The XNP remodeler targets dynamic chromatin in *Drosophila*

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Heterochromatic gene silencing results from the establishment of a repressive chromatin structure over reporter genes. Gene silencing is often variegated, implying that chromatin may stochastically switch from repressive to permissive structures as cells divide. To identify remodeling enzymes involved in reorganizing heterochromatin, we tested 11 SNF2-type chromatin remodelers in *Drosophila* for effects on gene silencing. Overexpression of five remodelers affects gene silencing, and the most potent de-repressor is the α-thalassaemia mental retardation syndrome X-linked (ATRX) homolog X-linked nuclear protein (XNP). Although the mammalian ATRX protein localizes to heterochromatin, *Drosophila* XNP is not a general component of heterochromatin. Instead, XNP localizes to active genes and to a major focus near the heterochromatin of the X chromosome. The XNP focus corresponds to an unusual decondensed satellite DNA block, and both active genes and the XNP focus are sites of ongoing nucleosome replacement. We suggest that the XNP remodeler modulates nucleosome dynamics at its target sites to limit chromatin accessibility. Although XNP at active genes may contribute to gene silencing, we find that a single focus is present across *Drosophila* species and that perturbation of this site cripples heterochromatic gene silencing. Thus, the XNP focus appears to be a functional genetic element that can contribute to gene silencing throughout the nucleus.

heterochromatin | nucleosome dynamics | chromatin remodelers

Chromatin in the eukaryotic nucleus consists of DNA wrapped around histone octamers into nucleosomes. Cytological and molecular features distinguish different kinds of chromatin within the nucleus (1). Gene-rich regions are usually packaged into euchromatin, which is decondensed in interphase nuclei, enriched for histone modifications associated with transcriptional activity, and often has high DNA accessibility. In contrast, gene-poor and repetitive sequences are packaged into heterochromatin, a condensed, relatively inaccessible chromatin organization that carries histone modifications associated with transcriptional repression.

The genetic phenomenon of position effect variegation first indicated that heterochromatin could affect gene activity (2). Heterochromatic gene silencing has served as a sensitive measure to identify mutations in components of chromatin-based regulation (3, 4). Transcriptional repression in heterochromatin is thought to be crucial to limit the accumulation of transposable elements and unstable repetitive sequences in genomes.

A major class of factors involved in altering chromatin structure is the SNF2-type chromatin remodelers (named after the founding sucrose nonfermenting 2 protein in *Saccharomyces cerevisiae*) (5). These nuclear enzymes use the energy of ATP binding and hydrolysis to manipulate histone–DNA contacts in nucleosomes. Individual remodelers also appear to differ in their activities; whereas some have been implicated in regulating promoter accessibility (6, 7), others are required for transcriptional elongation (8), recombination (9), DNA replication (10), and histone exchange (11, 12). These studies have led to the view that remodelers of different subfamilies have different biochemical properties. At least 14 subfamilies have been distinguished by phylogenetic analysis of the core SNF2-type domain (13), suggesting that novel activities for remodelers await discovery. *Drosophila melanogaster* has 17 SNF2-type proteins representing all 14 of the chromatin remodeler subfamilies.

Results

Overexpression of Chromatin Remodelers Alters Heterochromatic Gene Silencing. To determine the in vivo relationship between chromatin remodeling and gene silencing, we tested whether overexpression of specified remodelers in the eye could alter gene silencing caused by the *brown*^{Dominant} (*bw*^{D}) heterochromatic insertion (14). This piece of heterochromatin silences a *bw*$^{+}$ allele on the homologous chromosome and is sensitive to the dosage of heterochromatin proteins (15). We used this reporter system because *bw*^{D} consistently silences *bw*$^{+}$ in ≈95% of pigment cells in every animal and is not affected by the common *mini-white*$^{+}$ transgene marker. These features allow us to conduct an efficient F_1 screen of available overexpression insertions. We targeted overexpression specifically to the eye by using the GAL4 misexpression system (16, 17), thus minimizing potential lethal effects of overexpression. Suitable transposon insertions were available from public stock centers for 11 of the 17 SNF2-type chromatin remodelers in the *Drosophila* genome. We found that overexpression of five of these genes had detectable effects on silencing (Table 1 and Fig. 1*A*). Overexpression of *Ino80* and *Chd1* enhanced gene silencing, whereas the *kismet*, *Etl1*, and *xnp* [named after the mammalian homolog X-linked nuclear protein (XNP)] genes de-repressed the *bw*$^{+}$ allele. Notably, overexpression of *xnp* greatly relieved silencing, whereas other remodelers had more moderate but consistent effects on the frequency of silenced and expressing cells. The effects on gene silencing that we do observe likely result from overexpression of the adjacent gene, because each transposon lies close to or within the transcription unit, and had no effect on silencing without GAL4 induction. However, we have not verified that remodelers that do not affect silencing are overexpressed from these insertions.

