Seeing is believing

John E. Hearst

A new microscope described elsewhere in this issue by G. J. Puppels *et al.* (*Nature* **347**, 301–303; 1990) represents a milestone in the long history of cytology. The instrument can focus on a volume of less than $1\ \mu\text{m}^3$ (a volume approaching the theoretical diffraction limit of a microscope using light of wavelength 660 nm), and obtain a high-quality Raman spectrum. Because Raman-scattering spectra are sensitive to molecular vibration, such a spectrum allows one to 'see' chemical bonds, and therefore to distinguish between protein and nucleic acid.

As the technique becomes more completely developed and more rigorously applied, it could be used to explore far more subtle structural and compositional variations than are possible at the resolution of the conventional light microscope. As a new arrow in the quiver of procedures available to cytologists for determination of biological structure, confocal Raman microspectroscopy is an exceptional development.

The authors have used a combination of emerging technologies in a manner never before applied to microspectrophotometry. First, a charge-coupled device (CCD) is used as the photon detector. CCDs have been used primarily in astronomy because of their extremely high signal sensitivity achieved at low background intensities. Second, to achieve these low backgrounds, a chevron-type dielectric band-pass filter tuned to suppress the Rayleigh (or elastic) scattering of the incident 660-nm laser light with an efficiency of one part in 10^9 is used. Finally, the confocal optics allow for the tight localization of the detected inelastically scattered light to the $1\ \mu\text{m}^3$ -volume element. The authors have determined the changes in the ratio of DNA to protein in salivary chromosome bands, interbands and telomeres, demonstrating the extraordinary capabilities of Raman microspectroscopy.

Genetics has had a special relationship with cytology since the 1870s, when light microscopy first revealed the existence of chromosomes. Ever since then, the quality of cytological representations of chromosomes has always outclassed molecular understanding. The cytological maps of *Drosophila* salivary chromosomes pioneered by Bridges, for example, established the relationship between genetic linkage and microscopically observable bands and distances. This work preceded the observations in 1944 by Avery and his co-workers that established DNA as the genetic carrier. In the 1960s, Joseph Gall developed the technique of *in situ* hybridiza-

tion, by which many of the simple satellite DNA sequences, discovered by density gradient sedimentation and renaturation kinetics, were shown to be structurally unique to the chromosome, localizing at centromeres and telomeres. There is a major distinction between heterochromatin and euchromatin, a cytological density difference inferred to be related to biological activity. The banding patterns of salivary chromosomes and correlations between the numbers of bands and the numbers of genes in *Drosophila* make these issues even more intriguing.

The discovery of banding patterns in human chromosomes and their importance to karyotyping introduced the question again. What are the compositional and structural properties of the chromosome that create these observable cytological features, and why do they exist? Although tremendous in-

ONCOGENIC VIRUSES

Of marmots and men

Don Ganem

ONE of the great unsolved problems in the biology of human cancer is the reason for the close association of hepatocellular carcinoma (HCC) with chronic infection by hepatitis B virus (HBV). This link is no small matter: over 250 million humans are chronic carriers of HBV, and in the vast areas where HBV infection is endemic, HCC ranks among the most common human tumours. On page 294 of this issue, Fourel *et al.*¹ shed new light on this connection by showing that integrated viral DNA is often linked with known oncogenes in an animal model of liver cancer.

The fact that HBV carriers are between 100 and 200 times more likely than non-carriers to contract HCC is compelling epidemiological evidence for a link between HBV and HCC². Almost all HBV-related HCCs harbour integrated viral DNA, usually in multiple copies. Tumours are clonal with respect to these insertions, indicating that integration of viral DNA precedes or accompanies the transforming event.

Further evidence of a link comes from animal studies. Although HBV infection is largely restricted to humans, related viruses affect a number of other species, including woodchucks (marmots), ground squirrels and ducks. Remarkably, woodchucks infected from birth with woodchuck hepatitis virus (WHV) are almost guaranteed to develop HCC³.

The oncogenic sword of HBV is terrible

roads have been made at the level of DNA sequence, nucleosome structure and, to a lesser degree, chromatin coiling, we remain remarkably inept at mapping the compositional and structural properties of larger domains. Confocal Raman microspectroscopy is a most promising technique for alleviating our difficulties, for it provides *in situ* compositional and structural information without the use of invasive stains and fixatives that are common features of most cytological and microfluorometric analyses.

What remains to be seen is what else can be studied by this technique, for there are potential limitations associated with contrast and resolution. If the $1\text{-}\mu\text{m}^3$ volume contains too complex a mixture of chemically distinct molecules, assignment of Raman lines may be impossible. On the other hand, the sensitivity of this instrument is remarkable. □

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but not swift: tumours typically develop only after 20–30 years of persistent infection, and are usually accompanied by signs of hepatocyte necrosis and active inflammation (chronic active hepatitis) and fibroblastic proliferation (cirrhosis). Hepatoma induction by WHV is similar to that by HBV, except that tumours develop earlier in the course of infection and at a much higher frequency.

The molecular mechanisms underlying the connection between HBV and HCC have been elusive. The long incubation period of HCC argues against the existence of a dominant oncogene encoded by the viral genome, and most experiments designed to find one have been unsuccessful. In the absence of such a gene, most models for how HBV engenders HCC fall into two broad categories, according to whether viral DNA contributes directly or indirectly to hepatocellular proliferation. In the past year, evidence in support of both views has accumulated.

Models in which hepadnaviruses (the class of DNA virus that includes HBV) make a direct genetic contribution to the loss of growth control are influenced by studies of retroviruses (such as murine leukaemia virus, MuLV) that lack dominant oncogenes. These viruses often activate cellular proto-oncogenes by the integration of viral sequences in the flanking host DNA. But efforts to identify such oncogenes in the vicinity of integrated