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The *Drosophila* NURF remodelling and the ATAC histone acetylase complexes functionally interact and are required for global chromosome organization

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***Drosophila* Gcn5 is the catalytic subunit of the SAGA and ATAC histone acetylase complexes. Here, we show that mutations in Gcn5 and the ATAC component Ada2a induce a decondensation of the male X chromosome, similar to that induced by mutations in the Iswi and Nurf301 subunits of the NURF nucleosome remodelling complex. Genetic studies as well as transcript profiling analysis indicate that ATAC and NURF regulate overlapping sets of target genes during development. In addition, we find that Ada2a chromosome binding and histone H4-Lys12 acetylation are compromised in *Iswi* and *Nurf301* mutants. Our results strongly suggest that NURF is required for ATAC to access the chromatin and to regulate global chromosome organization.**

Keywords: Ada2a; ATAC; Gcn5; Iswi; NURF

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INTRODUCTION

Two classes of regulatory factor have been found to induce distinct states of gene activity by using the energy of ATP hydrolysis to physically remodel the nucleosomal arrangements (Cairns, 2005) or post-transcriptionally modify histones (Peterson & Laniel, 2004).

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Drosophila nucleosome remodelling complexes can be divided into two main classes, depending on whether the ATPase catalytic subunit involved is the SWI2/SNF2 homologue Brahma or the Iswi protein (Elfring *et al*, 1994). Iswi was purified in three complexes, ACF, CHRAC and NURF, which all increase accessibility to chromatin templates in biochemical assays (Tsukiyama & Wu, 1995; Ito *et al*, 1997; Varga-Weisz *et al*, 1997). Accordingly, genetic analyses pointed to a role of these complexes in transcriptional regulation (Deuring *et al*, 2000; Xiao *et al*, 2001; Badenhorst *et al*, 2002, 2005).

The yeast Gcn5 protein was the first transcriptional coactivator identified with histone acetyltransferase (HAT) activity (Brownell *et al*, 1996). From yeast to human, Gcn5 orthologues operate as a catalytic subunit in various multiprotein complexes that contain Ada2- and Ada3-related coactivators, Spt proteins and TATA-binding protein-associated factors. *Drosophila* Gcn5 was purified biochemically in two complexes, SAGA and ATAC, which preferentially acetylate nucleosomal histones H3 and H4, respectively (Guelman *et al*, 2006). The complexes include distinct Ada2 relatives: SAGA contains the Ada2b protein, whereas ATAC contains the Ada2a protein (Kusch *et al*, 2003; Muratoglu *et al*, 2003). Gcn5 was found to be essential for *in vivo* acetylation of larval polytene chromosomes at positions lysine 9 (K9)/K14 of histone H3 and K5/K12 of histone H4 (Carre *et al*, 2005; Ciurciu *et al*, 2006). In addition, mutations in the *Ada2b* gene result in a loss of acetylation of residues H3-K9/K14, whereas mutations in the *Ada2a* gene only affect acetylation of residues H4-K5/K12 (Qi *et al*, 2004; Pankotai *et al*, 2005; Ciurciu *et al*, 2006). These data indicate that SAGA and ATAC have distinct substrate specificity for histone residues, which in turn could determine distinct functions or downstream regulatory events.

Numerous studies established that HAT and nucleosome remodelling complexes act synergistically to regulate chromatin structure and gene expression in yeast and human (Featherstone, 2002). In *Drosophila*, H4-K16 acetylation by Mof was shown to antagonize Iswi function *in vivo* (Corona *et al*, 2002) and to negatively regulate interactions between Iswi and its nucleosomal

substrate *in vitro* (Clapier *et al*, 2002). Interactions between HAT and remodelling complexes remain otherwise poorly characterized in this organism. Here, we provide evidence for functional interactions between the ATAC and NURF complexes. In addition, our results show that NURF is required for proper histone acetylation of chromosomes by ATAC and indicate that this interplay is involved in the maintenance of higher order chromosome structure.

RESULTS

Genetic interactions between ATAC and Iswi

To test whether Gcn5 and Iswi act in the same gene regulatory pathway during development, we analysed genetic interactions between *Iswi* and *Gcn5* mutant alleles. We noticed first that homozygous $Iswi^{2}$ or $Gcn5^{E333st}$ animals die at the end of the third larval instar, whereas double homozygous $Iswi^{2}$ $Gcn5^{E333st}$ animals die during the first larval instar, indicating that the combination of zygotic *Iswi* and *Gcn5* loss of function impairs early development more severely than either mutation alone.

We then took advantage of a transgenic construct that expresses a dominant-negative form of Iswi. Expression of $Iswi^{K159R}$ in eye-antennal imaginal discs leads to small, rough eyes in about 20% of adults (Deuring *et al*, 2000; Fig 1). The occurrence of this phenotype was significantly increased in heterozygous *Gcn5*/+ mutant backgrounds as well as after *Gcn5* RNA interference (RNAi) knockdown ($P<0.0001$; Fig 1). A similar enhancement of the $Iswi^{K159R}$ eye phenotype was observed in an *Ada2a*/+ mutant background, whereas an *Ada2b* mutant allele had only a modest effect ($P<0.0001$ and $P=0.0137$, respectively). These results point to a functional interaction between ATAC and Iswi during development.

