

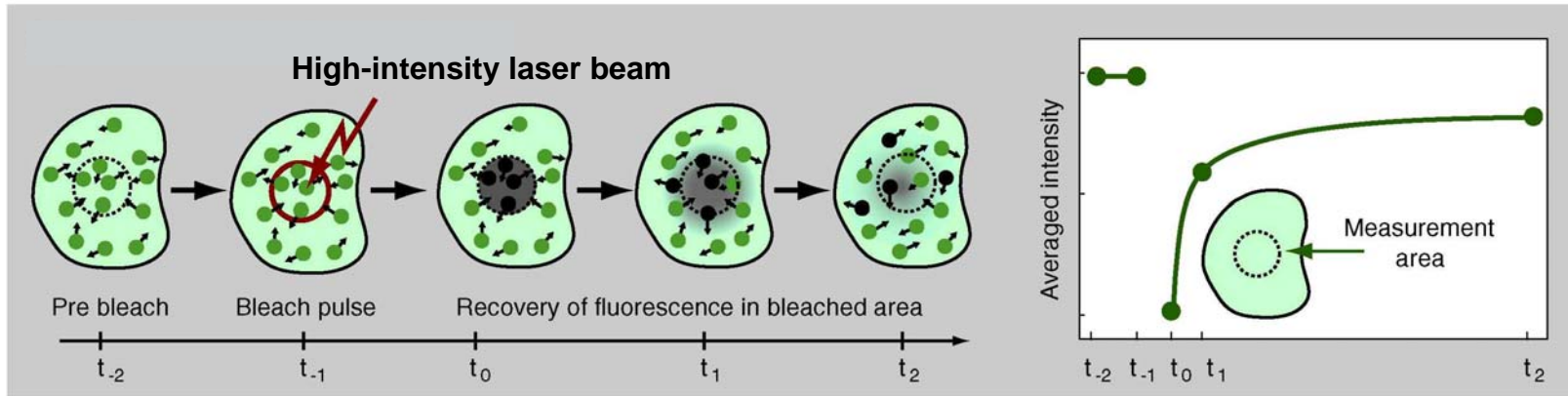
Dynamics of chromatin-related proteins measured by FRAP technique

