

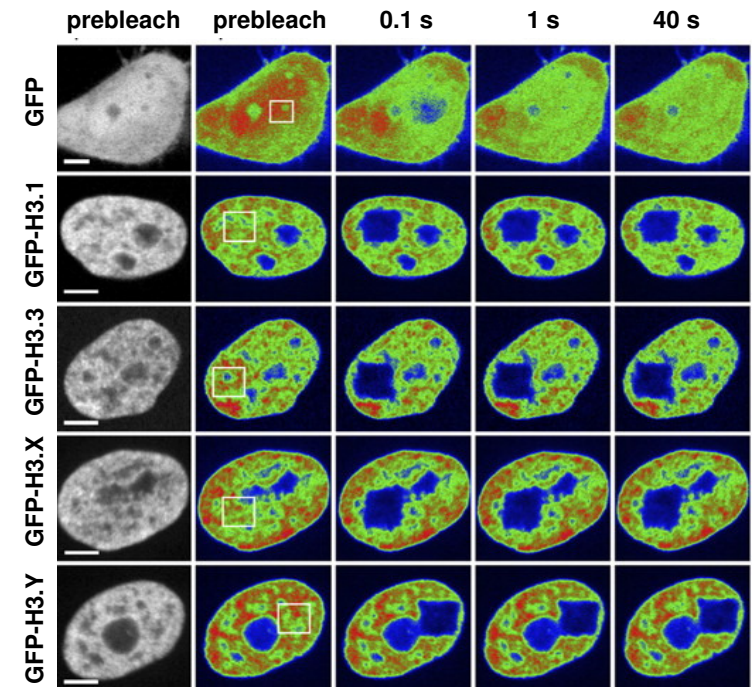
## Identification and characterization of novel histone variants: FRAP studies using the UltraVIEW VoX

Nucleosomes, the basic repeating elements of chromatin, are composed of DNA and the core histone proteins H2A, H2B, H3 and H4. In mammals, variants of the H2A, H2B and H3 families have been identified, whose inclusion results in nucleosomes with different functional and structural properties, thus allowing for diverse biological functions. It has long been thought that all mammalian variants of the histone H3 family had been identified (H3.1, H3.2, H3.3, tH3 and CENP-A), however this study describes the identification and characterization of two novel primate-specific histone H3 variants (H3.X and H3.Y).

As part of this study, researchers needed to assess whether the newly identified H3.X and H3.Y variants exhibited similar nucleosomal incorporation stability to nucleosomes containing the known variants H3.1 and H3.3. To investigate this, FRAP experiments were performed using an UltraVIEW®VoX 3D live cell imaging system with integrated FRAP PhotoKinesis accessory. Human HeLa Kyoto cells were transiently transfected with GFP-tagged histone H3 constructs and photobleaching experiments were performed (figure).

Several square bleach regions with a size of 5 x 5 µm were positioned on selected cell nuclei within the field of view. Photobleaching was performed using two iterations with the acousto-optical tunable filter of the 488 nm and the 514 nm laser line set to 100 % transmission. To determine long-term recovery kinetics, 3D image stacks of 8 µm height and a Z distance between image planes of 1 µm were recorded with an initial speed of 1 frame/min for the first 10 frames, followed by intervals of 10 min. The FRAP experiments demonstrated similar dissociation kinetics for the novel GFP-tagged H3 variants compared with GFP-H3.1 and GFP-H3.3. The GFP fluorescence recovery rates of all H3 variants were very slow in comparison to GFP alone, indicating that all expressed fusion proteins were stably incorporated into nucleosomes.

These observations supported the conclusion that H3.X and H3.Y are novel variants of H3. Additional experiments also led the researchers to conclude that H3.Y is involved in the regulation of cellular responses to extracellular stimuli.



**FRAP experiments to evaluate nucleosomal stability of novel H3 variants.** A small nuclear area was photobleached (box) and the recovery of the fluorescent signal was monitored. Depicted is a short-term FRAP series (selected time points are shown) of GFP-tagged H3 variants compared with GFP alone. Scale bar 5 µm.