Research Focus

Planning for remodelling: nuclear architecture, chromatin and chromosomes

J.S. (Pat) Heslop-Harrison

Department of Biology, University of Leicester, Leicester, UK LE1 7RH

DNA sequences occupy three-dimensional positions and their architecture is related to gene expression, gene–protein interactions and epigenetic processes. The recent analysis of chromosome 4 in *Arabidopsis* interphase nuclei reveals that gene-rich, undermethylated DNA is composed of active loops of 200 to 2000 kb associated with acetylated histones, providing a well-defined model system to study chromatin in its nuclear context.

Primary DNA sequence is likely to help answer many important questions in genetics, taxonomy, developmental biology and biochemistry. But the events leading to differentiation and development involve gene regulation and the interactions of gene networks in ways that are far more complex than can be explained by the control of gene expression via upstream, gene-specific promoters. During 2002, several laboratories made impressive progress in elucidating the nature of DNA packaging in chromatin and its organization within the nucleus during cell division, development and differentiation. Understanding the modulation of the organization of features of DNA and chromatin proteins [1–3], broadly classed as epigenetic processes, are crucial to development and are needed for modelling nuclear gene expression. Paul Fransz and colleagues [4] have studied the physical architecture of chromatin in the interphase nucleus, enabling us to examine functional properties of known chromatin domains. Within the cell nucleus, many fundamental features involve physical changes and movements of chromatin, such as enzyme complexes and promoters coming together, chromosomes pairing and chromatin becoming accessible to polymerases. In plants, as well as in animals [5], there are multiple, dynamic specialized domains within the nucleus.

Arabidopsis provides a fine model for molecular cytogenetics and the understanding of genome organization. In spite of initial doubts that cytological analysis was feasible in a species with such a small genome size, steady advances in microscopy, preparation techniques, probes and fluorescence methods in the past decade have made the system amenable to analysis. As in other species, chromatin within interphase nuclei shows domains of dense heterochromatin, which is condensed, and decondensed euchromatin in an extended form. From the earliest work on the *Arabidopsis* nucleus nearly 100 years ago, sites of heterochromatin, referred to as chromocentres, have been noted as conspicuous features, and are now known to correspond to the centromeric heterochromatin on each of the five sub-metacentric chromosome pairs, plus the four sites of the 45S rDNA genes (Fig. 1). Many of the heterochromatin blocks are either found to fuse or to overlap in microscope preparations, so few are regularly seen, and some of these groupings in *Arabidopsis* represent chromocentres from homologous chromosomes. The constitutive heterochromatin is largely inert and rich in repetitive DNA: in *Arabidopsis*, it comprises a 180 bp motif, representing

