Drosophila H1 Regulates the Genetic Activity of Heterochromatin by Recruitment of Su(var)3-9
Xingwu Lu et al.
Science 340, 78 (2013);
DOI: 10.1126/science.1234654

This copy is for your personal, non-commercial use only.
involves a series of crucial Arg residues. How-
version by SelA (depicted in Fig. 4 and ex-
Ser-tRNASer. Moreover, the large cleft created
reduced the activity in vivo (table S1), Asn218A
and J (Fig. 3, B and C). Notably, the interaction
pocket at the interface between the subunits A
Ala mutations of Asn218 and Phe224 drastically
formed by Arg86A (subunit A), Arg312B, a n d
and Phe224J might form part of the A76-binding
Fig. S8A), whereas the N-terminal domain
acceptor arm toward the catalytic site. Although
acceptor stem to the Ser-adenosine moiety of Ser-
S6C). Therefore, the large cleft can accommodate
binds to the linker DNA between nucleosomes
core histones, H2A, H2B, H3, and H4. Chroma-
base pairs of DNA wrapped around an octamer of
N66001-12-C-4020 and N66001-12-C-4211). The plasmids
for protein expression and tRNA transcription are available
in 2006). 2. J. E. Cone, R. M. Del Río, J. N. Davis, T. C. Stadtman,

References and Notes
2. J. E. Cone, R. M. Del Río, J. N. Davis, T. C. Stadtman,
3. A. Böck, M. Thanbichler, M. Rother, A. Resch, in Aminoacyl-tRNA
Synthetases, M. Iiba, C. S. Franklyn, S. Cusan, Eds. (Landes Bioscience,
Georgetown, TX, 2005), pp. 320–327.
325, 321 (2009).
9. A. Schön, A. Böck, G. Ott, M. Spiridal, D. Söll, Nucleic
10. Y. Itoh, S. Chiba, S. Sekine, S. Yokoyama, Nucleic Acids
Res. 37, 6259 (2009).
12. H. Engelhardt, K. Forchhammer, S. Müller, K. N. Goldie,

263, 1404 (1994).

Acknowledgments: Y.I. was supported by research fellowships
from the Japan Society for the Promotion of Science (JSPS).
M.B. holds a Feodor Lynen Postdoctoral Fellowship of the
Alexander von Humboldt Foundation (Bonn, Germany).
We thank the staff members of SPring-8 BL4XU and the Photon
Factory beam line for assistance with our data collection,
A. Ishii and T. Nakayama for assistance in the manuscript
preparation, and M. Simonovic (University of Illinois, Chicago)
for critical discussions. We are grateful to the National
Bioresource Project in Japan for providing the ASKA E. coli
K22 ORF library. This work was supported in part by JSPS
Grants-in-Aid for Scientific Research (A) to S.Y. and (C) to
S.S. and the Targeted Proteins Research Program of the
Ministry of Education, Culture, Sports, Science and Technology
(to S.Y.). D.S. acknowledges support from the Division of
Chemical Sciences, Geosciences, and BioSciences, Office of
Basic Energy Sciences of the U.S. Department of Energy
(DE-FG02-98ER201311) for funding the genetic experiments;
The National Institute of General Medical Sciences (GM022854);
and Defense Advanced Research Projects Agency contracts
N66001-12-C-4020 and N66001-12-C-4211. The plasmids
for protein expression and RNA transcription are available
from S.Y. under a material transfer agreement with RIKEN.
The atomic coordinates and structure factors have been
deposited in the Protein Data Bank (PDB IDs 3W1H, 3W1I,
3W1J, and 3W1K).