**Institute of Biophysics, AS CR, v.v.i., Brno
Department of Molecular Cytology and Cytometry**

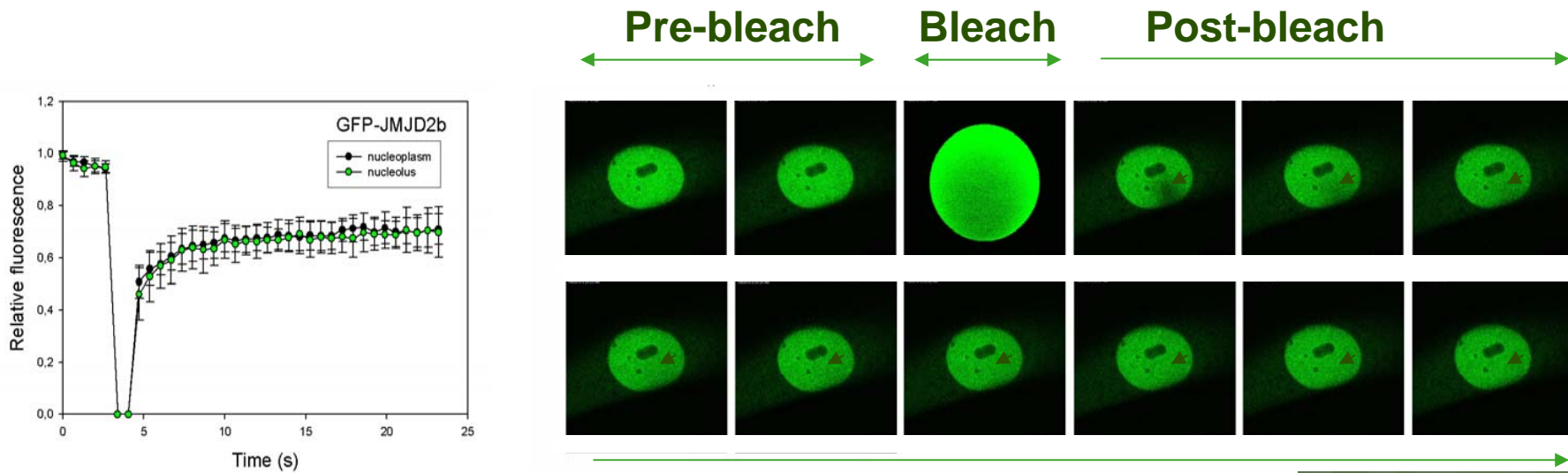
Lenka Stixová



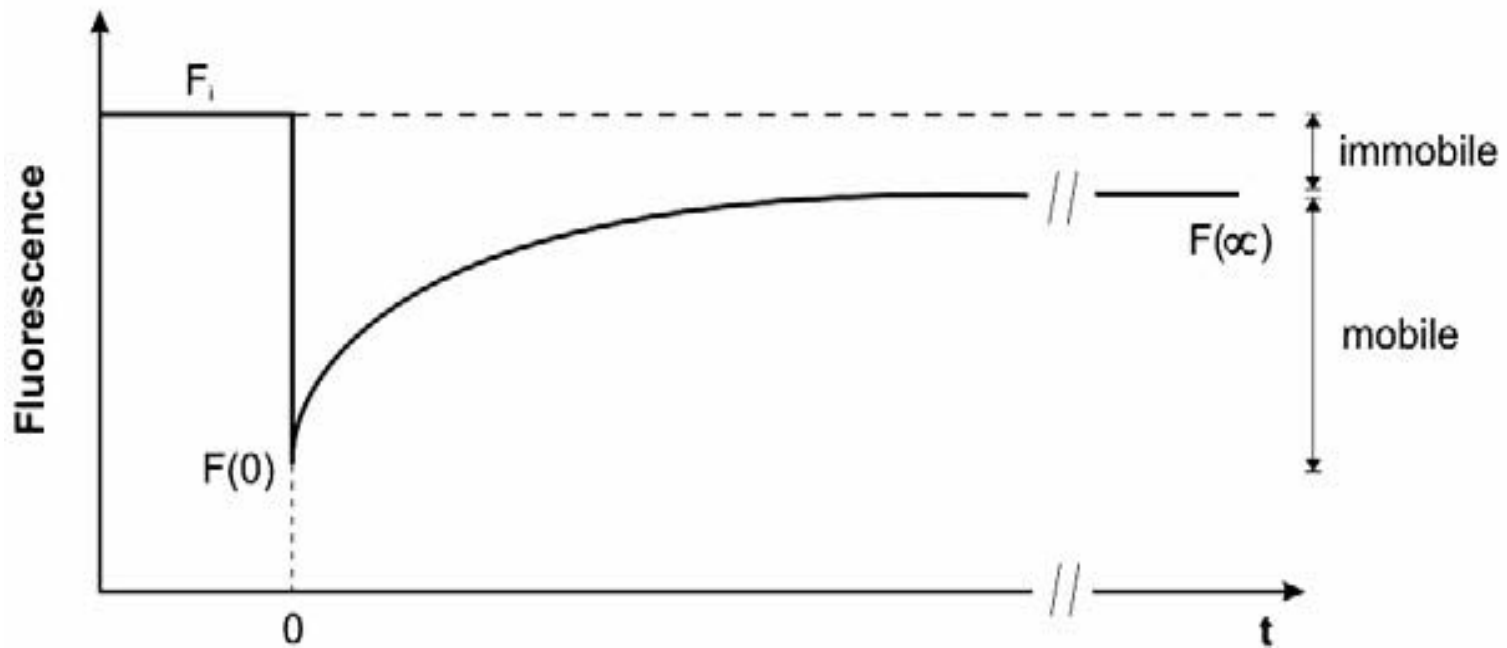
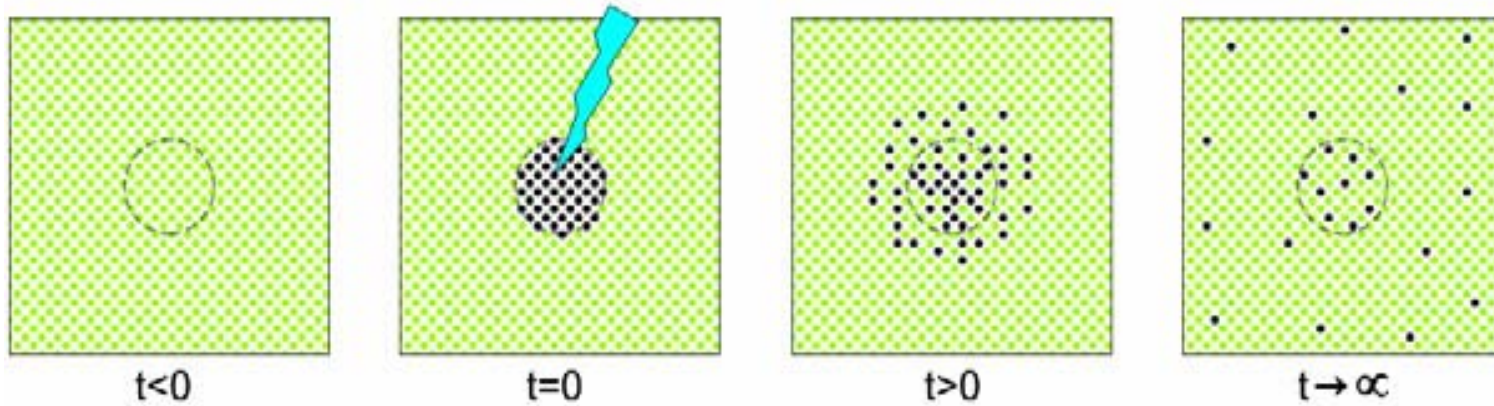
Fluorescence recovery after photobleaching (FRAP)