XNP Remodeler Is a General De-Repressor of Heterochromatin Gene Silencing. We generated deletion alleles for the *xnp* gene (*SI Text* and Fig. S1) and found homozygous null mutants for *xnp* to be viable and fertile. Both alleles recovered (*xnp*^{403} and *xnp*^{406}) are mild dominant de-repressors of *bw*^{D}-mediated silencing, and animals completely deficient for *xnp* had greatly de-repressed (Fig. 1*B*). Moreover, these mutations appear to be general

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Table 1. Effects of SNF2-type remodelers on heterochromatic gene silencing

Type	Gene	Synonym	Insertion	Distance from TSS*, bp	*GMR-GAL4*†
SNF2-like	*CG5942*	*brm*	f04692	−318	No effect
	CG8625	*iswi*	—	—	—
	CG3696	*kis*	EY12846	+1430	De-repressed
	CG3733	*Chd1*	EP^{Chd1-2}	−2	Enhanced
	CG8103	*Mi-2*	EY13252	−20	No effect
	CG9594	*Chd3*	—	—	—
SWR1-like	*CG9696*	*dom*	f00946	+327	No effect
	CG31212	*Ino80*	d10097‡	−265	Enhanced
	CG5899	*Etl1*	EP701	+587	De-repressed
RAD54-like	*CG3736*	*okr*	—	—	—
	CG4548	*xnp*	EP635	−20	De-repressed
	CG4049	*Arip4*	f01495	−475	No effect
RAD5/16-like	*CG26984*	*lds*	—	—	—
	CG10445	*CG10445*	—	—	—
	CG7376	*SHPRH*	—	—	—
Mot1-like	*CG4261*	*Hel89B*	d00861‡	−232	No effect
SMARCAL1-like	*CG3753*	*Marca1*	f05123	−486	No effect

*TSS, transcriptional start site.
†Eye pigmentation of *v/Y; P[GMR-GAL4]D* bw^D/bw^+ adult males with the indicated misexpression transposon was compared with *v/Y; P[GMR-GAL4]D* bw^D/bw^+ males.
‡*P[XP]* insertions with divergent promoters that overexpress *Ino80* and *CG5316* (d10097), or *Hel89B* and *moira* (d00861).

modifiers of heterochromatic silencing, because they also de-repress silencing in the $In(1)w^{m4}$ rearrangement (Fig. 1*C*) (18). This demonstrates that XNP is required for efficient heterochromatic silencing. However, models for XNP function must take into account that both the lack or the overexpression of the protein de-represses silencing.

XNP Marks Transcribed Genes and a Focus Near Heterochromatin. To characterize the normal function of XNP, we raised antisera directed against an XNP C-terminal peptide. We found that our antiserum recognizes both isoforms (18) of the protein in nuclear extracts from wild-type, but not *xnp* mutant animals (Fig. S1). The mammalian homolog XNP or α-thalassaemia mental retardation syndrome X-linked (ATRX) has been described as a component of heterochromatin and implicated in the epigenetic regulation of transcription (19). Strikingly, we found that most endogenous *Drosophila* XNP in diploid wing disc cells localizes to a single focus within the nucleus, always adjacent to heterochromatin, as marked by heterochromatin protein 1 (HP1) staining (Fig. 2*A*). Additional staining is present throughout euchromatin. To examine the localization of XNP with higher resolution, we examined the giant polytene chromosomes of larval salivary glands and found a single bright focus next to the heterochromatic chromocenter (Fig. 2*B*, arrow). The spreads also show clear, but weaker signals throughout the euchromatic arms. Both the focus and euchromatic signals must be due to endogenous XNP protein, because they are absent in *xnp* mutants (Fig. 2*C*). We costained the chromosomes for XNP and elongating RNA polymerase II (pol II) and observed considerable overlap between the two proteins in euchromatin, implicating XNP generally in transcription (Fig. 3 *D* and *E*; arrowhead marking an ecdysone-responsive puff). However, although all sites of pol II contain XNP, a limited number of XNP sites do not contain pol II. The XNP focus is a clear case in which a large fraction of XNP is localized, but there is no overlapping signal for elongating or initiating forms of pol II (Fig. 2*D*, arrow). Notably, a previous study claimed that *Drosophila* XNP is found throughout heterochromatin (18) but used overexpression of the protein to assess its localization. We found that overexpression of XNP changes its localization pattern and causes chromatin defects (Fig. S2).

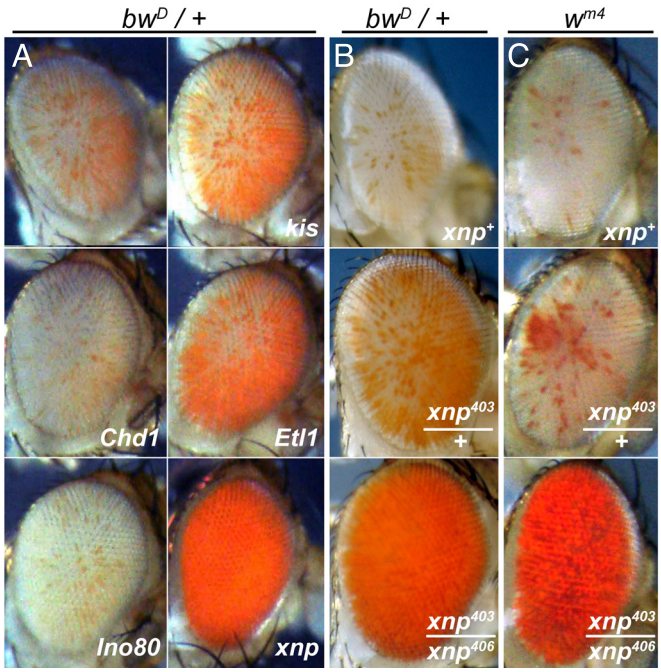

Fig. 1. Effects of chromatin remodelers on heterochromatic gene silencing. (*A*) Adult eyes showing variegated gene expression in $bw^{D/+}$ animals. The indicated remodeler was overexpressed with the GMR-GAL4 eye-specific driver. Overexpression of Chd1 or INO80 enhances silencing, whereas overexpression of Kismet, Etl1, or XNP de-repress. (*B* and *C*) Null alleles of the XNP remodeler are de-repressors of heterochromatic silencing. Heterozygotes show moderate dominant de-repression in both $bw^{D/+}$ (*B*) and $In(1)w^{m4}$ (*C*) animals, whereas XNP-deficient animals have greatly de-repressed silencing.

