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.

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 have 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 light microscopy, 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. 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 correlate to the C-banding patterns seen on chromosomes), have often been examined. Avivi et al. 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. At metaphase 1, 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 and at mitosis, particularly in the cereals. The newer techniques of confocal microscope reconstructions and computer processing of images of sections are also being increasingly used. The problem of chromosome identification, at both interphase and metaphase, is
association of the nucleolus organizing chromosome pair in hawk's-beard, *Crepis capillaris*. At metaphase, they found no evidence that other chromosome pairs were associated, although many chromosome pairs were associated at prophase. Horn and Walden 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. 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. 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. 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, 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.

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, 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, 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 nucleolar 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 peripheral tend to be lost. While peripheral positioning does not always lead to elimination of a genome's expression is 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 nucleolar 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 peripheral tend to be lost. While peripheral positioning does not always lead to elimination of a genome's expression.

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 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 distribution of whole genomes, chromosomes, repetitive DNA sequences and genes within active, and even differentiated, interphase nuclei.

Acknowledgements

We thank BP Venture Research Unit for enabling our work on the architecture of the nucleus. We also thank our collaborators, and in particular Drs Andrew Leitch and Trude Schwartzzacher, for their contributions to our research.

References

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Full References