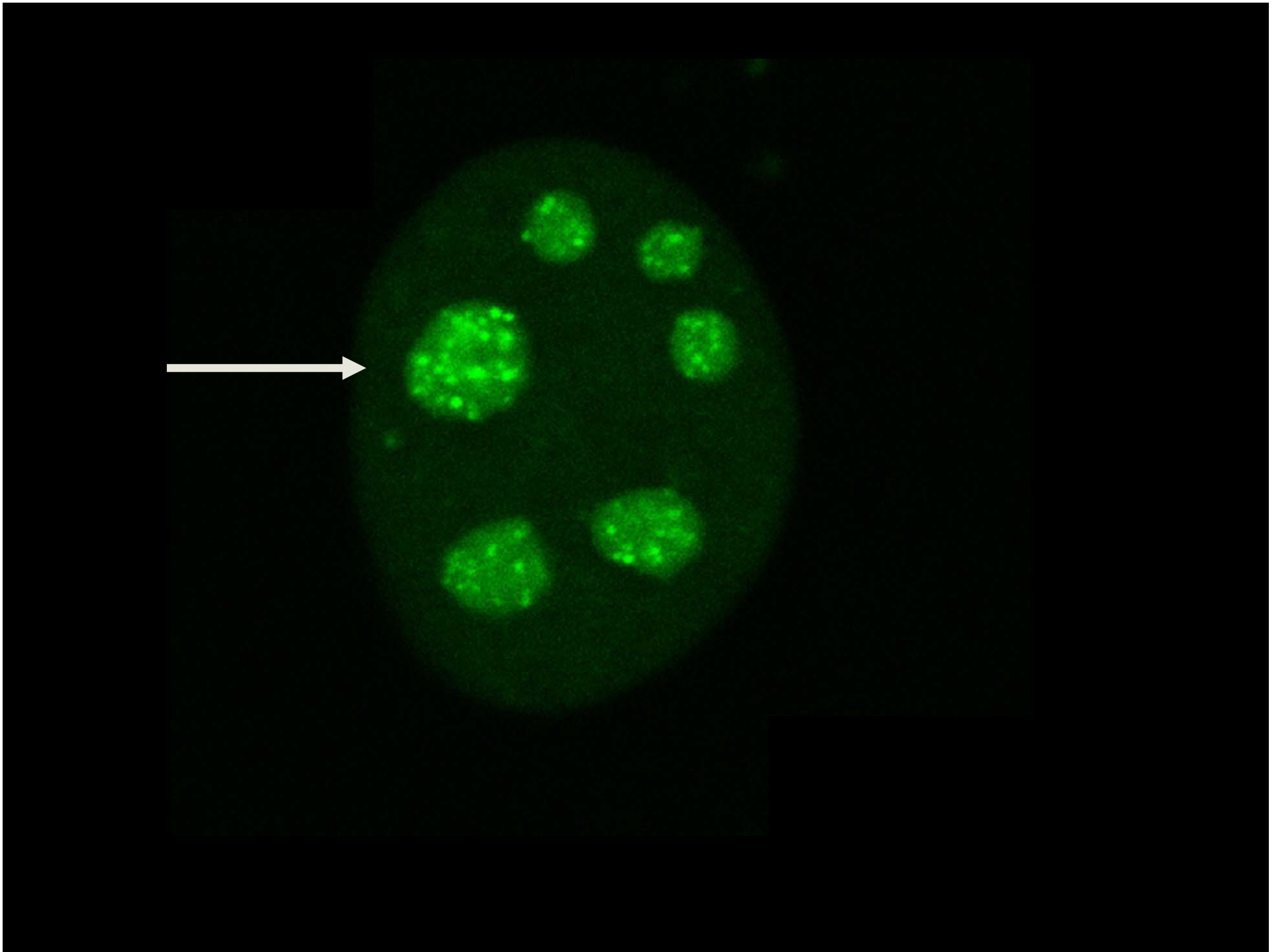


Mueller et al., Current Opinion in Cell Biology, 2010

