Studying single living cells and chromosomes by confocal Raman spectroscopy

G.J. PUPPELS *ET AL.*
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Confocal scanning light microscopy, which has revolutionized approaches to reconstructing three-dimensional images of living cells, has a new cousin: confocal Raman spectroscopy. This technique combines the use of confocal optics, to narrow detection to an extremely small volume (1 μm^3), with the ability of Raman spectroscopy to distinguish chemical bonds. Confocal Raman spectroscopy thus promises a high-resolution approach to examining the composition of, for example, chromosomal domains. Preliminary results from use of this technique indicate that the protein:DNA ratio varies widely along banded polytene chromosomes, being highest in the bands, lower (but very variable) in interband regions and lowest at the telomeres. Confocal Raman spectroscopy can be combined with immunofluorescence techniques to examine sites labelled by specific antibodies, and provides a useful check on the degree to which such labelling techniques perturb native structure. It may prove to be a vital link in correlating cytological observations with the results of genetic and biochemical analyses.

Gene interactions and epigenetic variation in transgenic plants

M.A. MATZKE AND A.J.M. MATZKE
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It is a well-established notion that interactions between genetic loci can have profound effects on the phenotype of an organism. However, the mechanistic basis of such interactions is poorly understood. A number of recent observations, particularly in plants, highlight an intriguing type of genetic interaction between genes introduced by transformation, and resident genes: an introduced transgene can alter the expression both of other homologous transgenes and of endogenous homologous genes. (In the current issue of *Trends in Biotechnology*, Richard Jorgensen discusses this phenomenon in detail.) Matzke and Matzke have carried out a series of experiments in which transgenic tobacco plants were sequentially transformed with two T-DNAs carrying different resistance markers. In about 15% of double transformants (which were all derived by re-transforming with T-DNA-II one singly transformed plant carrying T-DNA-I), expression of T-DNA-I was repressed. Repression correlated with methylation of the NOS promoter (which was shared by both T-DNAs) in T-DNA-I. In progeny of the double transformants in which T-DNA-I had segregated away from T-DNA-II, T-DNA-I could be reactivated and methylation reversed, at least in some cells. Matzke and Matzke suggest that these observations are consistent with there being competition between homologous sequences in the T-DNAs for binding to a particular nuclear site at which transcription is activated. This competition might be expected to depend on the relative chromosomal positions of the two genes, thus explaining why not all double transformants show the effect. An important next step will be to determine the chromosomal locations of the T-DNAs in plants in which the interaction is apparent and in those in which it is not.

A deletion of the human β-globin locus activation region causes a major alteration in chromatin structure across the entire β-globin locus

W.C. FORRESTER *ET AL.*
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The locus activation region (LAR) of the human β-globin locus on chromosome 11 influences β-globin gene expression from a position 10–20 kb upstream of the 5'-most gene in the β-globin cluster. The LAR contains DNase I hypersensitive sites that are erythroid specific and stable throughout development, and it can confer high levels of expression on linked β-globin genes in transfected cell lines and transgenic mice. Lesions in the human β-globin locus have been shown to cause different types of thalassaemia, for example Hispanic thalassaemia is associated with a deletion of 35 kb of DNA, including the LAR, 60 kb upstream of the β-globin gene. Forrester *et al.* examined the properties of the β-globin locus in somatic cell hybrids between lymphoid cells of a patient with Hispanic thalassaemia, and mouse erythroleukaemia (MEL) cells. (MEL cells, when fused with nonerythroid human cells, can activate the human erythroid programme of gene expression.) When hybrids containing only a normal chromosome 11 were compared with hybrids containing a chromosome 11 carrying the Hispanic thalassaemia deletion, the β-globin locus of the latter was found to be late-replicating and DNase I resistant, in contrast to the properties of the normal locus. More than 150 kb of DNA was affected in this way. The effect on replication extended both 5' and 3' of the deletion breakpoint, suggesting that there may be an origin of replication in the LAR. In contrast, DNase I resistance was affected only in the 3' direction, indicating that effects of the LAR on chromatin and on replication may be separable. In the normal β-globin locus, the LAR may act as a block to the 3' spread of DNase-I-resistant chromatin, or sequences upstream of the LAR may prevent 5' spread of DNase I sensitivity. The changes in chromatin structure reflected in the emergence of DNase I sensitivity and hypersensitive sites lead in turn to the utilization of elements that directly control globin gene expression.

Architectural organization in the interphase nucleus of the protozoan *Trypanosoma brucei*: location of the telomeres

H-M.M. CHUNG *ET AL.*
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The positioning of chromosomes in nuclei, although notoriously difficult to analyse, has been investigated in detail in *Drosophila* salivary gland cells with polytene chromosomes, and in cereals. Now, Van der Ploeg's group is mapping the arrangement of chromosomes in the protozoan parasite *Trypanosoma brucei*. Again, the techniques used are *in situ* hybridization and confocal scanning optical microscopy. *T. brucei* has ~100 minichromosomes (50–100 kb) and at least 18 larger chromosomes (up to ~6 Mb). Chung *et al.* used a biotinylated telomere repeat $(GGGTTA)_n$ to determine the position of telomeric DNA, which in most cases was found in clusters at the periphery of the nucleus. The minichromosomes carry a specific 177 bp repeat, and their distribution in the insect form of *T. brucei*, similarly detected, involved clustering specifically into less than half the nucleus, either in a narrow band at one pole or spread evenly across the half-nucleus. The confocal microscopy results suggest that the telomere repeats extend along the nuclear periphery, or form a network through the interior of the nucleus. For the minichromosomes, the microscopy confirmed that the repeat sequences are generally in a network tightly associated with the nuclear envelope. The significance of the apparent spatial order in the nucleus is unclear, although the authors speculate on roles in the control of gene expression and chromosome and telomere function.