XNP Focus Is a Site of Rapid Nucleosome Replacement. We used chromosomal rearrangements and different genetic backgrounds to map the XNP focus (Fig. S3). The focus coincides with a previously uncharacterized satellite block of ≈50 kb of the simple sequence TAGA, near heterochromatin on the X chromosome (Fig. 3*A*). Although the sequence of the block is a simple repeat, it is not enriched for HP1 (Figs. 2*B* and 3*B*). Furthermore, we find that the block stains poorly for DAPI and histones but is enriched for $H3^{K9}$ acetylation, a mark of active

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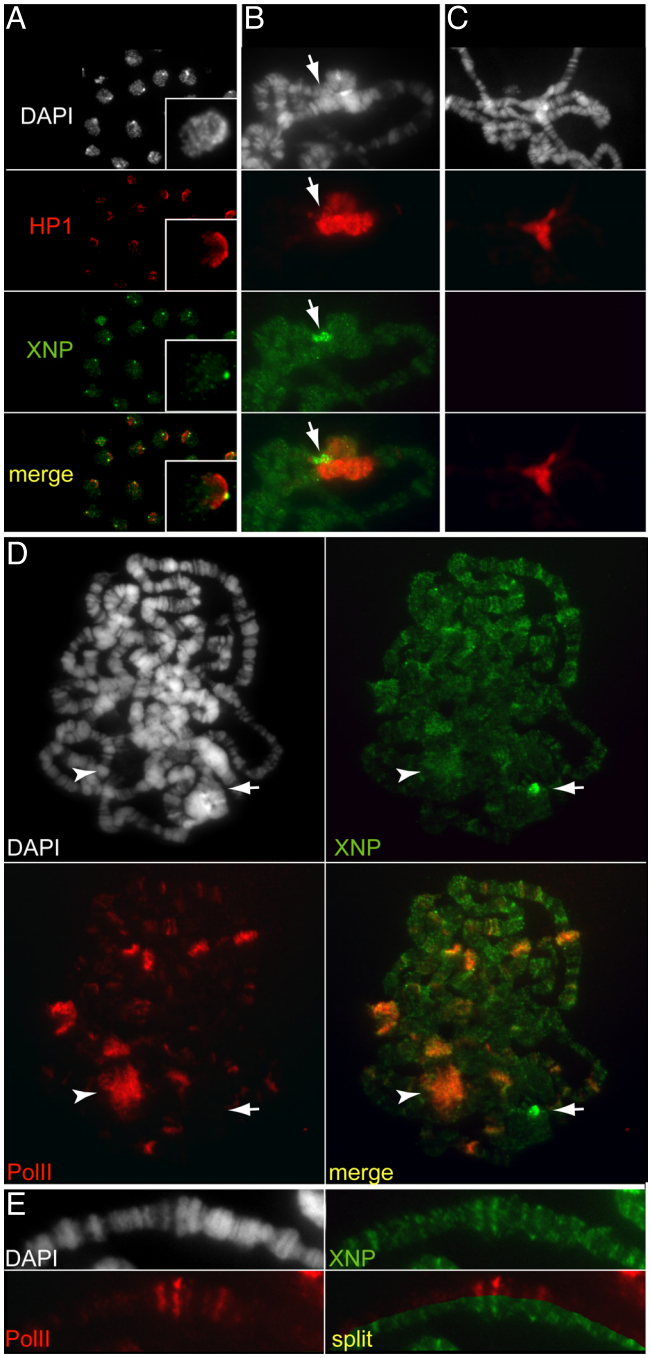

Fig. 2. XNP marks active genes and a single major focus in the nucleus. XNP staining is in green, and DAPI-stained DNA is in gray. (*A*) In larval imaginal wing disc nuclei, most XNP localizes to a single site near the HP1-rich chromocenter (red), and lower levels are broadly distributed throughout the nucleus. The *Inset* shows the staining pattern in a single nucleus. (*B*) XNP localizes to a major site (arrow) near the HP1-rich chromocenter (red) and to the chromosome arms in polytene chromosomes from larval salivary glands. (*C*) No XNP signal is observed in spreads from for xnp^{403} mutants. (*D*) XNP in euchromatin coincides with elongating RNA polymerase II (red) (arrowhead indicates a heavily transcribed ecdysone puff). The XNP focus near heterochromatin (arrow) does not contain RNA polymerase. (*E*) Color splits showing the correspondence between the distribution of XNP and that of elongating RNA polymerase II (red).

chromatin (Fig. 3*B*). These features suggest that the focus is either nucleosome-poor or relatively decondensed.

The low histone density and the enrichment for histone acetylation in the XNP focus prompted us to test whether it is a site of nucleosome replacement. Although most nucleosomes are assembled in S-phase during DNA replication, cells also use the H3.3 histone variant for replication-independent (RI) nucleosome replacement throughout the cell cycle. Nucleosome replacement can occur after transcription-associated displacement of nucleosomes, at enhancer elements where transcription factors bind, and at anti-nucleosomal sequences where nucleosomes are unstable (20, 21). We used a truncated H3.3 protein fused to green fluorescent protein ($H3.3^{core}$-GFP), a construct that can only be incorporated into chromatin by RI assembly to mark sites of nucleosome replacement in vivo (22). We produced a pulse of $H3.3^{core}$-GFP in larvae and compared the localization of the tagged histone and XNP in polytene chromosomes 24 h later (Fig. 3*C*). Strikingly, the XNP focus is a major site of H3.3 deposition, implying that nucleosomes are continuously being disassembled and reassembled at this site.