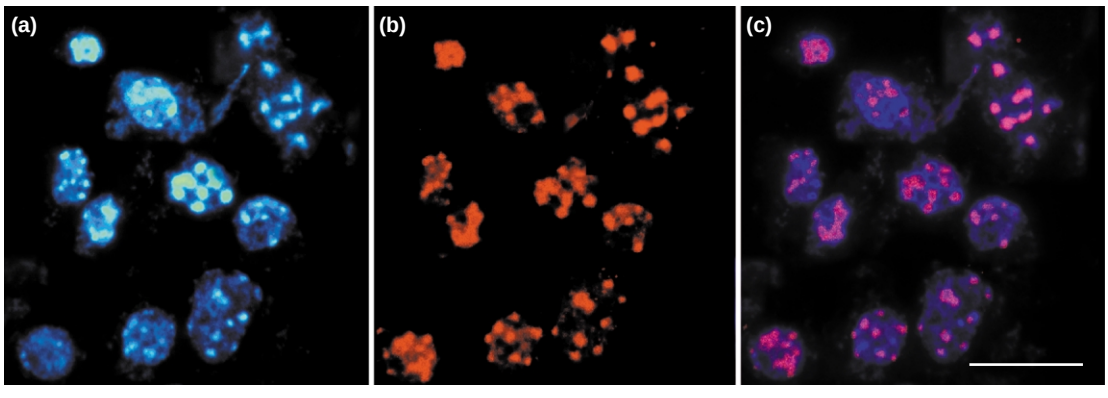

Fig. 1. Root tip nuclei from *Arabidopsis thaliana* stained with (a) fluorescent DNA stain DAPI (4,6-diamidino-2-phenylindole). Heterochromatin is seen as brightly staining chromocentres surrounded by more diffuse euchromatin in the interphase nuclei. (b) *In situ* hybridization (stained in red) of the major 180 bp repeat present at the centromeres shows that this sequence is present at the chromocentres and around the centromeres of the five pairs of chromosomes (metaphase top right of plate). (c) Overlay of the DAPI DNA stain and 180 bp sites. Chromocentres often fuse within interphase nuclei. Scale bar = 10 μm.

Corresponding author: J.S. (Pat) Heslop-Harrison (phh4@le.ac.uk).

several percent of the whole genome, and various transposable element sequences, as well as the rDNA. Facultative heterochromatin – a second type of heterochromatin – involves specific chromosome regions that are condensed and transcriptionally inactivated only in some cell types. This process is correlated with modification of the histone H3 protein with respect to acetylation and methylation: heterochromatin assembly is linked with both DNA and histone methylation and DNA replication [6]. Another twist is the replacement of H3 by a variant in *Arabidopsis*, as in other organisms, at the centromeric regions of chromosomes [7].

Interphase chromatin organization

In *Arabidopsis* nuclei, Fransz *et al.* [4] have carried out a study of the physical organization of chromosome 4 using DNA probes to the 45S and 5S ribosomal DNA (rDNA), the centromeric regions and several BAC clones covering a significant fraction of the genome. These probes enabled them to show exactly which sequences were associated with heterochromatin, and the nature of sequences that are dispersed around and between the chromocentres. Their experiments with antibody staining could show which DNA sequences are methylated, and relate the DNA sequences to acetylation of the associated histones. To acquire the data from the different DNA and antibody probes, many experiments, typically involving analysis of hybridization patterns in 50 to 200 nuclei spread in two-dimensions with multiple labels, is required. The results give a relatively simple and straightforward picture: the centromeric heterochromatic chromocentre is rich in the repetitive DNA sequences that are methylated at cytosine and show low levels of histone H3 acetylation in the chromatin proteins, all related to their transcriptional inactivity. From these inactive chromocentres, the researchers detected gene-rich, euchromatic loops from 200 to 2000 kb long with lower levels of DNA methylation and high levels of histone H3 acetylation. The complete model (Fig. 2) involves sequences moving in and out of the active euchromatin phase, almost certainly affecting the transcriptional activity in these regions, as is the case in animals. Heterochromatin at the core of chromosomes has major effects at meiosis too: Wolfgang Haupt *et al.* [8] have shown that the recombination rate in the chromocentre region of *Arabidopsis* chromosome 1 is $<0.5\%$ of that in euchromatin. Finally, heterochromatin has been implicated widely in chromosome pairing – or somatic transinteractions to use a more molecular terminology – and extensions of the work by Fransz *et al.* should show whether such interactions are important.

The model nucleus

The small quantity of nuclear DNA in *Arabidopsis* has assisted in analysing the packaging and modulation of the small amount of heterochromatin that is present. In nuclei with only a small change in the number of genes but with magnitudes more repetitive sequences, the arrangement of individual chromosomes at interphase would have been much harder to decipher. The *Arabidopsis* genomic DNA sequence shows a low density of genes within blocks of repetitive DNA sequences, and the apparent presence of some ESTs with no genomic sequence equivalent might suggest more are deeply buried in repeats. It will be fascinating to see how expression of such sequences occurs: are there transient decondensation events? Perhaps nuclear architecture can be included as a key aspect of the models of networks of gene expression [9], where small perturbations cause changes in expression of many genes. Ever-improving methodology should give us insight into the dynamics of three-dimensional nuclear organization: single-gene tags from transgenic green fluorescence protein constructs, improved DNA and antibody probes, live-cell imaging, and single-cell DNA and protein analysis are all beginning to yield results.

