Linker histone H1 functions as a liquid-like glue to organize chromatin

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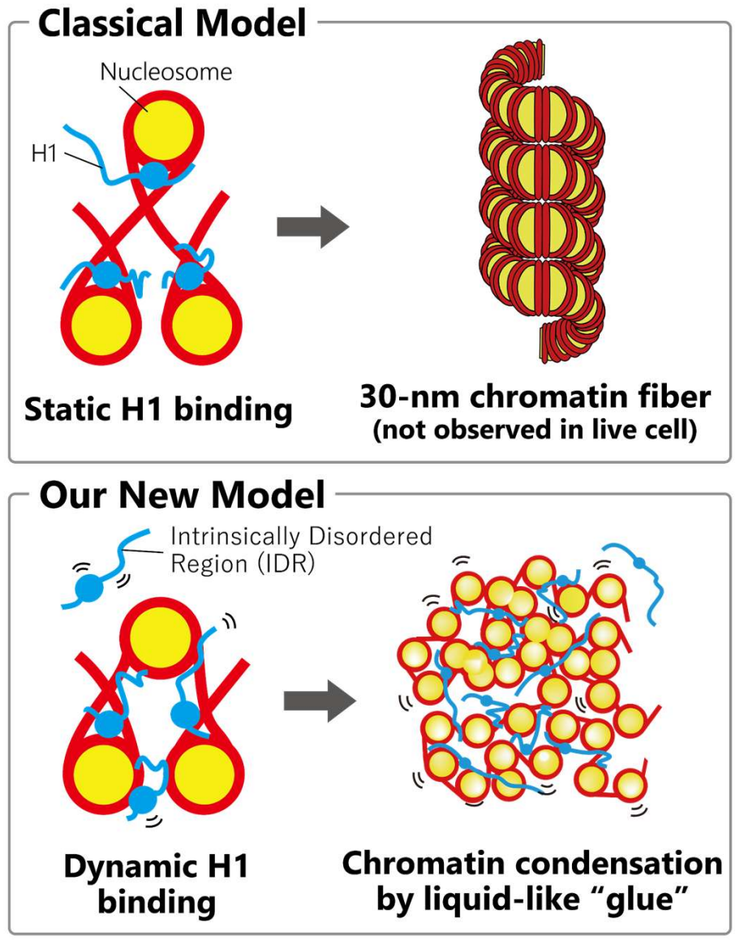
Abstract

- Linker histone H1 makes chromatin condensed.
- H1 helps 30-nm chromatin fiber formation *in vitro*.
  (J. Allan et al. *Nature* (1980))
- *In vivo*, chromatin folds into irregular "Chromatin domain", but not 30-nm chromatin fiber.
  (T. Nozaki et al. *Molecular Cell* (2017))
- H1 has dynamic interaction with chromatin in living higher eukaryotic cells.
  (T. Misteli et al. *Nature* (2000))

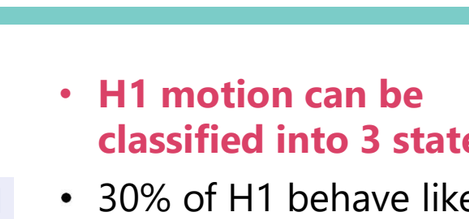
An alternative model for chromatin condensation by H1 is needed.



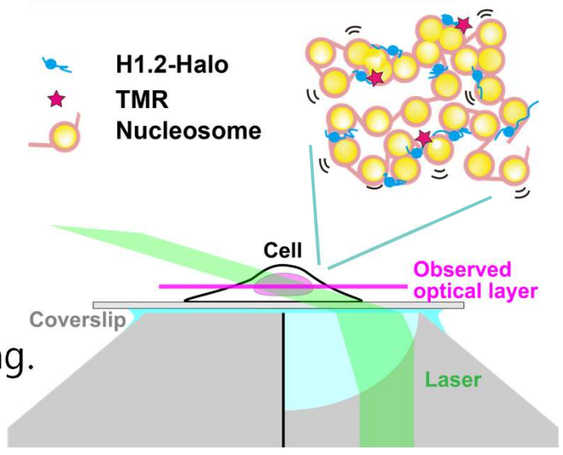
Method: Single molecule imaging of H1

- Human RPE-1 cells are used.
- Linker histone H1.2 is a major and replication-dependent subtype.
- By CRISPR/Cas9 system, HaloTag is inserted into the C-term of endogenous H1.2 with 3x GGGGS linker.
- Sparse labeling by HaloTag ligands and oblique illumination enables single-molecule imaging.
- TrackMate is used for tracking.
  (J. Tinevez *Methods* (2017))