We hypothesized that the XNP focus is a site of nucleosome replacement because the sequence of the satellite block is difficult to wrap around a histone octamer. The positioning and stability of nucleosomes is in large part due to the distribution of anti-nucleosomal sequences that bend poorly around histone octamers (23). Computer prediction based on the calculated structural properties of the TAGA sequence suggests that this satellite block will not favor nucleosome packaging (24). We conclude that the chromatin dynamics of the XNP focus is due to intrinsic sequence effects on nucleosome stability.

Active genes are the major sites of nucleosome replacement in *Drosophila* (25). Our conclusion that the nontranscribed XNP focus is also a site of nucleosome replacement suggests that this may be a common feature of all XNP target sites. To test whether XNP signal in euchromatin corresponds to regions of dynamic chromatin, we induced the $H3.3^{core}$-GFP construct with a constitutive driver, and stained chromosomes for GFP and XNP. The vast majority of XNP sites costain with the histone variant (Fig. 3 *D* and *E*).

XNP Is Not Required for Nucleosome Replacement. Animals deficient for XNP show strong de-repression of heterochromatic gene silencing, suggesting that chromatin structure may be altered in these mutants. We therefore examined nucleosome replacement at the normal site of the XNP focus in XNP-deficient larvae. Incorporation of $H3.3^{core}$-GFP in the 20E interval appeared indistinguishable from that in xnp^{+} cells (Fig. 3*C*). Additionally, the general appearance of the region was not visibly perturbed, remaining relatively decondensed, depleted for histone H3, enriched for $H3^{K9Ac}$, and lacking RNA polymerase II. This demonstrates that the rapid nucleosome replacement at this site does not require XNP but is consistent with the idea that intrinsic sequence features of the satellite destabilize nucleosomes. Finally, there were no apparent defects in chromatin morphology at transcribed sites in the genome. Thus, although XNP appears to target sites of nucleosome dynamics, it is not required to maintain these regions.

Perturbation of the XNP Focus Affects Gene Silencing. Staining for XNP in different *Drosophila* species revealed that the XNP focus is a conserved feature of the drosophilid nucleus;, however, the underlying sequence of the focus in *D. melanogaster* is not (Fig. S4). Thus, the conservation of an XNP focus cannot be explained by a simple sequence-specific DNA–protein interaction. Conservation implies a function for the focus. Available deletions in *D. melanogaster* that remove the TAGA satellite block also delete neighboring essential genes, and this prevents us from testing whether the XNP focus is required for silencing. However, a transposon insertion ($P[EY]^{TAGA}$) has been recovered adjacent to the satellite block. Although this insertion itself does not affect the bw^{D}/+ silencing assay, the construct contains a GAL4-inducible misexpression promoter directed into the

