Nuclear architecture in plants

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Structure within the nucleus of plants is becoming increasingly clear in both metaphase and interphase nuclei, although there are conflicting data about the relative positions of individual and pairs of chromosomes. At interphase, individual chromosomes may generally occupy discrete domains that are not intermixed with other chromosomes. Aspects of mechanical chromosome behaviour and even of gene expression may correlate with interphase chromosome position, and imply that a better understanding of nuclear architecture is required.

The interphase nucleus of plants is usually imagined, albeit unconsciously, as a bowl of spaghetti – strands of interphase chromosomes running at random through the volume of the nucleus. During cell division, these strands are organized into discrete metaphase chromosomes, but most preparations still give the impression of little or no suprachromosomal organization. Recent work, both in plants and animals, is questioning some of these hidden assumptions, and suggests that it may be possible to relate nuclear architecture to aspects of gene expression and chromosome behaviour.

Such assumptions were not made when chromosomes were first described. Early drawings show symmetrical arrangements of chromosomes and their arms throughout the cell cycle, and the physical disposition of chromosomes within the nucleus was regarded as important. For example, Fig. 1 shows a drawing from sections of nuclei in the desert plant *Yucca*, in which similarly sized chromosomes are together, and large chromosomes lie in a peripheral domain surrounding the smaller chromosomes[1].

Nuclear architecture describes the structure and pattern of the nucleus. To understand the architecture, we must know about the three-dimensional organization of the nucleus, including both the position and identity of each chromosome. These two simple ideals have rarely been achieved because the nucleus is dynamic (moving through the cell cycle, transcribing RNA and replicating DNA), small (typically 10 μm diameter) and its chromosomes thread-like (0.2 μm diameter at interphase and 1 μm at metaphase) and often with similar morphologies. The techniques of chromosome spreading pioneered in plants by Darlington and colleagues[2] largely changed the way chromosomes were examined. Spread and squashed preparations of metaphases are ideal for most cytogenetics: they enable the counting of chromosomes, and the examination of chromosome morphology. Chromosomes can be easily identified in banded, two-dimensional spreads of metaphases made for the light microscope, and three-dimensional position can be reconstructed from sections, but combining the two techniques is difficult.

Many attempts have been made to analyse the disposition of chromosomes at metaphase in spread preparations[3]. Although large numbers of dividing cells can be obtained easily, and almost any plant can be used, analysis of architecture is difficult because the three-dimensional nucleus has been reduced to two dimensions. Large sample sizes may only increase the chance of assessing artefacts of spreading. In order to overcome the difficulty of identifying chromosomes, chromosomes with particular morphologies, including heterochromatic blocks (large tandemly repeated DNA sequences, which may relate to the C-banding patterns seen on chromosomes), have often been examined. Avivi *et al.*[4] analysed the positions of mitotic wheat chromosomes that were missing a whole arm (telocentric chromosomes), and so could be easily identified. However, we reported that large systematic errors were introduced when positions of telocentric chromosomes paired with normal chromosomes ('marked' bivalents) were analysed in squashes of wheat meiotic preparations[5]. At metaphase I, we found that there was a strong tendency for any marked bivalent to lie near the edge of the metaphase spread preparation, regardless of which particular chromosome type gave rise to the marked bivalent.

Within the past ten years, interest has returned to examination of whole nuclei, from both plants and animals. In plants, extensive work has been carried out using serial section reconstructions at meiosis[6] and at mitosis[7,8], particularly in the cereals. The newer techniques of confocal microscope reconstructions and computer processing of images of sections[9,10] are also being increasingly used. The problem of chromosome identification, at both interphase and metaphase, is

FIG **1**

A drawing by Müller from 1909[1], showing sections of nuclei in the desert plant *Yucca*, where similarly sized chromosomes are together through the division. Large chromosomes lie in a peripheral domain, surrounding the smaller chromosomes.

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association of the nucleolus organizing chromosome pair in hawk's-beard, *Crepis capillaris*[9]. At metaphase, they found no evidence that other chromosome pairs were associated, although many chromosome pairs were associated at prophase. Horn and Walden[15] examined spreads of maize, and also found that the nucleolus organizing chromosomes were associated. The association of nucleolar organizing chromosomes in particular is not surprising because of the tendency for nucleoli to fuse by late interphase.