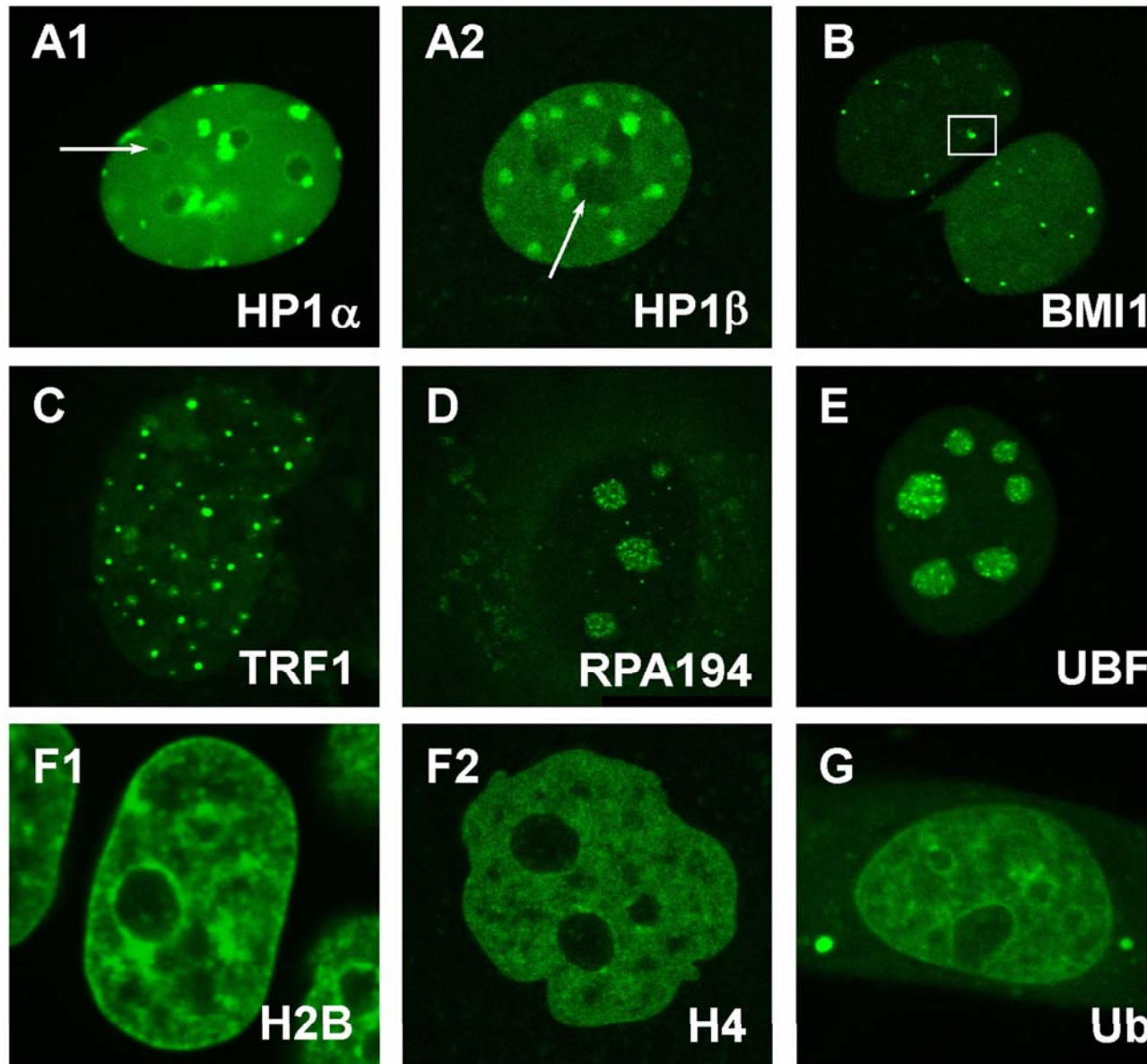


Fluorescence recovery after photobleaching (FRAP)