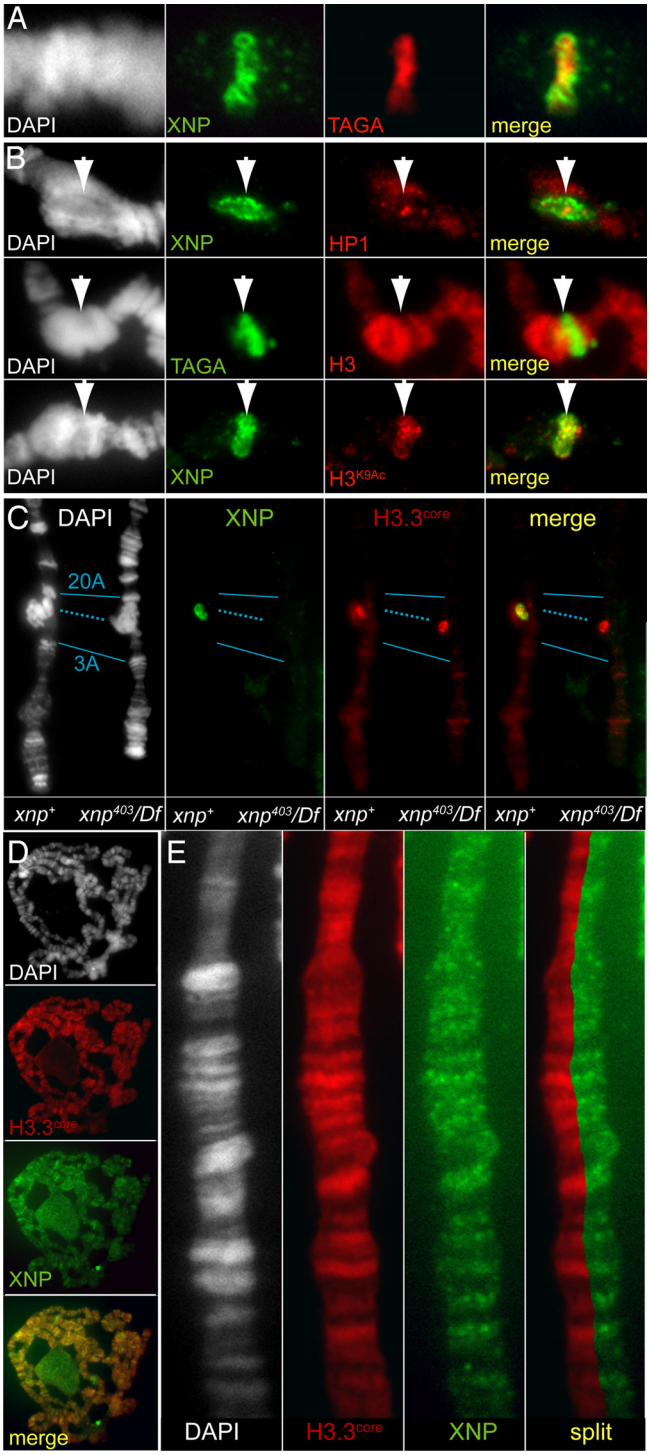

Fig. 3. The XNP focus is a satellite block with a dynamic chromatin environment. Chromatin features were assayed in polytene chromosomes from *In(1)w^{m4}; Su(var)3-9* larvae. XNP is in green, and DAPI-stained DNA is in gray. (*A*) XNP staining completely overlaps with a $(TAGA)_n$ probe, which stains a wide band at region 20E. (*B*) The XNP focus (arrow) is depleted for DAPI, HP1, and histone H3, but is enriched for acetylated H3^{K9} histones (H3^{K9Ac}). Each feature is indicated in red. (*C*) Sites of nucleosome replacement are marked by a pulse of H3.3^{core}-GFP (red). Rapid nucleosome replacement occurs at the focus in polytene chromosomes in *xnp*$^+$ (*Left*) and XNP-deficient (*Right*) larvae (*xnp^{403}/Df(3R)Exel6202*). Images are from two separate spreads. (*D*) XNP coincides with constitutively expressed H3.3^{core}-GFP (red), which marks nucleosome replacement throughout the genome. (*E*) Color splits showing the correspondence between XNP and H3.3^{core}-GFP (red) localization along the arms of polytene chromosomes.

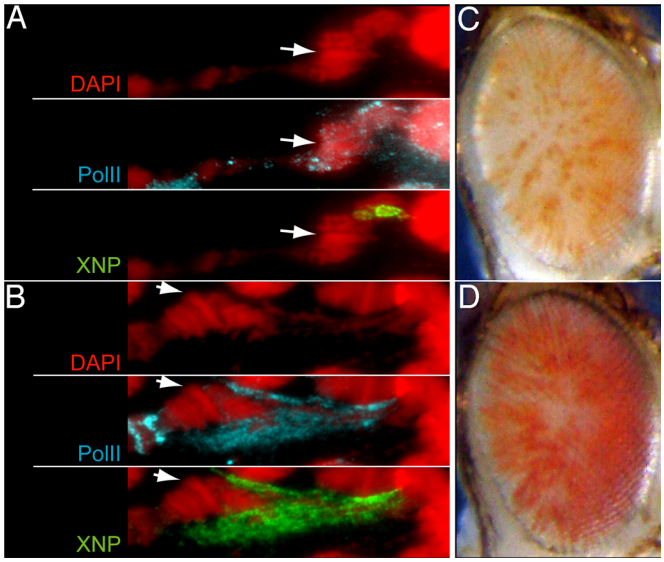

Fig. 4. The XNP focus is capable of modulating gene silencing. (*A*) Morphology of the base of the X chromosome in polytene spreads from a male carrying a transposon insertion (*P[EY]^{TAGA}*) adjacent to the TAGA satellite block. The transposon has no effect on the morphology of the XNP focus. DAPI-stained DNA is in red, and the elongating isoform of RNA polymerase II is in blue. (*B*) GAL4 induction of a misexpression promoter in *P[EY]^{TAGA}* causes massive decompaction of the XNP focus, which is also heavily stained for elongating RNA polymerase II (blue). DAPI-stained DNA is in red, and the arrow points to the same band. (*C* and *D*) Adult eyes showing variegated gene silencing in *GMR-GAL4 bw^D/+; st* males. (*C*) The *bw^D* allele induces severe silencing. (*D*) Silencing is de-repressed in males in which the *P[EY]^{TAGA}* insertion is induced and transcribes across the satellite block. The pigmented area of the eye increases threefold when *P[EY]^{TAGA}* is induced.

TAGA satellite block. We induced the misexpression promoter by using a constitutive GAL4 driver and examined the cytological appearance of the XNP focus. In the uninduced X chromosome, the XNP focus lies at the base of the X chromosome adjacent to the chromocenter and does not contain RNA pol II (Fig. 4*A*). In contrast, induction of the misexpression promoter in *P[EY]^{TAGA}* dramatically alters the morphology of the XNP focus (Fig. 4*B*). Chromatin at the base of the X decondenses, increasing the distance from the chromocenter and the first bands on the X chromosome. This decondensed region comprises the TAGA satellite block, because it is intensely stained with antibodies against both RNA pol II and with XNP. Thus, forcing transcription across the block results in massive decompaction, apparently allowing increased access of the XNP protein to its binding sites at the block.

We then assayed gene silencing by using the *bw^D*/+ system to determine whether the transcription-mediated perturbation of the focus has any functional outcome. Indeed, induction of *P[EY]^{TAGA}* by using an eye-specific GAL4 driver de-repressed silencing (Fig. 4 *C* and *D*). Although transcription within the XNP focus does not occur under normal circumstances, we interpret the effects of induced transcription to indicate that the XNP focus can contribute to heterochromatic silencing. Because *P[EY]^{TAGA}* and *bw^D* are on different chromosomes, decompacting the XNP focus hinders heterochromatic gene silencing throughout the nucleus.

Discussion

We show that five SNF2-type chromatin remodelers in *Drosophila* have dramatic effects on heterochromatic gene silencing when overexpressed. Although overexpression screens can create aberrant effects in cells, such screens are efficient for isolating

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candidate genes or processes involved in a biological function. The specificity of certain remodelers to affect silencing is presumably due to their individual activities, target sites, or features of their macromolecular complexes. Overexpression can enhance silencing (Chd1 and Ino80) or de-repress it (Kismet, Etl1, and XNP). The Kismet and XNP remodelers have been implicated in gene silencing phenomena from mutational studies (26, 27), and in our study overexpression of XNP was the strongest de-repressor of silencing. This demonstrates that overexpression can recover relevant factors involved in heterochromatic gene silencing. The role of the XNP remodeler in silencing appears to be conserved, because null mutations in the mammalian homolog ATRX also alter chromatin in heterochromatic regions (27). A large fraction of ATRX is localized to heterochromatin in mammalian cells, leading to the idea that it contributes to heterochromatin structure.