Supplemental Materials
www.sciencemag.org/cgi/content/full/340/6128/75/DC1
Materials and Methods
Figs. S1 to S9
Tables S1 and S2
References (15–28)
30 August 2012; accepted 28 January 2013
10.1126/science.1229521

Drosophila H1 Regulates the Genetic Activity of Heterochromatin by
Recruitment of Su(var)3-9

Xingwu Lu,1 Sandeep N. Wontakal,1 Harsh Kavi,2 Byung Ju Kim,2 Paloma M. Guzzardo,2
Alexander V. Emelyanov,3 Na Xu,2 Gregory J. Hannon,2 Jiri Zavadil,4 Dmitry V. Fyodorov,2,* Arthur I. Skoultchi2,*

Eukaryotic genomes harbor transposable elements and other repetitive sequences that must be silenced. Small RNA interference pathways play a major role in their repression. Here, we reveal another mechanism for silencing in these sequences in Drosophila. Depleting the linker histone H1 in vivo leads to strong activation of these elements. H1-mediated silencing occurs in combination with the heterochromatin-specific histone H3 lysine 9 methyltransferase Su(var)3-9. H1 physically interacts with Su(var)3-9 and recruits it to chromatin in vitro, which promotes H3 methylation. We propose that H1 plays a key role in silencing by tethering Su(var)3-9 to heterochromatin. The tethering function of H1 adds to its established role as a regulator of chromatin compaction and accessibility.

ukaryotic genomes are packaged into chromatin, which is composed of highly conserved repetitive units referred to as nucleosomes. The nucleosome consists of ~145 base pairs of DNA wrapped around an octamer of core histones, H2A, H2B, H3, and H4. Chromatin also contains the linker histone H1, which binds to the linker DNA between nucleosomes and facilitates folding of nucleosome arrays into more compact structures (1). Chromatin is organized into regions of euchromatin and more densely packed heterochromatin, which is generally silenced. The mechanisms leading to heterochromatic silencing are not well understood (2, 3).

Depleting H1 in Drosophila by RNA interference (RNAi) leads to marked disruption of salivary gland (SG) polytene chromosome structure, including pericentric heterochromatin, and a decrease in nucleosome spacing (4). We compared the RNA expression profiles of SGs depleted of H1 and control Nauitis (Nau) RNAi SGs. We found only a modest difference in the mRNA profile, with only 2174 (11.5%) protein-coding genes showing a change of twofold or more (P <
proximal heterochromatin-euchromatin transition zones (12). We hypothesized that H1 may silence TEs through its ability to regulate the activity of heterochromatin, with TEs responding differently to H1 depletion depending upon their insertion site. Stellate (Ste) (13) exhibits several features similar to TEs, with multiple tandem copies at two distinct loci, one euchromatic (Eu Ste) and the other in pericentric heterochromatin (Het Ste) (14). Ste expression is regulated by Su(Ste) encoding piRNAs that silence Ste (14, 15). Heterochromatic and euchromatic copies of Ste exhibit single-nucleotide polymorphisms that allow discrimination between transcripts originating from either locus. Depletion of H1 strongly up-regulates only Het Ste transcripts, whereas Eu Ste transcripts are not substantially affected (Fig. 1D). Although transcripts from both loci are negatively regulated by Su(Ste)-derived small RNAs, H1 specifically silences Het Ste, presumably through its role in regulation of heterochromatin function.

H1 depletion causes a reduction in dimethylation of histone H3 lysine 9 (H3K9Me2) (4). Quantitative chromatin immunoprecipitation (QChIP) in H1-depleted and control larvae at the regulatory regions of several TEs—including copia, gypsy, and ZAM—and at Het Ste, revealed a marked decline in the presence of H3K9Me2 accompanying the loss of H1 (Fig. 2, A and B). Although H1 depletion also leads to reduced H3K9Me2 at Eu Ste and other euchromatic loci like yellow (Fig. 2B), other heterochromatic loci are derepressed (Fig. 1, B and D), consistent with the existence of additional, H3 methylation–independent silencing mechanisms outside of heterochromatin. H3K9Me2 modification is catalyzed primarily by the histone methyltransferase (HMT) Su(var)3-9 (16). Su(var)3-9 null mutation also leads to strong...
and presented as in (A). (UAS:Su(var)3-9 RNA was prepared from SGs from control, H1-depleted, and H1-depleted may function in concert. Although H3K9Me2 is (Fig. 2C), which suggests that H1 and Su(var)3-9 severely reduced in (36x260)up-regulation of TEs and Het Ste but not Eu Ste (Fig. 2C), which suggests that H1 and Su(var)3-9 may function in concert. Although H3K9Me2 is (36x250)chromatin was measured by qCh IP. (36x260)and wild-type SGs. The data were analyzed as in Fig. 1B. (D) RNA was prepared from SGs from control, H1-depleted, and H1-depleted UAS:Su(var)3-9-eGFP larvae. QRT-PCR assays were performed as in (C). Black bars, H1-depleted SGs; gray bars, H1-depleted UAS:Su(var)3-9-eGFP SGs. (E) SGs from control (top), H1-depleted (middle), and H1-depleted UAS:Su(var)3-9–eGFP (bottom) larvae were dissected, and polytene spreads were stained with 4’,6’-diamidino-2-phenylindole (DAPI) and the indicated antibodies.