Most studies have been on metaphase or anaphase chromosomes, because this is the stage at which chromosomes are condensed, easy to study and can be identified. A few recent studies have looked at nuclei in interphase, which is the most important stage of the cell cycle for gene expression because the chromosomes are being actively transcribed. The analyses show, in general, that the positions of whole chromosomes at metaphase do reflect their interphase disposition[12,16]. Hence, valid work on metaphases can to some extent be extrapolated to chromosome disposition at interphase.

In situ hybridization is providing an important method to look at interphase chromosomes. In human nuclei, each decondensed chromosome has been shown to occupy a domain or restricted volume within the nucleus[11]. We have examined nuclei in hexaploid wheat varieties that include two chromosome arms originating from rye; these arms are present as a translocation between the 1B chromosome from wheat and the 1R from rye[17]. When rye DNA is used as a probe, the two single rye chromosome arms can be clearly seen at interphase in root tip nuclei, and occupy distinct domains that do not ramify throughout the whole nuclear volume. Figure 2 shows an interphase from one of the 1B/1R wheat varieties, in which the rye chromosome arm has been probed. The rye arms clearly occupy restricted domains, and the two homologous chromosome arms are not together[17], even though the rye arm contains an active nucleolus organizing region. We must wait for further information before concluding that all chromosome arms occupy individual domains, but it seems that most evidence now indicates that homologous chromosomes are not closely associated in somatic tissues of plants.