A previous study identified that *Drosophila* XNP is required for heterochromatic gene silencing and used overexpression genotypes to visualize that XNP localized to heterochromatin (18). Surprisingly, we find that endogenous *Drosophila* XNP is not localized throughout heterochromatin, and the difference appears to be that overexpression changes the localization of the protein. We find that endogenous XNP targets active genes and a single satellite block on the X chromosome. If both ATRX and XNP are required for efficient heterochromatin function, why does the nuclear localization of this conserved remodeler differ between *Drosophila* and mammals? First, the N-terminal portion of ATRX contributes to heterochromatin targeting (28), and *Drosophila* XNP lacks homology to this region. This can account for the absence of XNP in heterochromatin. Second, the distribution of mammalian ATRX in the nucleus is more complex than that of XNP. ATRX is found at low levels throughout the nucleus, at heterochromatic regions, and in discrete nuclear foci called promyelocytic leukemia (PML) bodies (29). PML bodies are functionally diverse, with connections to transcriptional regulation, DNA damage response, and genome stability (29). A subclass of PML bodies contain transcription factors and associate with gene-rich regions in a transcription-dependent manner. Mutational studies implicate PML-localized ATRX in at least some of its regulatory functions (30). *Drosophila* lacks a PML homolog, and XNP does not show a speckled appearance akin to mammalian PML bodies. However, XNP localized to active genes may play an equivalent role to ATRX localized in PML bodies. Alternatively, these may simply be lacking in *Drosophila*.

However, there is one situation in which a mammalian PML body has similarities to the *Drosophila* pattern. PML bodies do not normally coincide with satellite blocks, but in mutants lacking the Dnmt3 DNA methyltransferase a hypomethylated repetitive sequence nucleates the formation of a giant PML body (31). This is thought to form as part of a response when the hypomethylated satellite fails to compact. In *Drosophila*, the XNP focus is a constitutive feature of the nucleus but also corresponds to an unusually decondensed satellite block. The functions of PML bodies are mysterious, but such foci at satellite blocks may form as proteins are recruited to package the site and avoid DNA damage (29).

How might XNP promote packaging of decondensed satellite blocks? All XNP target sites in the *Drosophila* genome are sites of ongoing nucleosome replacement, where the H3.3 histone variant is incorporated. For active genes, this is a consequence of transcription that displaces histones from DNA (20). At the XNP focus, the intrinsic properties of the TAGA satellite appear to destabilize nucleosomes, resulting in decompaction and nucleosome replacement. XNP is recruited to both of these sites, suggesting that it has affinity for a shared aspect of dynamic chromatin. We speculate that XNP targets these regions by binding H3.3-containing nucleosomes or by complexing with other factors involved in replacement. XNP is not required for nucleosome replacement, because H3.3 deposition continues in *xnp* mutants. Furthermore, XNP is not essential, suggesting that it is redundant with other remodelers localized at active genes or that it has more significant functions in DNA repair or recombination. However, XNP may contribute indirectly to nucleosome dynamics. Nucleosome replacement requires that old histones dissociate from DNA and that new histones repackage the exposed DNA. In vivo, this process requires factors that manipulate DNA–histone contacts to unwrap the nucleosome, and protein chaperones to remove and deliver histones (32). Chromatin remodelers can contribute to nucleosome assembly by unwrapping and rebuilding nucleosomes (33). XNP belongs to the RAD54 (radiation-sensitive 54) subfamily of SNF2-type remodelers (34). These enzymes have DNA translocase activity, but little or no effect on nucleosomal accessibility (18, 35, 36). ATRX in particular shows no preference for free DNA or nucleosomes (28). However, a translocase in dynamic chromatin regions could adjust nucleosome positions by trapping exposed DNA. Alternatively, translocases could modulate nucleosome occupancy by introducing supercoils that promote nucleosome stability (37). Either of these functions would affect DNA accessibility in highly dynamic chromatin. At the XNP focus, these functions could promote the packaging of the satellite despite its intrinsic properties. It is reasonable to consider that XNP similarly modulates nucleosome dynamics at active genes. This can also account for why a lack or overexpression of XNP de-represses silencing, if less efficient packaging or excessive remodeling leave regions accessible for transcription factor binding.

Although satellite PML bodies are thought to be aberrant structures (31), our data imply that the properties of the XNP focus are more complex. The XNP focus is a constitutive and conserved feature of the *Drosophila* nucleus, but because satellite sequences rapidly evolve, a detrimental aberrant block should not be retained. Our experiments perturbing the XNP focus by induced transcription across the satellite block suggest that the XNP focus has a role in regulating gene silencing throughout the nucleus. We propose two models to explain how the XNP focus can affect silencing. First, the XNP focus may sequester the remodeler away from active genes. If XNP attenuates gene activity by limiting nucleosome dynamics in transcribed regions, sequestering more XNP at the focus would increase gene activity. This model parallels functions proposed for mammalian PML bodies, in which transcription factors may be sequestered away from their target sites as a mechanism of gene regulation (29). A second possibility is that the XNP focus is part of a larger genetic element that regulates silencing. Recent studies have identified a region adjacent to the XNP focus on the X chromosome as a major source of noncoding RNAs that direct global repression of retrotransposons and repetitive sequences (38, 39). Perhaps the XNP focus acts as a transcriptional enhancer for RNAs that are required for heterochromatic silencing. Regardless of how the XNP focus modulates silencing, its conservation in multiple *Drosophila* species implies that this structure is a functional element in the nucleus.

