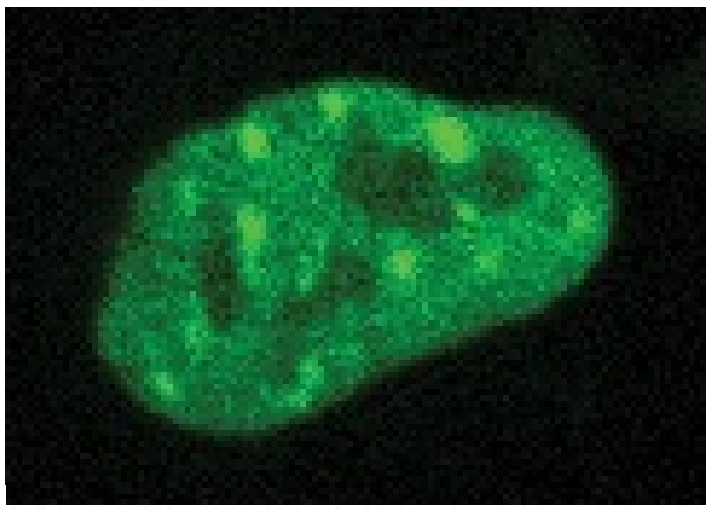
Study of Dynamics of Chromatin Binding Proteins

Using photobleaching (FRAP) to understand cellular dynamics

As imaging technologies advance, researchers are able to observe more biological events in real time with increasing spatial and temporal resolution.

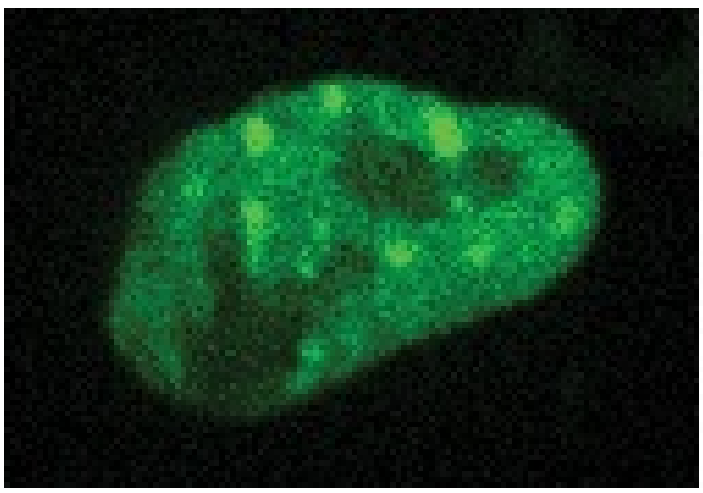
Recently, Nissim-Rafinia and Meshorer (ref. 1), from the department of genetics in the Alexander Silberman Institute of Life Sciences at the Hebrew University of Jerusalem, used Photostimulation and the technique known as FRAP (fluorescence recovery after photobleaching) to study the dynamics of chromatin binding proteins in mouse embryonic stem (ES) cells. This is of particular interest because other methods of studying chromatin binding proteins use purified chromatin from populations of cells, or from fixed cells. The ability to observe the real-time dynamics of chromatin binding proteins in single cells allows them to extract biological information that is otherwise unavailable using purified proteins.



A FRAP experiment in an embryonic stem cell nucleus expressing H1e chromatin-YFP fusion proteins.

Most chromatin binding proteins, such as HP1 (heterochromatin protein 1) and H1 (linker histone H1), are bound to DNA at any given time, but not for long. The binding is transient, such that these chromatin binding proteins associate and dissociate with DNA with a half-life in the order of seconds. This high turnover of residence on chromatin is a key factor in the ability to generate plasticity of gene expression. Furthermore, ES cells are known to have an even higher rate of chromatin binding protein exchange than differentiated cells.

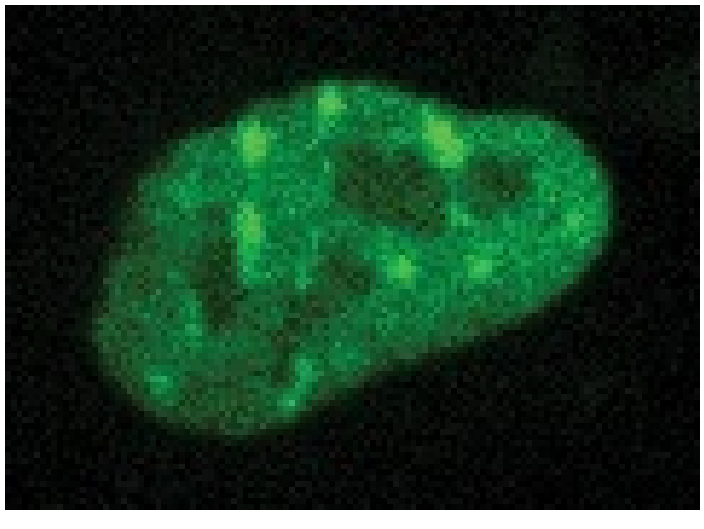
Nissim-Rafinia and Meshorer used transfected cells to express fusion proteins comprised of the DNA-binding proteins HP1 or H1 along with a fluorescent tag (such as green fluorescent protein (GFP), or yellow fluorescent protein (YFP)). To perform their FRAP experiments they took advantage of Andor Technology's FRAPPA.