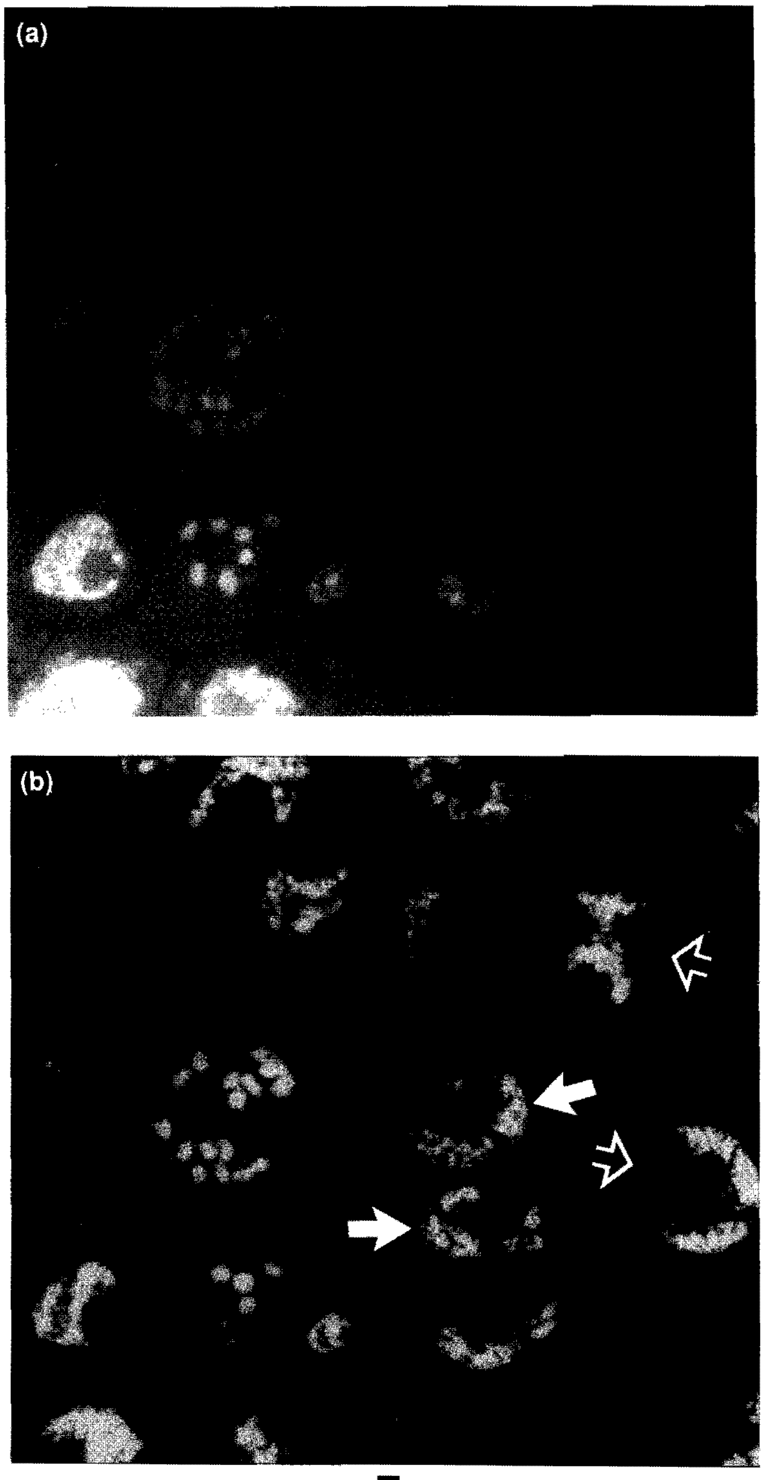

FIG 1

Semi-thin (0.25 μm) sections of interphase nuclei and a metaphase from the hybrid *H. chilense* (a wild barley) x *S. africanum* (wild rye). (a) DAPI staining shows that the DNA can be seen to fill the volume of the interphase nuclei relatively uniformly. (b) After probing with labelled rye DNA and detection of sites of hybridization with Texas red fluorescence, dark (DNA of barley origin; open arrows) and brightly labelled areas (DNA of rye origin; closed arrows) are differentiated within the nuclei. The chromatin originating from the two parents is not intermixed[23]. Magnification, x1900.

Meiosis involves the spatial reorganization of the nucleus. Homologous chromosomes come together and pair closely before crossing over and separation occurs. In the cereals, homologous chromosomes do not associate at the last metaphase before meiosis[18], but other evidence indicates that the chromosomes are

Consequences of ordering in the nucleus

Chromosome elimination

The loss of single chromosomes, or whole genomes, is a clear consequence of their positioning at a critical time. Their position within the cell at division excludes them from the daughter nuclei. In six different hybrids between various cereals that we have investigated[8], the chromosomes of the genome that is peripheral tend to be lost. While peripheral positioning does not always lead to elimination of a genome's chromosomes, at least in cereal hybrids it does predispose to this mitotic instability.

Gene expression

The consequences of nuclear order for gene expression are as yet unclear. Transformation experiments suggest that, in general, chromosomal position may have little or no effect on gene expression, but other observations have revealed at least some situations in which the nuclear position of a gene can influence its expression. In plants, there is only one set of genes that can be easily visualized by microscopy when they are being expressed – the nucleolar organizing genes that are transcribed within, and give rise to, the nucleolus. In root tips, the nucleolus is normally central within the interphase nucleus. In the cereal hybrids that show strong parental genome separation, with one genome around the other, the nucleolar organizing genes in the peripheral genome (for example the rye nucleolar organizing genes in Figs 3 and 4) are not expressed, but the nucleolar organizing genes from the central genome, which surrounds the nucleolus, are active[8,29]. In addition, many hybrid plants show a strong tendency to resemble one parent much more than the other in their gross appearance and behaviour. Where the hybrid shows strong concentric genome separation, the hybrid resembles the parent contributing the outer genome[8]. Thus the intranuclear position of a parental genome at interphase may affect the extent to which its genes are expressed.

Conclusions

Interest in the architecture and organization of interphase nuclei and metaphases is increasing. Whether ordering has implications for plants beyond those discussed above (including, perhaps, genomic imprinting) is not yet known. However, in human nuclei, Borden and Manuelidis[34] have shown that the relative position of the X chromosome alters in patients suffering from epilepsy – an important discovery indicating that order may directly correlate with cell and organism behaviour. The potential importance of chromosome position, because of its effect on gene expression, is being recognized in plants, although further studies are required.

Technical advances in fluorescent light microscopy, confocal microscopy, and *in situ* hybridization that have been made within the past three years are now enabling us to attack the problems of nuclear architecture directly. For the first time, we can study the disposition of whole genomes, chromosomes, repetitive DNA sequences and genes within active, and even differentiated, interphase nuclei.

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