**Fig. 2.** H1 represses repetitive elements in conjunction with Su(var)3-9. (A) The occupancy of H1 in larval chromatin was measured by qChIP. The ordinate indicates the amounts of qChIP DNA samples relative to input DNA. All experiments were performed in triplicate. Error bars, standard deviation. (B) The occupancy of the H3K9Me2 was measured by qChIP and presented as in (A). (C) QRT-PCR assays were performed in homozygous Su(var)3-9 flies and presented as in (A). (D) RNA was prepared from SGs from control, H1-depleted, and H1-depleted UAS:Su(var)3-9–eGFP larvae. QRT-PCR assays were performed as in (C).
but, rather, strongly inhibited the occupancy of purified recombinant fusion of yeast GAL-4 and herpes simplex virus VP16 proteins (GAL4-VP16), which can bind GAL-4 sites present in the template, and MBP-TRR-His6, a fusion protein of Trithorax-related, H3K4-specific HMT (Fig. 3E, fig. S3A). These results indicate that H1 can specifically recruit Su(var)3-9 to chromatin where it methylates histone H3 in nucleosomes.

Our results indicate that Drosofila H1 and Su(var)3-9 work together in repressing the transcriptional activity of TEs and TE-like sequences in heterochromatin. Su(var)3-9 physically associates with H1 and is recruited to H1-containing chromatin, where it mediates H3K9 methylation. Considering the previously observed interactions between Su(var)3-9, HP1 and H3K9Me2/3 [reviewed in (20)], HP1 and HP1 (Fig. 3A, C and D) (17–19, 21) and the physical interaction and joint activities of H1 and Su(var)3-9 reported here, we propose that these known heterochromatin effectors and components additionally require linker histone H1 for the establishment of heterochromatin identity and for repression of its genetic activity.

H1 is thought to be nearly ubiquitous in the genome, but several studies report its consistently higher abundance in heterochromatin (22–24). We propose that higher concentrations of H1, equal in stoichiometry to nucleosomes, along extended chromatin domains may be essential to achieve its optimal function as a repressor, whereas substoichiometric or local deposition may only allow for a limited ability to repress genetic activity in euchromatin (Fig. 1A, left). In the future, it will be interesting to compare H1 abundance in various parts of the genome by physical fractionation of chromatin and to study the effects of H1 on activity of other chromatin-modifying enzymes.

References and Notes

4. X. Lu et al., Genes Dev. 23, 452 (2009).

Acknowledgments: We are grateful to S. Elgin, A. Imhof, T. Kornberg, and G. Reuter for fly stocks and DNA constructs; J. Kadonaga, M. Khour, and T. Kusch for purified recombinant GAL4-VP16 and MBP-TRR; A. Losser for critical reading of the manuscript; and E. Vershilova for technical assistance. This work was supported by grants from the NIH to D.V.F. (GM074233) and A.I.S.

Supplementary Materials

www.sciencemag.org/cgi/content/full/340/6128/78/DC1 Materials and Methods Figs. S1 to S3 Tables S1 to S3 References (26–35)

24 October 2012; accepted 8 February 2013.

10.1126/science.1234654.