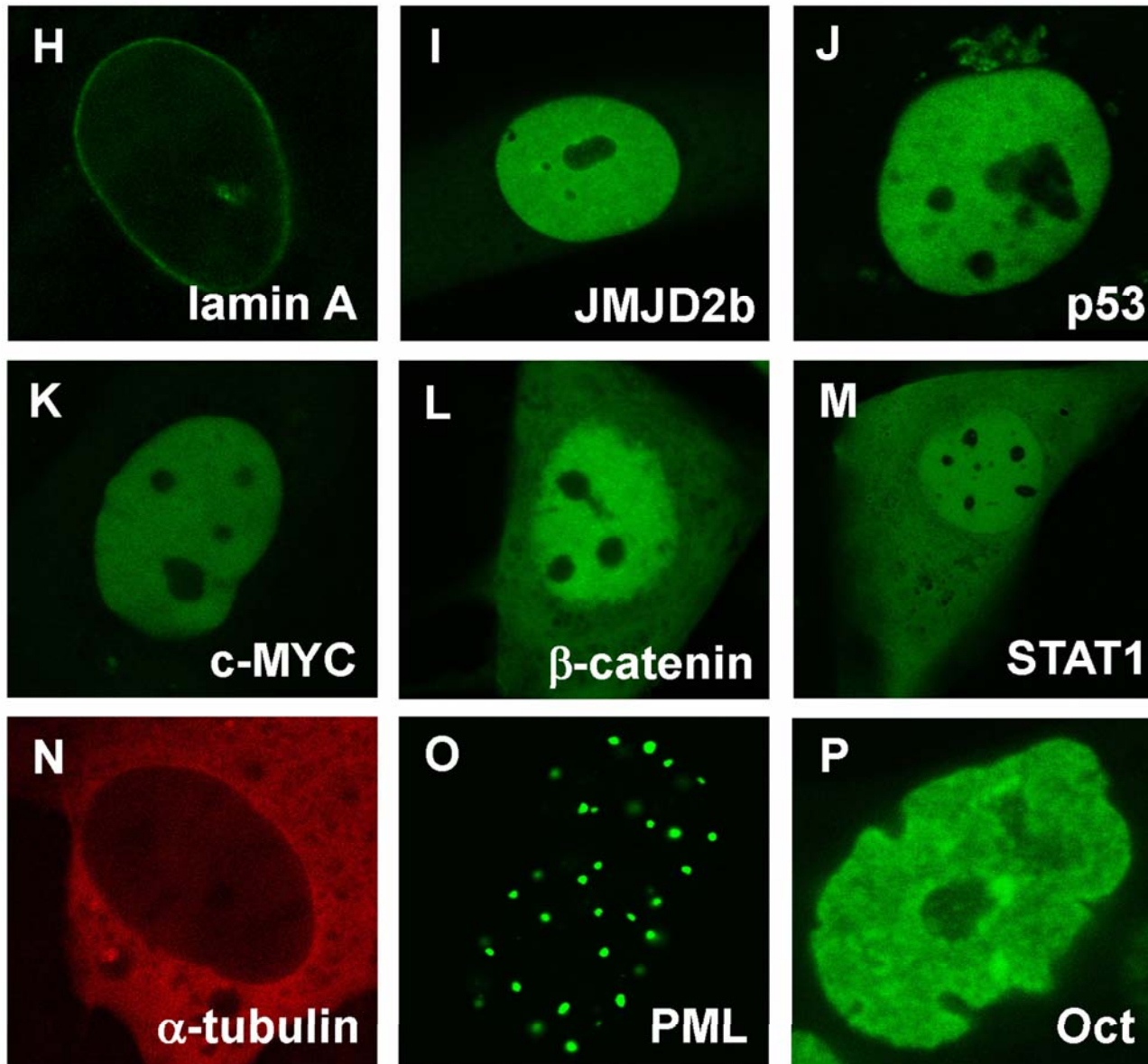




Cellular patterns of proteins studied

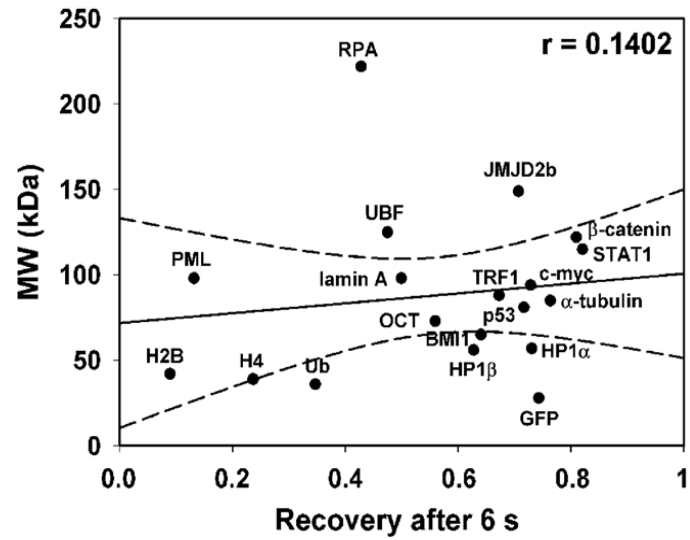


Cellular patterns of proteins studied



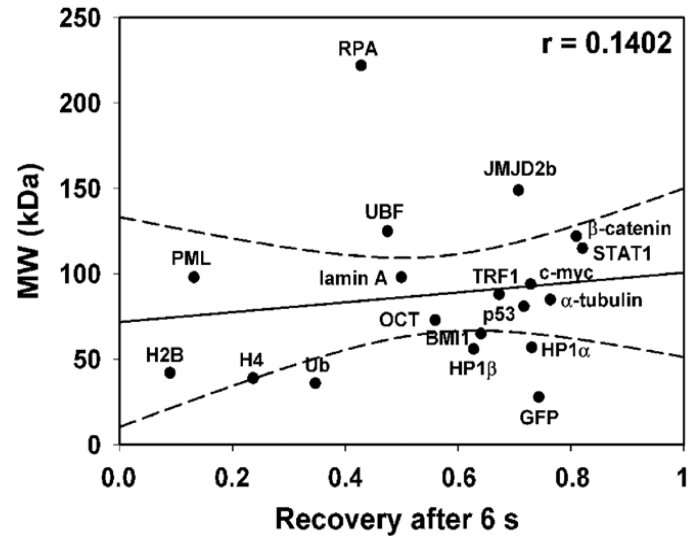
Correlation between molecular weight and kinetics of chromatin-related proteins