Materials and Methods

Fly Crosses. All stocks and crosses were grown at 25 °C. Strains used here are described in Flybase (www.flybase.org). Transposon insertions upstream of most chromatin remodeler genes were obtained from the Bloomington and Harvard stock centers. The *Chd1*^{EP} insertion line was obtained from J. Armstrong (Claremont College, Claremont, CA). We obtained *P[EY]09137* from the Bloomington stock center and designated this as *P[EY]*^{TAGA} as it is adjacent to the TAGA satellite block. The eye-specific (*GMR-GAL4*) and the constitutive (*A5C-GAL4*) driver lines are described in ref. 40, and the salivary-gland-specific driver in ref. 41. For scoring effects of chromatin remodelers on heterochromatic gene silencing, *v*^{36f}*; P[GMR-GAL4]D bw*^{D}*/CyO* females were crossed to males from the various insertion lines. Curly-winged and Cy^{+} male progeny were collected from crosses, aged for 3 days, and photographed as described

in ref. 42 using a Sony digital camera mounted on a Nikon SMZ1500 stereomicroscope. Effects on gene silencing were consistent in all progeny from each cross, and representative pictures are shown. Dependence of modification effects on GAL4-induced overexpression of a remodeler and not the transposon insertion itself was tested by crossing v^{36f}; bw^{D}/*CyO* females to male from the insertion lines, and comparing silencing in Curly-winged and Cy+ male progeny.

Antisera. Commercial primary antisera used were as follows: anti-HP1 (C1A9; DSHB), anti-H3K9-dimethyl (catalog #07-212; Upstate), anti-H3K9-acetyl (Ab12179; Abcam), anti-pol II H5 (MMS-129R; BabCo), anti-pol II H14 (MMS-134R; BabCo), and anti-GFP (FL; catalog #8372-2; Clontech). Polyclonal antisera to XNP were raised in rabbits to a C-terminal peptide of both isoforms (CAAPAPGFEPDKVYEID). Sera were affinity-purified and used at 1:2,000–5,000 dilution for cytology, and 1:40,000 for Western detection. Fluorescently labeled secondary antibodies (Jackson ImmunoResearch) were used at 1:100 dilution for cytology, and HRP-conjugated secondary antibody (Jackson ImmunoResearch) were used at 1:10,000 for Western detection.

Cytology. For polytene spreads, salivary glands were fixed and spread as described in ref. 43. Slides were incubated with primary antibodies overnight at 4 °C, with secondary antibodies for 1 h at room temperature, and then stained with DAPI. Air-dried slides were mounted with Vectashield, imaged by using a 100× objective, and photographed as described in ref. 20. *In(1)*w^{m4}*; Su(var)3-9*^{1}*/Su(var)3-9*^{2} were used for mapping and cytological characterization of the TAGA block. To detect sites of rapid replication-independent nucleosome replacement, we heat-shocked wild-type (w^{m4}) or XNP-deficient (*In(1)*w^{m4}*;* xnp^{406}*/Df(3R)Exel6202)* larvae carrying a *HS-H3.3*^{core}*-GFP* transgene for 1 h (20). Larvae were allowed to recover for 24 h at room temperature before dissection and polytene spread preparation. To visualize all sites of H3.3 deposition, the *HS-H3.3*^{core}*-GFP* transgene was constitutively induced in salivary glands by using the *SGS3-GAL4* driver. For diploid nuclear spreads, imaginal wing discs from third-instar larvae were fixed in methanol/acetic acid/water (10:2:1) for 2 min, and then squashed as described in ref. 44. Slides were then processed for immunostaining.

