GENOMICS

Seeing heterochromatin in early embryos

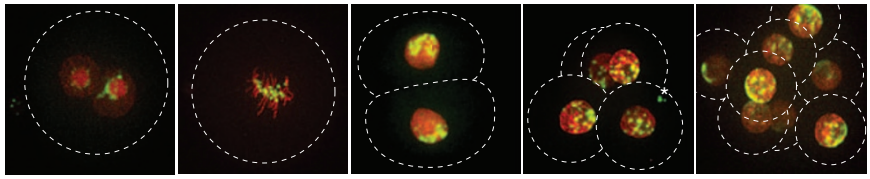
GFP targeted to satellite repeats via transcription activator–like effectors (TALEs) allows the imaging of nuclear dynamics early in mouse development.

In 1986, the developmental biologist Lewis Wolpert is reported to have said that "it is not birth, marriage or death but gastrulation, which is truly the most important time in your life." Maria-Elena Torres-Padilla from the University of Strasbourg, France, could argue that events even earlier are critical, namely those immediately after fertilization. "The formation of heterochromatin in an embryo is an integral and important part of the epigenetic reprogramming that occurs at this stage, and that is essential for development," she says.

Torres-Padilla has been studying the dynamics of heterochromatin formation in early mammalian embryos for years, relying on molecular techniques such as chromatin immunoprecipitation to profile chromatin signatures soon after fertilization. Her team, and other research groups working on this subject, found specific molecular signatures for active and inactive chromatin that are placed during early embryogenesis. What Torres-Padilla and her colleagues wanted to do next was to see and follow these changes as the embryo develops.

In 2012, Yusuke Miyanari, a postdoc with Torres-Padilla, took on the challenge of visualizing nuclear remodeling during early mouse development. He was inspired by work in which repetitive DNA had been imaged via GFP-tagged zinc-finger proteins (Lindhout et al., 2007). Instead of zinc-finger proteins, which are challenging to target specifically, Miyanari used TALEs to target a fluorescent protein to genomic repeat regions.

"Nuclear structure is drastically remodeled in the reprogramming process during mammalian early development, and we believe that this process is crucial for substantial lineage allocations," says Miyanari.



TALE-GFP (green) allows imaging of MajSat regions in mouse preimplantation embryos. Reprinted from *Nature Structural and Molecular Biology*.

Some of the regions that are remodeled into heterochromatin early on are major satellite (MajSat) repeats surrounding the centromere. The team decided to target a monomeric GFP to these regions. They optimized TALEs so that they would specifically bind to 15-nucleotide sequences in the MajSat repeats and showed in embryonic stem cells that the staining was identical to that seen using fluorescence *in situ* hybridization with short oligonucleotides in fixed cells. The researchers also showed that the MajSat repeat probes did not interfere with chromatin configuration.

The team was then ready to try their system, named TALE-mediated genome visualization (TGV), in a living organism. They microinjected RNA for TALE-GFP into mouse zygotes where the MajSat regions are still being transcribed; TGV allowed the researchers to follow the nuclear positions of these repeats from the fertilized egg through the process of heterochromatin formation all the way to the blastocyst stage. "The ability to see [heterochromatin formation] opens the door to address nuclear dynamics, localization of heterochromatin in the nucleus and to analyze how this changes as development proceeds," says Torres-Padilla.

Excitement about the method notwithstanding, Miyanari cautions people eager to adopt TGV to carefully evaluate side effects of overexpressing exogenous TALEs, as their binding could affect genome function. In fact, when he targeted TALE-mRuby to telomeric repeats in mouse embryonic stem cells, he observed shortened telomeres compared to controls. One explanation was that high levels of TALEs may affect chromatin function. He also suggests constructing several TALEs for each target sequence because not every TALE works as expected.

Extending the applications of TGV further, Torres-Padilla's team used TGV to track parental chromosomes. "Each parental genome occupies distinct nuclear spaces and displays differential epigenetic states," Miyanari explains. "This suggests a functional difference between paternal and maternal genomes in this developmental stage." The researchers designed a TALE-GFP against the MajSat region of *Mus musculus* and a TALE-mRuby against minor satellite repeats in *M. spretus*, and then expressed them in an embryonic stem cell line derived from embryos of a cross between the two mouse strains. The team could clearly distinguish each parental allele, even when they differed by only a single nucleotide per TALE target sequence.

Speaking of her team's results, Torres-Padilla highlights "the amazing ability to actually be able to see these fascinating regions in the genome as the embryo develops: they move, they pull out, they are timely localized to different nuclear spaces; and all this happens again and again and again, in all embryos."

Nicole Rusk

RESEARCH PAPERS

Lindhout, B.I. *et al.* Live cell imaging of repetitive DNA sequences via GFP-tagged ZFN proteins. *Nucleic Acids Res.* **35**, e107 (2007).

Miyanari, Y. *et al.* Live visualization of chromatin dynamics with fluorescent TALEs. *Nat. Struct. Mol. Biol.* **20**, 1321–1324 (2013).