All measured proteins

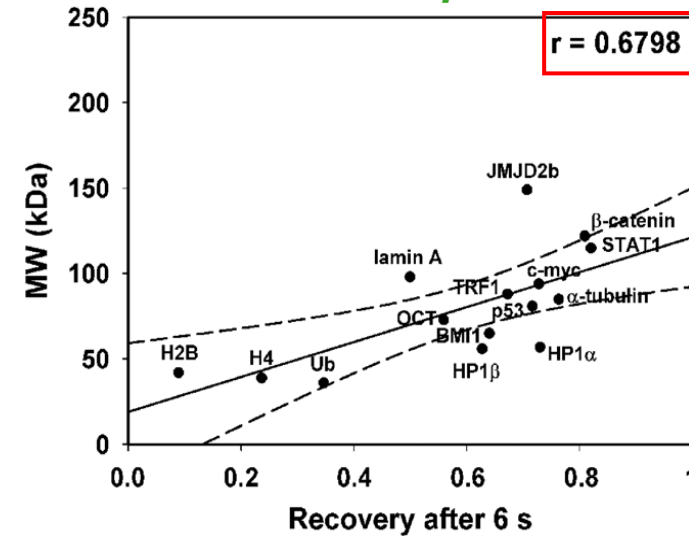


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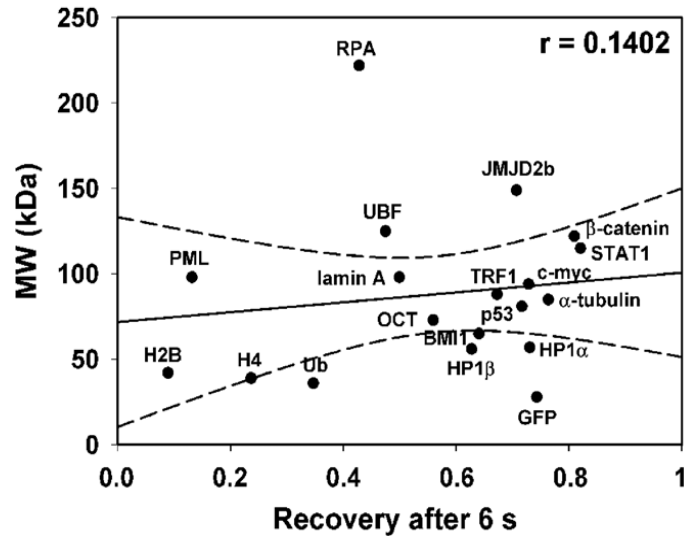


Proteins evenly dispersed throughout the nucleoplasm

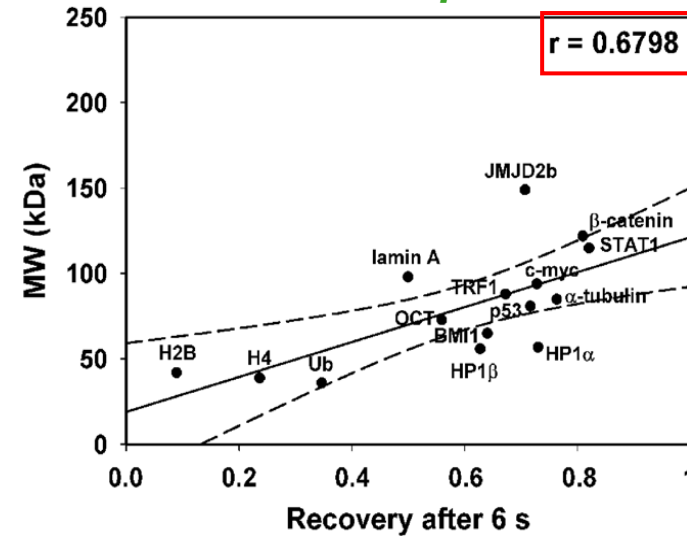


Correlation between molecular weight and kinetics of chromatin-related proteins

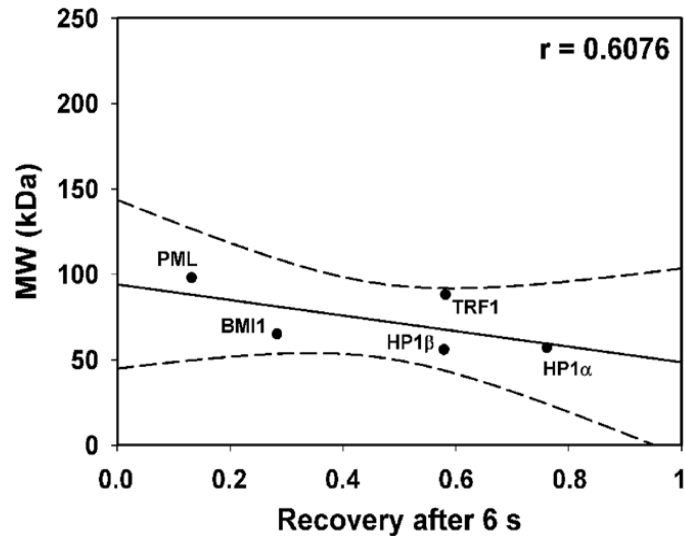
All measured proteins



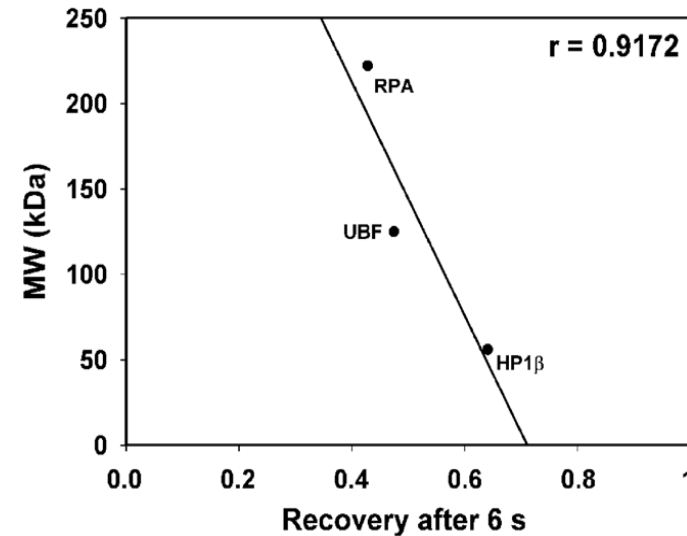
Proteins evenly dispersed throughout the nucleoplasm