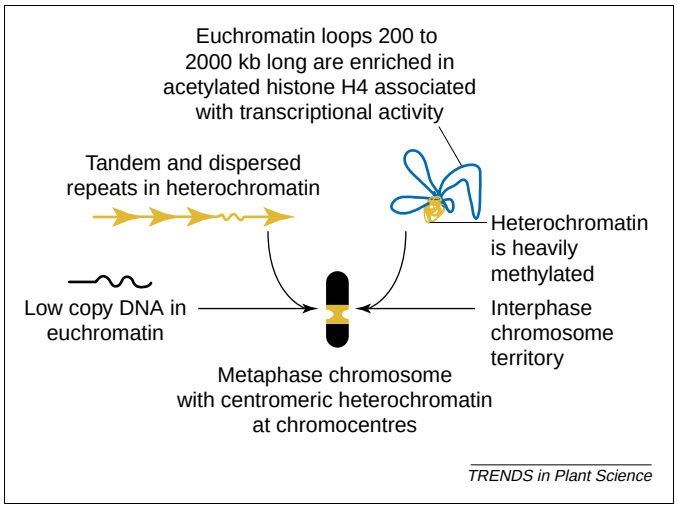

Fig. 2. The chromocentre model of *Arabidopsis* chromosome decondensation [4]. The heterochromatic chromocentre, rich in repetitive DNA sequences, shows heavy DNA methylation but low levels of histone H3 acetylation in the chromatin proteins, features that are correlated with transcriptional inactivity. Gene-rich, euchromatic loops with high levels of histone H3 acetylation emanate from the methylated chromocentres, and there can be dynamic interchange with the inactive state.

Much information in the nucleus is present at other levels beyond that of the primary DNA sequence. Big questions are still difficult to answer at the level of DNA: crop yield, drought tolerance or why an oil palm plant regenerated from tissue culture first shows flowering abnormalities five years after regeneration. The *Arabidopsis* reference genome is showing its value by enabling us to understand nuclear modulation. Individual cells now need to be analysed to show how each nucleus regulates its genes: no longer is a tube of 'genomic DNA' or 'embryo RNA' sufficient for an experiment. Plants have not had the advantages of flow cytometry and surface markers for cell-type analysis, but fortunately, microscopy and immunocytogenetics is now letting us tackle nuclear differentiation in plants.

References

1 Jackson, J.P. *et al.* (2002) Control of CpNpG DNA methylation by the KRYPTONITE histone H3 methyltransferase. *Nature* 416, 556–560
2 Gendrel, A.V. *et al.* (2002) Dependence of heterochromatic histone H3 methylation patterns on the *Arabidopsis* gene DDM1. *Science* 297, 1871–1873
3 Manzanero, S. *et al.* (2002) Alterations in the distribution of histone H3 phophorylation in mitotic plant chromosomes in response to cold treatment and the protein phosphatase inhibitor cantharidin. *Chromosome Res.* 10, 467–476

4 Fransz, P. *et al.* (2002) Interphase chromosomes in *Arabidopsis* are organized as well defined chromocenters from which euchromatin loops emanate. *Proc. Natl. Acad. Sci. U. S. A.* 99, 14584–14589
5 Spector, D.L. (2001) Nuclear domains. *J. Cell Sci.* 114, 2891–2893
6 Soppe, W.J.J. *et al.* (2002) DNA methylation controls histone H3 lysine 9 methylation and heterochromatin assembly in *Arabidopsis*. *EMBO J.* 21, 6549–6559
7 Talbert, P.B. *et al.* (2002) Centromeric localization and adaptive evolution of an *Arabidopsis* histone H3 variant. *Plant Cell* 14, 1053–1066
8 Haupt, W. *et al.* (2001) The CENTROMERE1 (CEN1) regions of *Arabidopsis thaliana*: architecture and functional impact of chromatin. *Plant J.* 27, 285–296
9 Hasty, J. *et al.* (2001) Computational studies of gene regulatory networks: in numero molecular biology. *Nat. Rev. Genet.* 2, 268–279

