MICROSCOPY

# Magnificent myelin

**Spectral confocal reflectance microscopy helps visualize myelinated axons *in vivo* without any labeling.**

Myelin wraps tightly around axons to insulate them and to increase conductance speed. Defects in myelination can impair signal propagation along the axon and lead to nerve damage. For studying myelin *in vivo*, genetically encoded reporters or complicated microscopy techniques have so far been necessary. Jaime Grutzendler and his colleagues at Yale University recently developed a simple, label-free imaging technique based on the reflective properties of myelin. "This technique opens a way to study cortical myelin *in vivo*, to understand its development, maintenance in adulthood and different pathologies," explains Grutzendler.

The new technique, called spectral confocal reflectance microscopy (SCoRe), is simple yet powerful. Under a confocal microscope, myelinated axons reflect light when illuminated with laser beams of different wavelengths. These reflections are discontinuous but cover the whole axon when merged into a single image, and they arise at the interphase between axons and the surrounding myelin sheaths, probably because of the difference in refractive index between axons and lipid-rich myelin. Owing to the high levels of reflection even at low laser powers, the researchers could visualize myelinated axons up to 400 micrometers deep in the cortex in an anesthetized mouse.

In the sciatic nerve and spinal cord of mice, SCoRe generates colorful images of myelinated axons. For each axon, the color pattern is patchy but one particular hue dominates. Grutzendler thinks that "the hue … has something to do with the overall diameter of the axons and the number of layers of myelin that are wrapping around the axon processes. The intrinsic patchiness … is probably some local heterogeneity of the myelin." These characteristics distinguish an axon from its neighbors and can be used to identify axons at different time points, as the color pattern stays almost constant over time. Grutzendler's team found that, in contrast to the

GENOMICS

## PREDICTING ENHANCERS BY THEIR SEQUENCE

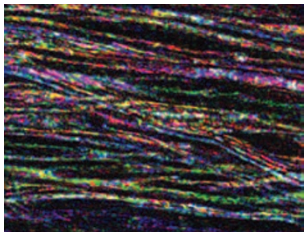
**Dinucleotide repeat motifs and transcription factor–binding sites are sufficient to define enhancers.**

The grand theme of Alexander Stark's work at the Research Institute of Molecular Pathology in Vienna, Austria, is to find out how DNA sequences encode regulatory information. "It is a passion of mine," says Stark, "to understand what a sequence needs to make an active enhancer in one cell type versus another."

When Walter Schaffner and colleagues first described enhancer elements in 1981, they were referring to DNA sequences that could activate genes. Thus, for Stark, "the term enhancer is intimately linked to DNA sequence properties." Although several epigenetic marks have been described that are often found at enhancer sites, none of these marks shows a perfect correlation. Even one of the best predictive histone modifications, an acetyl group at lysine 27 of histone 3, does not mark every enhancer and is therefore not a perfect predictor. The best predictor should be the enhancer sequence, but we do not yet know how to read or use it.

In order to find such predictive sequence features, the Stark team first needed to identify a large number of enhancers that they could then mine for motifs. The STARR-seq (self-transcribing active regulatory region sequencing) method, developed in 2013 by Cosmas Arnold in Stark's lab, allowed the researchers to detect over 10,000 active enhancers in three different *Drosophila melanogaster* cell lines.

J. Omar Yáñez-Cuna, a former PhD student in Stark's group, then computationally dissected the sequences. He found that transcription factor motifs, known to be present in enhancers, explained only a fraction of the motifs seen in active enhancers. He started to look for novel patterns and noticed that enhancers active in all three cell types contained dinucleotide repeat motifs. Intrigued as to why a feature that was so prominent in their data had not been seen before, he went back to the literature and examined raw data from previous studies of regulatory sequences—and there he saw the



Myelinated axons in the mouse spinal cord, captured with SCoRe. Figure from Schain *et al.*, Nature Publishing Group.

heterogeneity of myelinated axons in the peripheral nervous system, axons in the cortex are more homogeneous in diameter and color pattern.

When imaging the sciatic nerve *in vivo*, the researchers observed structures with a vertical reflection pattern within the myelinated axons. These structures were revealed to be Schmidt-Lanterman incisures, which are channels that are important for myelin maintenance and are rich in gap junctions. Grutzendler believes that SCoRe will be a useful technique to study myelin pathologies caused by mutations in gap-junction proteins.

Grutzendler and his team used SCoRe microscopy for several applications. In so-called shiverer mice, myelin is not compacted owing to a mutation in the gene encoding myelin basic protein; consequently, the researchers observed very little reflectance present in the cortex of these mice. In addition, they found that SCoRe could detect changes in myelin after nerve injury in the peripheral nervous system. The researchers also observed reflection from myelinated axons in fixed human brain tissue.

In the future, Grutzendler wants to use the technique to study cortical myelin, which is less well studied than subcortical myelin. He is interested in pathologies in the nervous system but also plans to address simple questions such as what the turnover of myelin is and what role neural activity has in myelin formation. SCoRe might be useful not only for myelin research but also in other areas: for example, for tracing neurons. Finally, it could be interesting to follow traumatic brain injuries in humans via SCoRe. Grutzendler concedes that "this is fairly futuristic at this point, but not completely impossible."

**Nina Vogt**

**RESEARCH PAPERS**

Schain, A.J. *et al.* Label-free *in vivo* imaging of myelinated axons in health and disease with spectral confocal reflectance microscopy. *Nat. Med.* **20**, 443–449 (2014).

same repeats. "They have been ignored," speculates Stark, "because they look repeat-like: they look like they were junk."

But for Stark's team, the statistical evidence that those repeats are real features of enhancers was too strong to ignore. They mutated the repeats in three broadly active enhancers and saw that the mutations drastically reduced enhancer activity.

Given the importance of these repeats, Stark believes that researchers should rethink how to annotate genomes. The common practice of masking, and effectively ignoring, repeats could exclude important parts of the genome.

These repeats are not exclusive to broadly active enhancers, nor are they exclusive to flies. The researchers also performed the computational analysis for enhancers that had been predicted in human cells by the presence of histone marks or DNase-hypersensitive sites and again saw that repeat motifs were overrepresented. When they mutated the motifs in HeLa cells, they observed a decrease in enhancer activity. The team concluded that the combination of repeat motifs with those for transcription factor binding was necessary for cell type–specific enhancer activity. In fact, when they added both types of motifs—or only the repeat motifs—to a nonfunctional sequence, the sequence gained enhancer activity.

The identification of a set of motifs that is sufficient to engineer an enhancer has far-reaching implications for several fields, but Stark cautions that it is still early days. "We have shown that a set of transcription factor motifs with dinucleotide repeats can be sufficient to enhance transcription, but it is still not possible to make a synthetic enhancer with a particular property," he says.

Although the goal of designing enhancers with activity in only a particular cell type or tissue that can replicate nature's intricate control of gene expression is not yet within reach, the insight of which sequence features to combine points to how this could be obtained.

**Nicole Rusk**

**RESEARCH PAPERS**

Yáñez-Cuna, J.O. *et al.* Dissection of thousands of cell type-specific enhancers identifies dinucleotide repeat motifs as general enhancer features. *Genome Res.* doi:10.1101/gr.169243.113 (8 April 2014).