Design of end-tagging

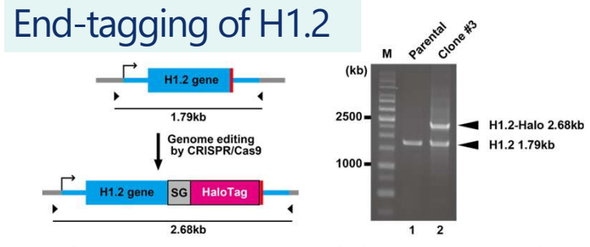


Sparse labeling & Oblique illumination



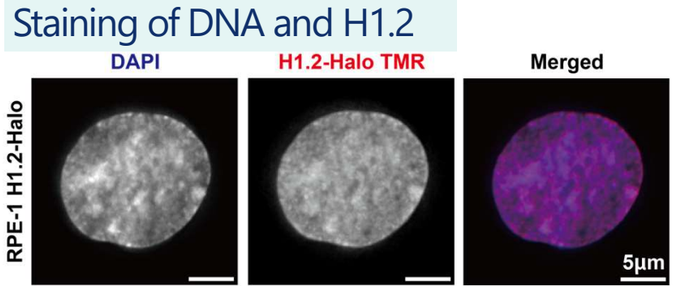
Result 1: Motion of H1

End-tagging of H1.2



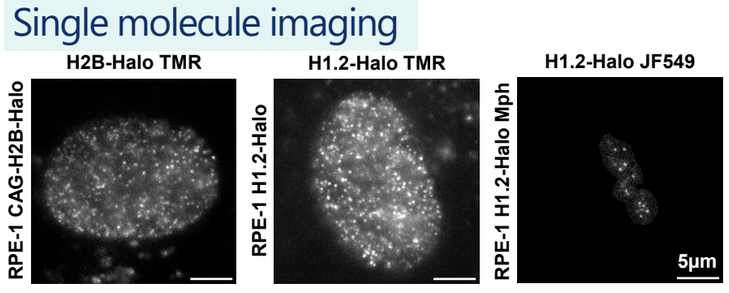
- One of the end-H1.2 is tagged with HaloTag.

Staining of DNA and H1.2

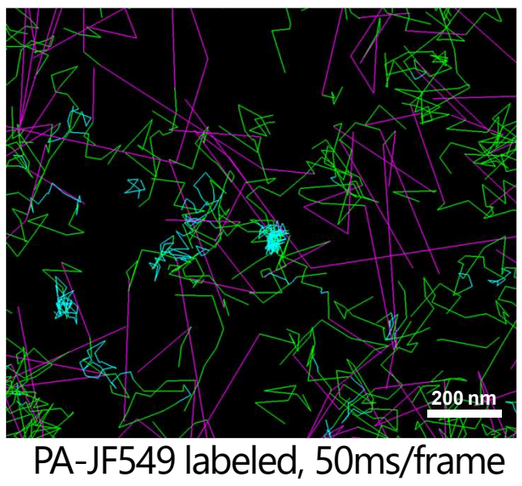


- H1.2-Halo localize similarly to DNA.