1360-1385/03/$ - see front matter
doi:10.1016/S1360-1385(03)00054-2

Two genetically discrete pathways convert tryptophan to auxin: more redundancy in auxin biosynthesis

Jerry D. Cohen[1], Janet P. Slovin[2] and Angela M. Hendrickson[1]

[1]Department of Horticultural Science, University of Minnesota, Saint Paul, MN 55108, USA
[2]United States Department of Agriculture, Agricultural Research Service, Fruit Laboratory, Beltsville, MD 20705, USA

The answer to the simple question of how plants make auxin has proven to be inordinately complex. Recent *in planta* studies in *Arabidopsis* have uncovered additional complexity in auxin biosynthesis. Two distinct pathways from tryptophan to the intermediate indole-acetaldoxime were identified. Genic, as well as functional redundancy, appear to be characteristic for auxin biosynthesis and plants might have evolved many different solutions for making and regulating auxin.

It has taken scientists >100 years to make marked progress in answering the question of how plants make auxin, a hormone that is not only essential for growth but also plays important roles in many developmental processes and in environmental responses. We cannot begin to ask crucial questions of agricultural significance about how auxin biosynthesis is regulated until the process is defined. Recently, Yunde Zhao and colleagues [1] have defined a key step in the biosynthesis of auxin from tryptophan. These results reveal a complex situation where two genetically discrete pathways operating in a single plant both start from the same precursor and result in the formation of the same signal messenger. These results are an impressive example of why it has taken so long to understand such an essential process.

New pathway defined

The classical genetic approach, screening for auxin-deficient mutants, yielded few positive hits. This was attributed either to such mutations being lethal or that multiple biosynthetic pathways and/or functional redundancy [2] in multiple genes in the same pathway resulted in silent mutations. Two different biosynthetic pathways are involved in indole-3-acetic acid (IAA) biosynthesis, one which uses tryptophan as a precursor, and another, discovered about ten years ago [3,4], which bypasses tryptophan and uses indole as a precursor for IAA biosynthesis. Using gain-of-function approaches in Arabidopsis, redundant pathways have now been defined within the tryptophan-dependent pathway. Activation tagging resulted in the identification of the YUCCA gene, which encodes a novel flavin monooxygenase that catalyzes the rate-limiting N-hydroxylation of tryptophan to create N-hydroxyl tryptamine [5]. This important advance defined a new pathway that had not been predicted for indole-3-acetic acid (IAA) biosynthesis from tryptophan. The identification of two Arabidopsis YUCCA paralogs extends the idea of redundancy to the gene level within a single pathway.

As with other Cruciferae species, *Arabidopsis* contains a class of secondary compounds known as glucosinolates that because of their flavor and medicinal properties have attracted much interest in their mode of production within plants. Recent attention has focused on the role of cytochrome P450s in the reactions leading to indolic glucosinolates [6,7]. Overexpression of *superroot2* (*sur2*) [8], an *Arabidopsis* gene encoding the cytochrome P450 CYP83B1, or overexpression of a related cytochrome P450, CYP83A1, results in increased indolic glucosinolate levels, but the morphological phenotype is consistent with underproduction of IAA. Mutations of *sur2* cause increased adventitious rooting and epinasty, consistent with IAA overproduction. It has been suggested that CYP83B1 serves as a branch point between IAA and indolic glucosinolate biosynthesis, but functions downstream from the most likely branch point from IAA production [9] where indoleacetaldoxime (IAOx) is an intermediate (Fig. 1).

The conversion of tryptophan to IAOx was already known to be catalyzed by two other cytochrome P450s, CYP79B2 and CYP79B3 in *Arabidopsis*, but whether IAA or only glucosinolates were produced from IAOx was unclear [10]. The CYP83B1 and CYP83B2 results suggested that supplying IAOx for conversion to 1-*aci*-nitro-2-indolyl-ethane, the committed step of indolic glucosinolate biosynthesis, was the most likely role for

Corresponding author: Jerry D. Cohen (cohen047@tc.umn.edu).