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1. Grewal SI, Elgin SC (2002) Heterochromatin: New possibilities for the inheritance of structure. *Curr Opin Genet Dev* 12:178–187.
2. Muller HJ (1930) Types of visible variations induced by X-rays in *Drosophila*. *J Genet* 22:299–334.
3. Reuter G, Werner W, Hoffmann HJ (1982) Mutants affecting position-effect heterochromatinization in *Drosophila melanogaster*. *Chromosoma* 85:539–551.
4. Reuter G, Wolff I (1981) Isolation of dominant suppressor mutations for position-effect variegation in *Drosophila melanogaster*. *Mol Gen Genet* 182:516–519.
5. Kingston RE, Bunker CA, Imbalzano AN (1996) Repression and activation by multiprotein complexes that alter chromatin structure. *Genes Dev* 10:905–920.
6. Hirschhorn JN, Brown SA, Clark CD, Winston F (1992) Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. *Genes Dev* 6:2288–2298.
7. Goldmark JP, Fazzio TG, Estep PW, Church GM, Tsukiyama T (2000) The Isw2 chromatin remodeling complex represses early meiotic genes upon recruitment by Ume6p. *Cell* 103:423–433.
8. Srinivasan S, et al. (2005) The *Drosophila* trithorax group protein kismet facilitates an early step in transcriptional elongation by RNA polymerase II. *Development* 132:1623–1635.
9. Alexeev A, Mazin A, Kowalczykowski SC (2003) Rad54 protein possesses chromatin-remodeling activity stimulated by the Rad51-ssDNA nucleoprotein filament. *Nat Struct Biol* 10:182–186.
10. Collins N, et al. (2002) An ACF1-ISWI chromatin-remodeling complex is required for DNA replication through heterochromatin. *Nat Genet* 32:627–632.
11. Mizuguchi G, et al. (2004) ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science* 303:343–348.
12. Konev AY, et al. (2007) CHD1 motor protein is required for deposition of histone variant H3.3 into chromatin in vivo. *Science* 317:1087–1090.
13. Eisen JA, Sweder KS, Hanawalt PC (1995) Evolution of the SNF2 family of proteins: Subfamilies with distinct sequences and functions. *Nucleic Acids Res* 23:2715–2723.
14. Henikoff S, Dreesen TD (1989) Trans-inactivation of the *Drosophila* brown gene: Evidence for transcriptional repression and somatic pairing dependence. *Proc Natl Acad Sci USA* 86:6704–6708.
15. Sass G, Henikoff S (1998) Comparative analysis of position-effect variegation mutations in *Drosophila melanogaster* delineates the targets of modifiers. *Genetics* 148:733–741.
16. Brand AH, Perrimon N (1993) Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. *Development* 118:401–415.
17. Rorth P (1996) A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. *Proc Natl Acad Sci USA* 93:12418–12422.
18. Bassett AR, Cooper SE, Ragab A, Travers AA (2008) The chromatin remodelling factor dATRX is involved in heterochromatin formation. *PLoS ONE* 3:e2099.
19. McDowell TL, et al. (1999) Localization of a putative transcriptional regulator (ATRX) at pericentromeric heterochromatin and the short arms of acrocentric chromosomes. *Proc Natl Acad Sci USA* 96:13983–13988.
20. Schwartz BE, Ahmad K (2005) Transcriptional activation triggers deposition and removal of the histone variant H3.3. *Genes Dev* 19:804–814.
21. Mito Y, Henikoff JG, Henikoff S (2005) Genome-scale profiling of histone H3.3 replacement patterns. *Nat Genet* 37:1090–1097.
22. Ahmad K, Henikoff S (2002) Histone H3 variants specify modes of chromatin assembly. *Proc Natl Acad Sci USA* 99(Suppl 4):16477–16484.
23. Lee W, et al. (2007) A high-resolution atlas of nucleosome occupancy in yeast. *Nat Genet* 39:1235–1244.
24. Kaplan N, et al. (2009) The DNA-encoded nucleosome organization of a eukaryotic genome. *Nature* 458:362–366.
25. Henikoff S, Ahmad K (2005) Assembly of variant histones into chromatin. *Annu Rev Cell Dev Biol* 21:133–153.
26. Kennison JA, Tamkun JW (1988) Dosage-dependent modifiers of polycomb and antennapedia mutations in *Drosophila*. *Proc Natl Acad Sci USA* 85:8136–8140.
27. Gibbons RJ, et al. (2000) Mutations in ATRX, encoding a SWI/SNF-like protein, cause diverse changes in the pattern of DNA methylation. *Nat Genet* 24:368–371.
28. Xue Y, et al. (2003) The ATRX syndrome protein forms a chromatin-remodeling complex with daxx and localizes in promyelocytic leukemia nuclear bodies. *Proc Natl Acad Sci USA* 100:10635–10640.
29. Bernardi R, Pandolfi PP (2007) Structure, dynamics and functions of promyelocytic leukaemia nuclear bodies. *Nat Rev Mol Cell Biol* 8:1006–1016.
30. Berube NG, et al. (2008) Patient mutations alter ATRX targeting to PML nuclear bodies. *Eur J Hum Genet* 16:192–201.
31. Luciani JJ, et al. (2006) PML nuclear bodies are highly organised DNA-protein structures with a function in heterochromatin remodelling at the G2 phase. *J Cell Sci* 119:2518–2531.
32. Park YJ, Luger K (2008) Histone chaperones in nucleosome eviction and histone exchange. *Curr Opin Struct Biol* 18:282–289.
33. Haushalter KA, Kadonaga JT (2003) Chromatin assembly by DNA-translocating motors. *Nat Rev Mol Cell Biol* 4:613–620.
34. Flaus A, Martin DM, Barton GJ, Owen-Hughes T (2006) Identification of multiple distinct Snf2 subfamilies with conserved structural motifs. *Nucleic Acids Res* 34:2887–2905.
35. Petukhova G, Van Komen S, Vergano S, Klein H, Sung P (1999) Yeast Rad54 promotes Rad51-dependent homologous DNA pairing via ATP hydrolysis-driven change in DNA double helix conformation. *J Biol Chem* 274:29453–29462.
36. Amitani I, Baskin RJ, Kowalczykowski SC (2006) Visualization of Rad54, a chromatin remodeling protein, translocating on single DNA molecules. *Mol Cell* 23:143–148.
37. Ristic D, Wyman C, Paulusma C, Kanaar R (2001) The architecture of the human Rad54-DNA complex provides evidence for protein translocation along DNA. *Proc Natl Acad Sci USA* 98:8454–8460.
38. Brennecke J, et al. (2007) Discrete small RNA-generating loci as master regulators of transposon activity in *Drosophila*. *Cell* 128:1089–1103.
39. Chung WJ, Okamura K, Martin R, Lai EC (2008) Endogenous RNA interference provides a somatic defense against *Drosophila* transposons. *Curr Biol* 18:795–802.
40. Ahmad K, Henikoff S (2001) Modulation of a transcription factor counteracts heterochromatic gene silencing in *Drosophila*. *Cell* 104:839–847.
41. Cherbas L, Hu X, Zhimulev I, Belyaeva E, Cherbas P (2003) EcR isoforms in *Drosophila*: Testing tissue-specific requirements by targeted blockade and rescue. *Development* 130:271–284.
42. Ahmad K, Golic KG (1996) Somatic reversion of chromosomal position effects in *Drosophila melanogaster*. *Genetics* 144:657–670.
43. Deuring R, et al. (2000) The ISWI chromatin-remodeling protein is required for gene expression and the maintenance of higher order chromatin structure in vivo. *Mol Cell* 5:355–365.
44. Pimpinelli S, Bonaccorsi S, Fanti L, Gatti M (2000) in *Drosophila Protocols*, eds Sullivan W, Ashburner M, Hawley RS (Cold Spring Harbor Lab Press, Cold Spring Harbor, NY), pp 3–23.