A map for integration

Mindful that where a retroviral vector integrates into the genome is critical to the safety and success of gene therapy, Mitchell and colleagues have documented the preferred integration sites for three retroviral vectors. The researchers analyzed data sets assembled from new experiments and published work (Wu *et al. Science* **300**, 1749–1751, 2003) for vectors derived from human immunodeficiency virus (HIV), avian sarcoma-leukosis virus (ASLV) and murine leukemia virus (MLV) to see how gene density, gene activity, proximity to CpG islands, G/C content and location within genes affect integration. HIV strongly prefers integration in active genes, whereas MLV prefers integration near transcription start sites. Both preferences spell trouble for therapeutic applications as integration at those sites can disrupt normal gene function. In contrast, ASLV showed no bias towards integration at those sites, indicating it may be a better therapeutic vector than the others. Although knowledge about site of integration is likely to be important in choosing a vector, other issues such as yet-to-be discovered DNA-binding proteins will also need to be considered. (*PLoS Biol.* **2**, doi:10.1371/journal.pbio.0020234, published online 17 August 2004) *TM*

FedEx gene delivery

Achieving gene transfer in striated muscles, such as heart and skeletal muscles, via systemic delivery of viral vectors has proven tricky because of the barrier imposed by the vascular endothelium. Chamberlain and colleagues have effectively transferred a gene throughout the skeletal and heart muscles of an adult mouse using only a single intravascular coadministration of a recombinant adeno-associated virus pseudotype 6 vector (rAAV6) and the vascular permeabilizing agent, vascular endothelial growth factor. To validate their system, the authors demonstrated phenotypic rescue of the Duchenne muscular dystrophy mouse model when rAAV6 was used to deliver microdystrophin cDNA throughout the skeletal muscles of mice. Future applications of this approach may be suitable for any disease in which genetic transfer to the skeletal muscle fibers or myocardium is necessary, such as muscular dystrophy or heart disease. (*Nat. Med.* 10, 828–834, 2004) *NC*

Mind the gap

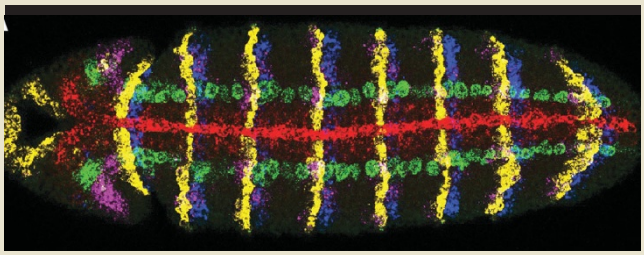
Several optical methods are available for measuring the distance between two fluorophores. Conventional microscopy—wide-field, confocal or multiphoton—is constrained by a diffraction-limited resolution on the order of 100 nm. Superresolution microscopy improves on this limit, with a resolution of ~50 nm. FRET imaging is suitable for gauging smaller distances, in the range of 1–10 nm. Now, Qu *et al.* describe a technique that tackles the gap between 10 nm and 100 nm. Nanometer-localized multiple single molecule (NALMS) fluorescence microscopy takes advantage of fluorophores' spontaneous photobleaching. By combining analysis of step changes in fluorescence intensity with centroid localization, the authors can resolve multiple fluorophores that are otherwise too close to be distinguished. The approach is demonstrated using two identical fluorophores separated by 24 or 40 base pairs of DNA (~8 or 13 nm). In its present form, NALMS fluorescence microscopy is limited to samples in which the fluorophores are immobile and sparse. The authors suggest that it could find immediate application in genomic mapping studies. (*Proc. Natl. Acad. Sci. USA* **101**, 11298–11303, 2004) *KA*

Research Highlights written by Kathy Aschheim, Nadia Cervoni, Laura DeFrancesco, Teresa Moogan and Gaspar Taroncher-Oldenburg.

Silence please!

Transcriptional gene silencing (TGS) is a process by which transcription of a gene is suppressed at the nuclear level through targeting of specific promoters. TGS via small interfering RNAs (siRNAs) has been described before in a variety of organisms, including plants and insects, but only now have scientists observed this phenomenon in mammals. Looney and colleagues report siRNA-induced TGS in human embryonic kidney cells after transfection with 21-nucleotide long siRNAs targeting the promoter of a human translation elongation factor (EF1A). The authors reduced the expression levels of both an EF1A promoter–reporter construct as well as endogenous levels of EF1A. Effective gene silencing was dependent on methylation of the DNA target and translocation of the siRNAs into the nucleus. Besides the potential of siRNA-mediated TGS as a tool for directed inhibition of virtually any gene activity in human cells and thus its promise for therapeutic applications, these observations further demonstrate the existence of siRNA-mediated gene regulation at the nuclear level in mammals and therefore the conservation of this process throughout nature. (*Science* published online 5 August 2004, doi:10.1126/science.1101372) *GTO*

Kaleidoscopic network visualization



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In vivo analysis of complex transcription networks has been hampered by a lack of high-resolution *in situ* methods that allow simultaneous visualization of multiple mRNAs. Kosman *et al.* describe a method for detecting as many as seven different transcripts in developing *Drosophila melanogaster* embryos. They used Alexa fluorescent dyes (Molecular Probes, Eugene, OR) in three different ways: directly attached to RNA probes, attached to secondary antibodies for the indirect detection of RNA species and amplified with a tyramidine amplification system. Using this method, transcription patterns can be visualized from nascent mRNA chains associated with chromatin separated by as little as 20 kilobases of DNA. In addition, genotyping and transcription mapping can be accomplished simultaneously using a two-color coding scheme. The method detects transcripts in 99.8% of nuclei. The authors point out that this technique facilitates the description of molecular patterns of gene expression in individual embryonic cells and in decoding regulatory networks that control embryogenesis. (*Science* **305**, 846, 2004) *LD*