CHROMATIN

Eukaryotic genomes in complete control

A recently published report shows that eukaryotic genomes encode preferences for nucleosome positioning, which can be modulated to facilitate specific chromosomal functions.

Nucleosomes — comprising 146 base pairs of DNA wrapped around a histone protein octamer — form the first level of genome compaction in eukaryotes. This organization has important implications for gene expression, because nucleosome-bound DNA is less accessible to various regulatory factors than nucleosome-free DNA. But how is the positioning of the nucleosomes regulated? Could the genome itself encode a preference for their distribution?

Segal *et al.* addressed this question in a series of experiments that combined experimental and computational approaches. Working in yeast, the authors isolated regions of DNA that were stably wrapped around nucleosomes on a genome-wide scale. On the basis of the isolated sequences, they used a probabilistic model to predict the sequences that preferentially wrapped around the histone octamer. It is known that certain nucleotide combinations that recur with a certain frequency facilitate DNA bending; Segal *et al.* not only found such motifs and periodicities using their model, but, by experimentally manipulating the motif sequence and their patterns, they could enhance or reduce the binding affinity of DNA to the nucleosome in accordance with the predictions of the model. The results also showed that the preferences of DNA for nucleosome occupancy vary from one genomic region to another. For example, centromeric sequences have the highest predicted nucleosomal occupancy; conversely, highly expressed genes such as ribosomal or transfer RNA genes show a preference for low occupancy.

Genome-wide scans showed that the high-affinity sequences occur more frequently than predicted by chance. Using a computational approach, the authors showed that various genomic regions favoured one or more nucleosome positioning patterns. Such a preference can have important functional effects; for example, a preference for a strong affinity at a transcription site would make it less accessible to transcription factors, whereas a preference for weak affinity would have the opposite effect. Using various approaches to compare *in vivo* nucleosome-positioning data to their predictions, Segal *et al.* confirmed the validity of their models and showed that ~50% of nucleosomal organization *in vivo* can be explained by these sequence preferences alone.

On the basis of their data, the authors suggest that by encoding weak nucleosomal affinity eukaryotic genomes might guide transcriptional machinery to functional sites. If correct, this hypothesis could also account for why some transcription-factor binding sites remain unoccupied *in vivo*.

Although Segal *et al.* admit that more accurate DNA–nucleosome interaction models are needed, this report represents the first step towards integrating the effects of chromatin structure into models of gene regulation.

Magdalena Skipper, Chief Editor,
Nature Reviews Genetics

ORIGINAL RESEARCH PAPER Segal, E. *et al.* A genomic code for nucleosome positioning. *Nature* **442**, 772–778 (2006)

Technology watch

NANOSCALE MICROSCOPY GOES GREEN

Fluorescence microscopy has become a popular mainstay of subcellular visualization, but its resolving power is limited by the diffraction of light. Several approaches have been taken to break the 'diffraction barrier', including an approach called stimulated emission depletion (STED) microscopy. In this technique, the area of the focal spot of a fluorescence-excitation beam is tightened by precisely overlaying a second doughnut-shaped beam that is tuned to de-excite fluorophores by stimulated emission. By this means, the focal spot area can be reduced below the diffraction barrier of a conventional fluorescence microscope.

Imaging applications of STED microscopy until now have used organic dye staining to visualize cell organelles. Now, the authors have applied STED microscopy to samples labelled with green fluorescent protein (GFP). They achieved a resolution of 70 nm when analysing GFP-tagged virus-like particles and fixed mammalian cells that expressed GFP. A comparison of confocal images and STED images clearly showed the improved resolution in STED images. STED microscopy is compatible with other fluorescent proteins and multicolour labelling, and future technical advances are expected to further improve the resolution. The authors anticipate that nanoscale fluorescent-protein microscopy will "open up a new avenue for answering many key questions in biology."

ORIGINAL RESEARCH PAPER Willig, K. I. *et al.* Nanoscale resolution in GFP-based microscopy. *Nature Methods* **3**, 721–723 (2006)

STOCHASTIC IMAGING

Single fluorescent molecules can be detected with sub-diffraction-barrier resolution, but accurately resolving multiple fluorophores that are in close proximity poses a problem. However, a new imaging technique — stochastic optical reconstruction microscopy (STORM) — overcomes this drawback by repeated high-resolution detection of individual fluorophores within a sample. STORM uses fluorophores that can be switched reversibly between a fluorescent and a dark state. A cycle of imaging intentionally turns on only a fraction of these fluorophores at a time, so that each fluorophore can be optically resolved from the rest, which allows the position of the fluorophore to be determined with high accuracy. The key is that a stochastically different subset of fluorophores is turned on in each cycle. The final image of the sample is then reconstructed by combining a set of multiple high-resolution images.

Using STORM, the location of proteins bound to a single DNA plasmid could be mapped with a sub-diffraction-barrier resolution of 20 nm. The method should therefore be a valuable tool for high-resolution fluorescence *in situ* hybridization and immunofluorescence imaging. Although the concept was tested with a cyanine dye, Cy5, it is also applicable to other photoswitchable fluorophores and fluorescent proteins.

REFERENCE Rust, M. J. *et al.* Sub-diffraction-limit imaging by stochastic optical reconstruction microscopy (STORM). *Nature Methods* 9 Aug 2006 (doi:10.1038/nmeth929)