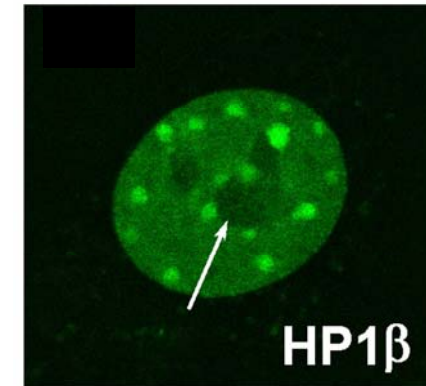
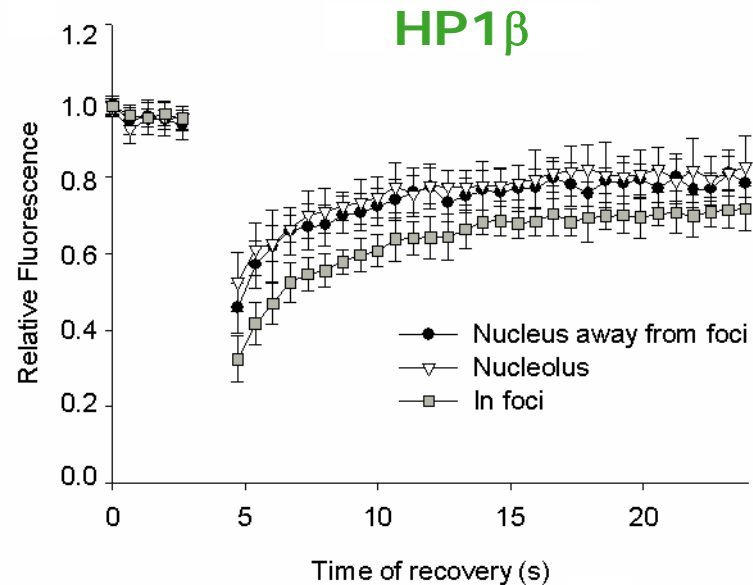
Proteins accumulated into foci



Proteins accumulated into nucleoli



Recovery of fluorescence after photobleaching of HP1 β protein with HDAC inhibition and suppression of transcription by actinomycin D



- In centromeric clusters
- In nucleoli (Harničarová Horáková et al., 2010)

Protein density and affiliation with heterochromatin or euchromatin influence protein mobility.

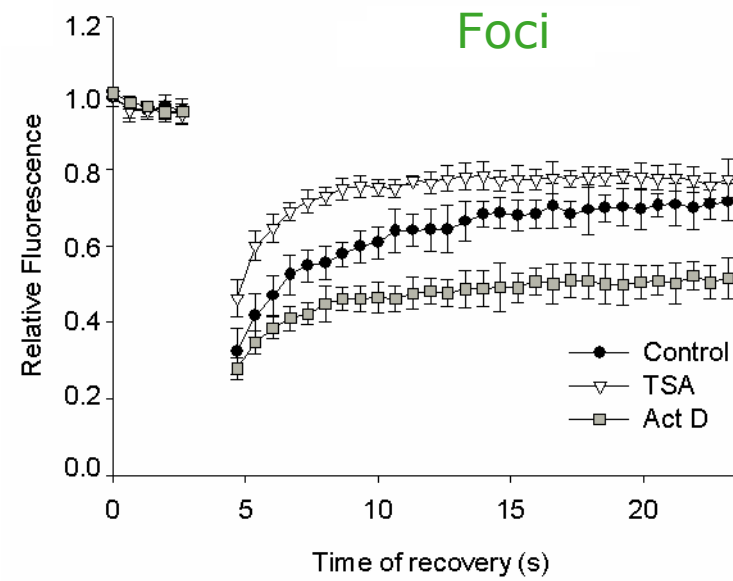
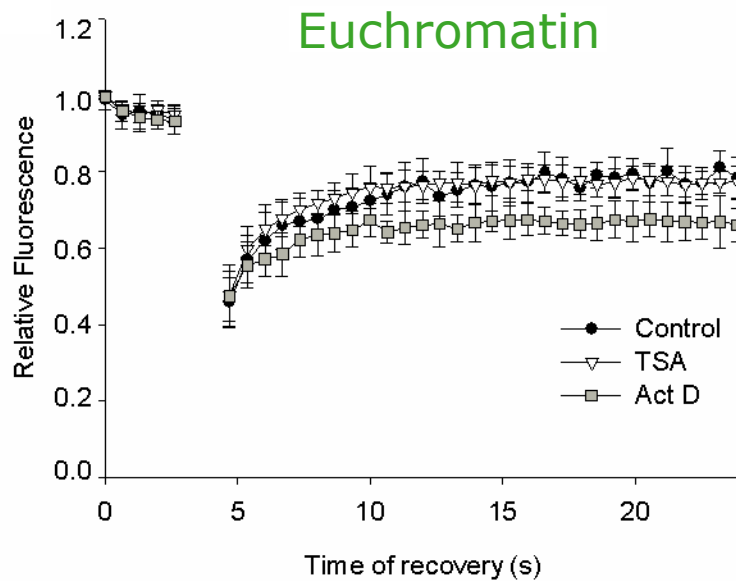
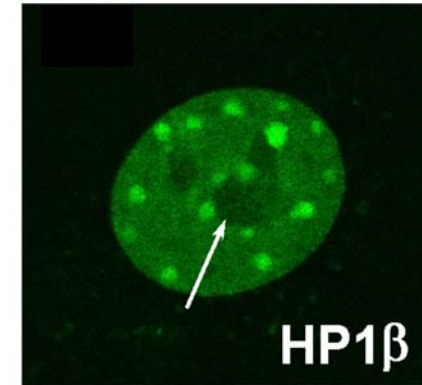
Recovery of fluorescence after photobleaching of HP1 β protein with HDAC inhibition and suppression of transcription by actinomycin D

