytene chromosomes display a characteristic differentiation of heterochromatin, euchromatin, centromeres and nucleolar organiser regions (NORs) allowing accurate FISH of probes for karyotyping and physical mapping and genome studies (de Jong *et al.* 1999). Concurrent with the development of pachytene FISH,

Fransz *et al.* (1996) introduced extended DNA fibres and on isolated DNA molecules immobilized on coated slides as targets for FISH. Calibration experiments revealed that with this technique a stretching degree of about 3.27 kb/ μm could be obtained, which implies a microscopical resolution of 1 kb, which close to that of the native Watson-Crick conformation. The combination of high-resolution FISH on pachytene and extended DNA fibre targets has since then been found highly successful for numerous small genome size plant species. For large genome representatives like rye, wheat and chickpea a novel high resolution FISH strategy was published recently using super-stretched flow-sorted chromosomes, with lengths up to 100x their native size and resolution values of up to 70 kbp compared to 5-10 MB for their metaphase counterparts (Valarik *et al.* 2004).

Repetitive DNA sequences constitute a major fraction of plant genomes and can be subdivided into classes of tandem and dispersed repeats. Tandem repeats often correspond to the constitutive heterochromatin regions around the centromeres, and at telomeres and NORs, and their position and molecular organisation can be highly variable even between accessions and subspecies. In addition to the ubiquitous ribosomal and telomere repeats, species have their specific tandem repeats which together can be used as diagnostic FISH markers for the identification of the chromosomes in the complement, as we showed for *Arabidopsis thaliana* (Koornneef *et al.* 2003), *Medicago truncatula* (Kulikova *et al.* 2001), and tomato (Chang 2004). The second class are the dispersed repeats, including transposons, retrotransposons and microsatellites that occur both in heterochromatin and euchromatin regions. For tomato we isolated Cot1, Cot10 and Cot100-fractions containing high-, middle and low-copy repetitive sequences from the reassociation of sheared and denatured genomic DNA and used them as probes in a FISH to establish chromosome distribution of these pooled repeats on spread mitotic and pachytene complements. Comparative hybridisations with different Cot fractions on pachytene complement revealed that the Cot100 entirely covers all heterochromatin regions and so could be used for blocking repetitive sequences in a FISH using BACs as probes, even when they occur in pericentromeric heterochromatin regions (Zhong *et al.* 1999; Budiman *et al.*, 2004; Chang 2004). The Cot-100 as probe in a Southern to BAC filters was also most effective to annotate those clones that are relatively rich in repetitive sequences and should therefore better omitted for DNA sequencing programs (Chang, 2004).

We optimised fluorescence microscopy and digital image acquisition for multicolour FISH of single copy (BAC) and repeat sequences on pachytene chromosomes of tomato. For best heterochromatin differentiation we applied a Hi-Gaussian sharpening of the DAPI images and used a combinatorial labelling scheme for FISH images based on FITC, Cy3 and Cy5 to produce pseudocolours for up to seven different repeats or BACs simultaneously used in a single hybridisation experiment. Based on these technical innovations we were able to map various tandem and dispersed repeats, which revealed six different chromatin classes for the tomato genome (Chang 2004). We also mapped a large number of BACs, based on genetic data for the chromosomes 6 and 12, of which various were shown to be located on one of the other chromosomes, and of which the chromosome positions dramatically deviated from the relative genetic map positions. The discrepancies between chromosomal and genetic maps, and the erroneous genetic mapping of some of the BACs were ascribed to the abundant occurrence of the repeats and the suppression of recombination in the pericentromeric heterochromatin regions, which in most cases encompass more than 70% of the total chromosomal DNA.

For a deeper understanding of the molecular organisation of euchromatin and heterochromatin we have chosen to work with the model species Arabidopsis, whose small genome size and consequently relatively low proportion of repetitive sequences is outstanding material for chromatin organisation, DNA sequences and gene expression. The five chromosomes of Arabidopsis have clear blocks of heterochromatin at the centromeres and at the NORs of the chromosomes 2 and 4 with distinguishable structural, molecular, and functional properties (Koornneef *et al.*, 2003). In Columbia and related other accessions we have described a conspicuous heterochromatic knob of approximately 700 kb that accommodates a tandem repeat and several dispersed pericentromeric-specific repeats. Moreover, our data provide evidence for an inversion event that relocated pericentromeric sequences to an interstitial position, resulting in the heterochromatic knob (Fransz et al. 2000).

The same simplicity of "clean" heterochromatin is also recognised in the way how interphase nuclei are organised. In a study on young parenchyma cells we have shown how heterochromatin segments are organized as clearly distinguishable, condensed chromocenters (CCs) with heavily methylated, mostly

repetitive DNA sequences. The remaining euchromatin domains are weakly stained and contain less methylated DNA. Chromosome painting with 131 selected BACs for chromosome 4 showed the interphase domain for this chromosome was organised with a variable number of chromatin loops spanning 0.2-2 Mbp and emanating from the CCs. These loops are rich in acetylated histones, in contrast to the CCs that are relatively low in acetylated histones (Fransz *et al.* 2002). Morever, the well-defined organisation of the methylated CC inspired Soppe *et al.* (2002) to study chromocenter morphology in the Arabidopsis *ddm1* and *met1* methylation mutants. Both show a reduction of heterochromatin due to dispersion of pericentromeric low-copy sequences away from heterochromatic chromocenters. Analysis of F_1 hybrids between wild- type and hypomethylated mutants revealed that DNA methylation is epigenetically inherited and represents the genomic imprint that is required to maintain pericentromeric heterochromatin.

We now have extended this study to a larger number of mutants affecting DNA methylation, histone acetylation, chromatin assembly factors involved in the chromocentre-loop arrangement of interphase chromosomes. The advantage of this approach is that we can integrate microscopical analysis of DNA organisation, loop formation with specific gene expressions in nuclei of cells at subsequent developmental stages in a developing organ in different mutant backgrounds. Part of our research will be focused on the root tip in which all individual cells can be identified and their chromatin and gene expression pattern followed (Sabatini *et al.* 1999; Birnbaum *et al.* 2003). The relative simplicity of the Arabidopsis nucleus (compared to for example the human nucleus) makes Arabidopsis a very suitable model to perform such studies on nuclear architecture and positioning of genes in relation to development. This research will have an impact that is likely to go beyond plant systems only. It has shed new light to the potential of modern chromosome research that now promise new, hitherto unpredicted links to chromatin genomics, developmental processes and epigenetics.

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