Single molecule imaging

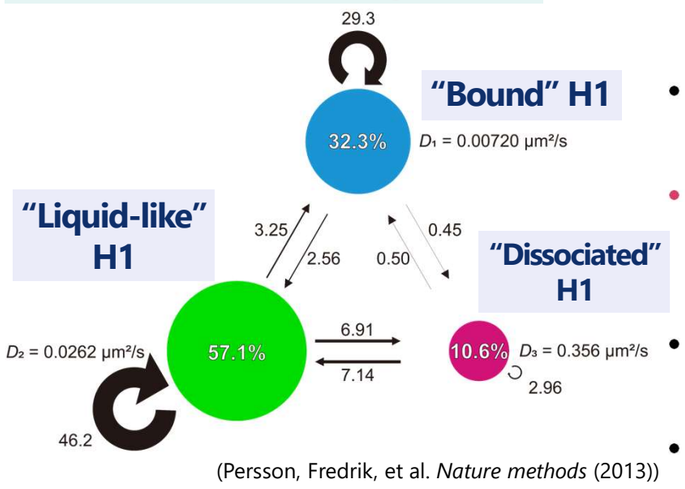


Trajectories of H1



PA-JF549 labeled, 50ms/frame

Motion classification by vbSPT

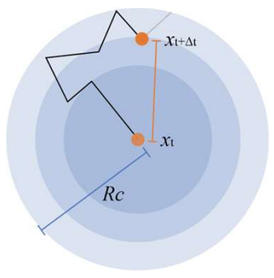


(Persson, Fredrik, et al. *Nature methods* (2013))

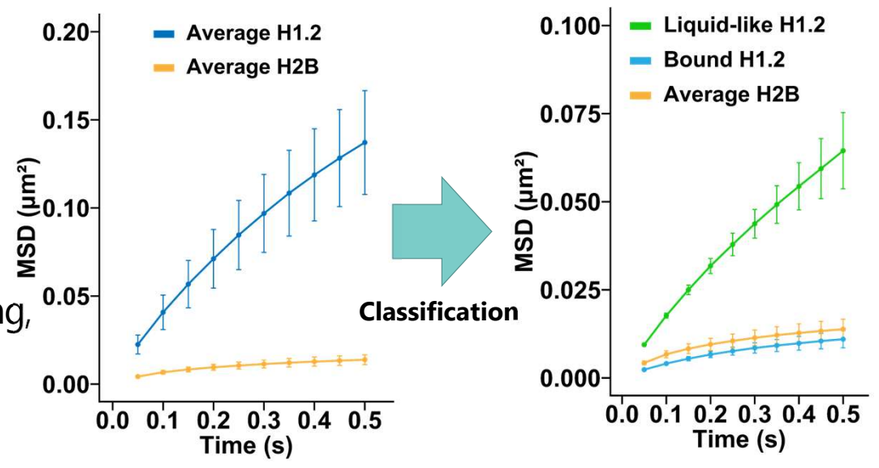
- **H1 motion can be classified into 3 states.**
- 30% of H1 behave like nucleosomes ("Bound").
- **Major H1 shows dynamic behavior than nucleosome.**
- 60% of H1 behave like liquid (linear MSD plot).
- H1 rarely shows "Jumps".

MSD plot of H1 and H2B

$$MSD \equiv \langle |x_t - x_{t+\Delta t}|^2 \rangle$$

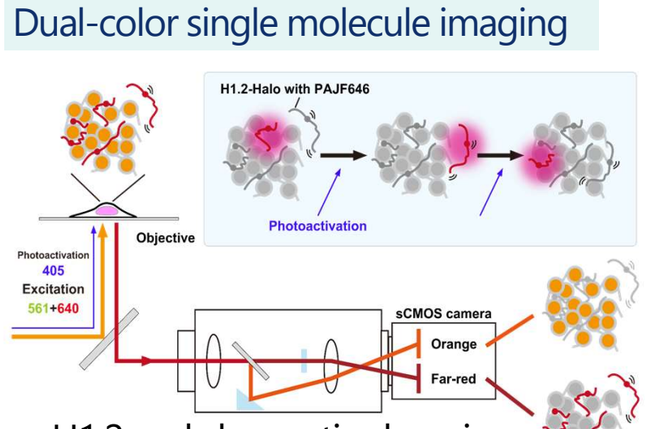


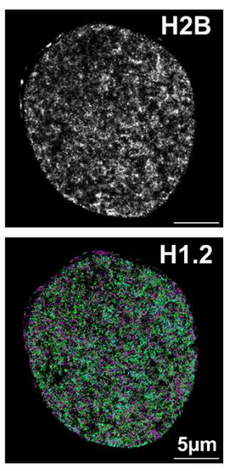
- Mean Square Displacement (MSD) indicates how much molecules explored the area during Δt.
- If molecules are freely diffusing, that result in a linear increase in the MSD plot.