HP1 β

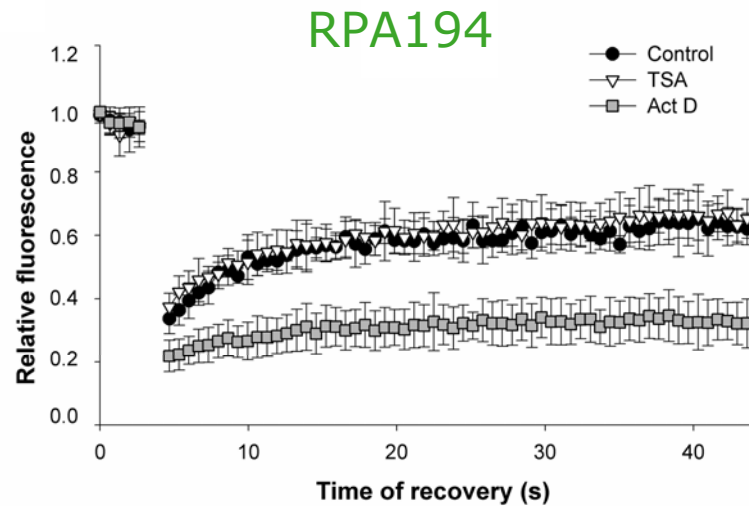
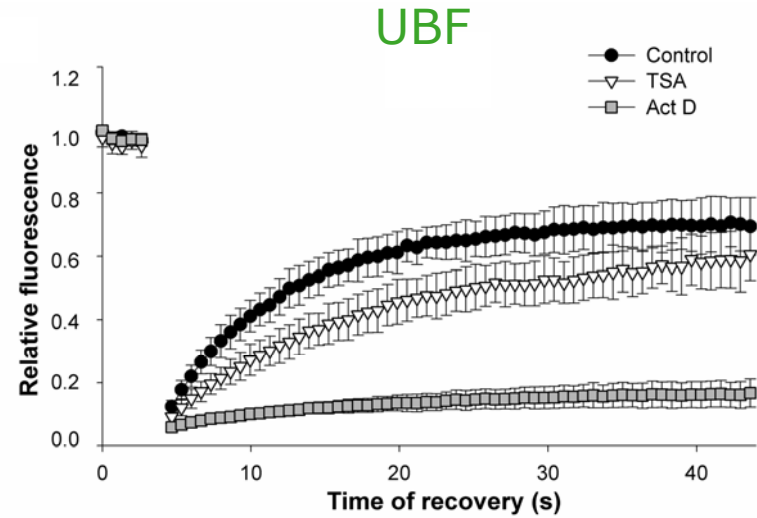
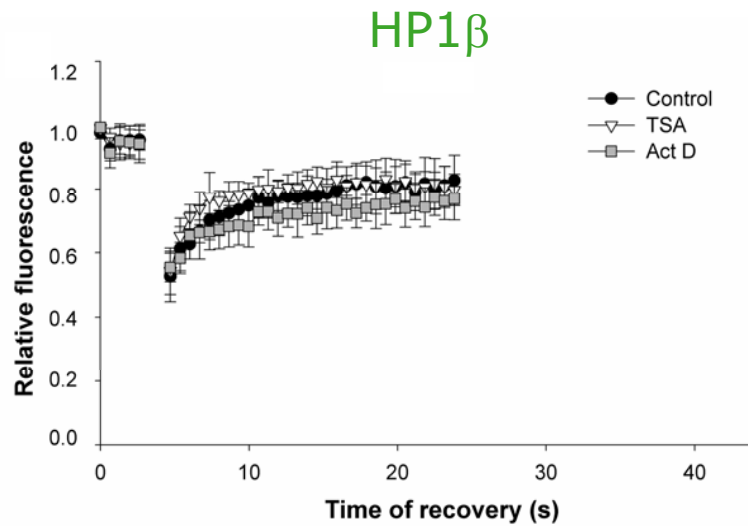
TSA – inhibitor of histone acetylases
→ induction of transcription

×

Actinomycin D - inhibition of transcription



Kinetic modeling of HP1 β , RPA194, and UBF dynamics with modulated transcription levels



Conclusions

- We showed that protein dynamics are influenced by many factors and events, including nuclear pattern and transcription activity.
- A slower recovery after photobleaching was found when proteins, such as HP1 β and others accumulated at specific foci. In identical cells, proteins that were evenly dispersed throughout the nucleoplasm recovered more rapidly.





Thank you!