The nucleus immediately after photobleaching.

FRAPPA is a photobleaching and photoactivation device which can besupplied stand alone or integrated with Andor's Revolution XD laser spinning disk confocal microscope system. Revolution XD is ideal for work with live cells providing outstanding sensitivity and low phototoxicity. FRAPPA's in-line configuration allows the researcher to use the same wavelengths for both imaging and photo-stimulation. Its switching design (switching speed is approximately 10 ms) allows it to be configured in the imaging path in either "bypass mode," where it projects an image to the detector, or in "scanning mode," where it scans a target region of the sample.

Nissim-Rafinia and Meshorer used the FRAPPA scanned laser beam (with a wavelength appropriate to the particular fluorescent tag) to photobleach the fusion proteins in user-selected regions of interest (ROIs). Immediately afterward, they used time-lapse imaging to study the time course of recovery of fluorescence in the photobleached ROI. Presumably, this recovery is due to unbleached fluorescent fusion proteins moving into the ROI. If no recovery after photobleaching was observed, then the fusion proteins (at least the unbleached ones) must have little or no mobility. If fast recovery was observed, then the fluorescent fusion proteins are likely to be very mobile within the cell. The rate of recovery allows the mobility to be calculated and add weight to the hypothesis that chromatin binding protein dynamics are involved in plasticity of gene expression.

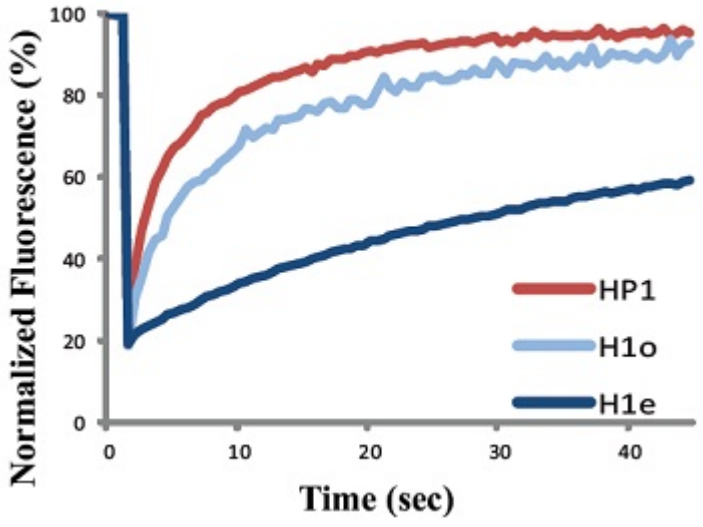


The nucleus after recovery.

The Revolution XD allowed the researchers to perform photobleaching fast enough to allow accurate measurement of the recovery half-time, while at the same time enabling highly sensitive time-lapse confocal imaging that minimized observation photobleaching. "Combining the high acquisition speed and the low phototoxicity of the spinning disk with photobleaching capacity makes FRAPPA superior to any other system," says Eran Meshorer, senior lecturer in the department of genetics at the Hebrew University of Jerusalem. "It is unique by allowing rapid acquisition, long-term imaging and photobleaching experiments."

Nissim-Rafinia and Meshorer found that both of the chromatin binding proteins they studied, HP1 and H1, have a chromatin binding half-life of a few seconds. However, they also were able to differentiate between different types of chromatin: FRAP was slower in heterochromatin than in euchromatin, indicating that chromatin binding proteins are less dynamic in heterochromatin. The authors surmise that this difference is due to a higher concentration of binding sites for HP1 and H1 in heterochromatin, along with molecular crowding. They also used a computer simulation to fit their experimental data with mathematical models. They used a single exponential equation I(t) = A(1-e-koff•t), where t is time, A is the mobile fraction of chromatin binding proteins, 1-A is the immobile fraction, and koff is the dissociation constant. The authors calculated a direct estimate of the off rate of binding (koff), as well as the parameter A, which can be used to calculate the association rate.

Meshorer says that the chromatin binding proteins move slowly relative to the fast biological events that Andor's Revolution system equipped with FRAPPA can handle. In future experiments, they look forward to studying faster events that will make full use of FRAPPA's capacity. "So far we haven't used the system in maximum acquisition speed because we concentrated on the dynamic behavior of chromatin binding proteins". Meshorer notes in regard to FRAPPA. "However, I envision that we will investigate some highly dynamic properties of the cell nucleus, which will require faster imaging after photobleaching. In addition, we will perform long time-lapse experiments during which we will introduce photobleaching experiments every so often to monitor gradual changes in the dynamic properties of proteins during cellular differentiation. This can only be achieved using such a system."



The FRAP recovery curves for different chromatin binding proteins, which reveals quantitative information about the binding dynamics of the proteins.