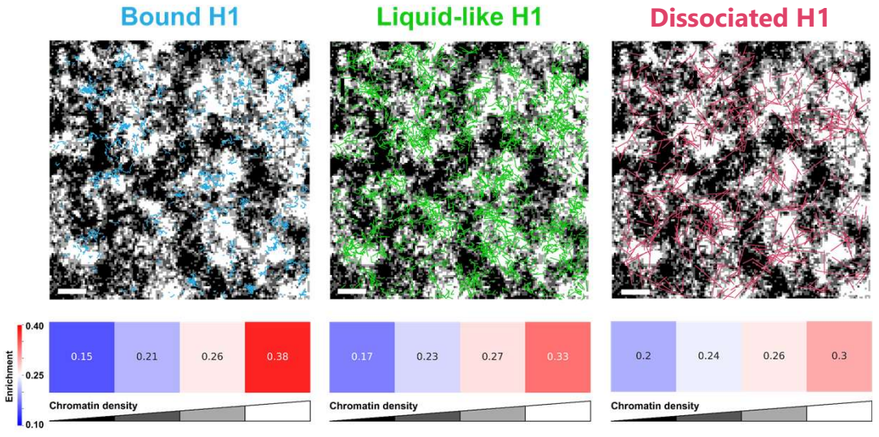
Result 2: Liquid-like H1 diffuse within chromatin domains

Dual-color single molecule imaging





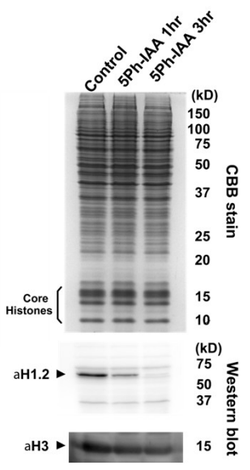
- H1.2 and chromatin domains are observed in the same cell.

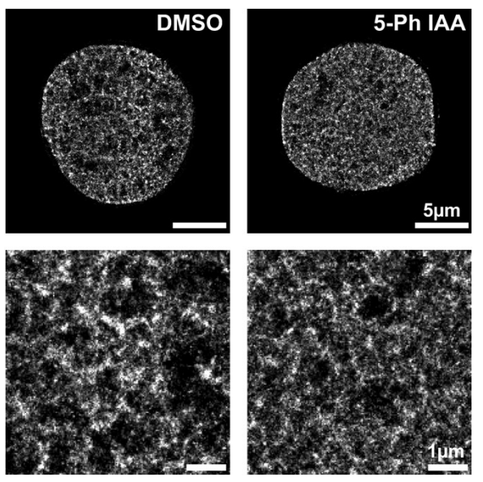


Result 3: H1 depletion decondenses chromatin

PALM imaging of chromatin domain with rapid depletion of H1.2

- With AID2* system, mAID-tagged end-H1.2 can be rapidly (~3hrs) depleted by addition of 5-Ph IAA.
  (*A. Yesbolatova et al. *Nat. Comun.* (2020))
- Chromatin domain structure get decondensed by H1.2 depletion.

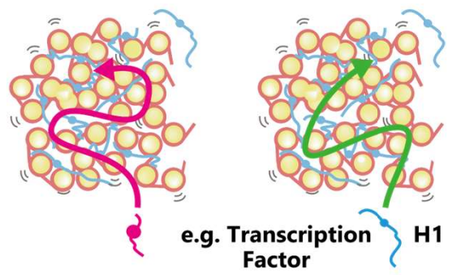




Conclusion & Discussion

"Bound" H1	30%	Nucleosome-bound H1 "Dyad" binding of H1
"Liquid-like" H1	60%	Dynamic binding on nucleosomes **Seems to be Liquid-like "glue"**
"Dissociated" H1	10%	Temporally dissociated Immediately back to "Liquid"

Major fraction of H1 moves like liquid within chromatin domains, while interact with several nucleosomes.



H1 as Liquid-like "glue" can make chromatin compact and accessible at the same time!!!

Acknowledgement

Masaki Sasai, S. S. Ashwin, Kana Sato, Kayo Hibino, Hiroko Ochi, Katsuhiko Minami, Shiori Iida, Adilgazy Semeigazin, Aoi Otsuka, Jason Hernandez, Yu Nagata, and Kako Nakazato