Gcn5, Ada2a and Nurf301 regulate common target genes

In an attempt to define ATAC target genes, we compared whole-genome transcript profiles of homozygous *Gcn5* and *Ada2a* mutant larvae with those of corresponding heterozygous larvae at the end of the third larval instar (supplementary Table 1 online). Among the genes significantly affected in mutants as compared with control samples ($P<0.05$), a total of 284 and 1,625 genes were decreased by at least a factor of three in *Gcn5* and *Ada2a* mutants, respectively. Previous whole-genome expression profile analyses have shown that loss of function of Nurf301, a specific subunit of the Iswi-containing NURF complex, results in a threefold downregulation of 274 genes at the end of the third larval instar (Badenhorst *et al*, 2005). Strikingly, among these 274 genes, 55 ($P=2.6\times10^{-6}$) and 120 ($P=8.0\times10^{-7}$) genes are also downregulated in *Gcn5* and *Ada2a* mutants, respectively (Fig 2). Moreover, 43 genes are repressed in all three mutants (supplementary Table 2 online).

To validate our data set, we further analysed *Ultrabithorax* (*Ubx*), *engrailed* (*en*) and heat-shock protein 70 (*hsp70*) candidate gene expression in *Gcn5* mutants. All three genes were shown to have reduced expression in *Nurf301* mutants (Badenhorst *et al*, 2002). We also found that they are downregulated in *Gcn5* mutant larvae. In addition, heat-shock induction of *hsp70* was reduced to 50% and 35% of the wild-type induction level in $Gcn5^{E333st}$ and $Gcn5^{f02830}$ mutant larvae, respectively—an effect similar to that observed in *Nurf301* mutants (supplementary Fig S1 online).

A

Genotype	% class 1	% class 2	Total count	χ^2	*P*
$Iswi^{K159R}$/*Tm3, Sb*	22	78	117		
$Iswi^{K159R}$/$Gcn5^{E333st}$	72	28	36	30.6757	<0.0001
$Iswi^{K159R}$/$Gcn5^{f02830}$	70	30	54	36.5739	<0.0001
$Iswi^{K159R}$/IR[Gcn5]	59	41	44	19.8770	<0.0001
$Iswi^{K159R}$/$Ada2a^{\Delta189}$	67	33	44	27.1330	<0.0001
$Iswi^{K159R}$/$Ada2b^{\Delta842}$	42	58	43	6.0823	<0.0137

B

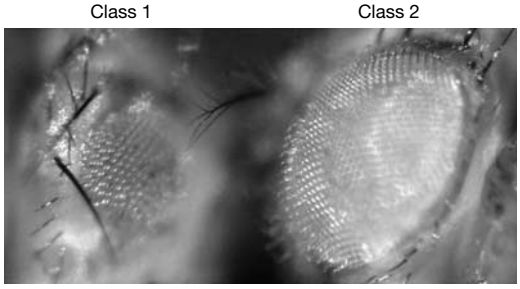

Fig 1 | ATAC and Iswi interact functionally during *Drosophila* development. Decreased *Gcn5* or *Ada2a* expression enhances the *Iswi* dominant-negative phenotype induced by expression of $Iswi^{K159R}$ in the eye. (**A**) Enhancement of $Iswi^{K159R}$ dominant-negative phenotype by *Gcn5* and *Ada2a* mutant alleles. *eye*-Gal4, UAS-$Iswi^{K159R}$/*Tm3*, *Ser* females were crossed with RNAi trigger UAS-IR[Gcn5] transgenic males or *Tm3*, *Sb* balanced $Gcn5^{E333st}$, $Gcn5^{f02830}$, $Ada2a^{\Delta189}$ or $Ada2b^{\Delta842}$ heterozygous males. Progenies of the indicated genotype were scored for eye defects as class 1 (>50% reduction in size) or class 2 (<50% reduction in size). The *eye*-Gal4, UAS-$Iswi^{K159R}$ chromosome is indicated in the table as $Iswi^{K159R}$. Results are expressed as percentages relative to the total count of siblings of the indicated genotype. Control $Iswi^{K159R}$/*Tm3*, *Sb* siblings were counted from the progeny of the *eye*-Gal4, UAS-$Iswi^{K159R}$/*Tm3*, *Ser* × $Gcn5^{E333st}$/*Tm3*, *Sb* cross. Equivalent counts were obtained for $Iswi^{K159R}$/*Tm3*, *Sb* siblings from the other crosses. Statistical significance was first assessed by a χ^2 homogeneity test with 5 degrees of freedom for error, which indicated that class-1 and class-2 frequencies vary with the tested genotypes ($P<0.0001$). Pairwise χ^2 tests were then performed for each mutant genotype as indicated, taking the $Iswi^{K159R}$/*Tm3*, *Sb* as reference. χ^2 and *P*-values indicate that class-1 and class-2 frequencies differ significantly from the control in all mutant combinations except in $Ada2b^{\Delta842}$ ($P=0.0137$). (**B**) A representative class-1 *eye*-Gal4, UAS-$Iswi^{K159R}$/$Gcn5^{E333st}$ adult fly is shown together with a representative class-2 *eye*-Gal4, UAS-$Iswi^{K159R}$/*Tm3*, *Sb* adult fly.

These data indicate that ATAC and NURF complexes are involved in the transcriptional regulation of a common set of target genes. In agreement, HAT-containing and nucleosome remodelling complexes were suggested to act synergistically at promoters to regulate target gene transcription (Featherstone, 2002). Although we cannot rule out that our genetic and transcript profiling data reflect indirect interactions, a similar interplay between NURF and ATAC might exist at *Drosophila* target promoters.

A role of ATAC and NURF in X chromosome structure

Genetic loss of function of Iswi as well as of the NURF subunit Nurf301 leads to a marked decondensation of the male X chromosome, indicating a role of this remodelling complex in

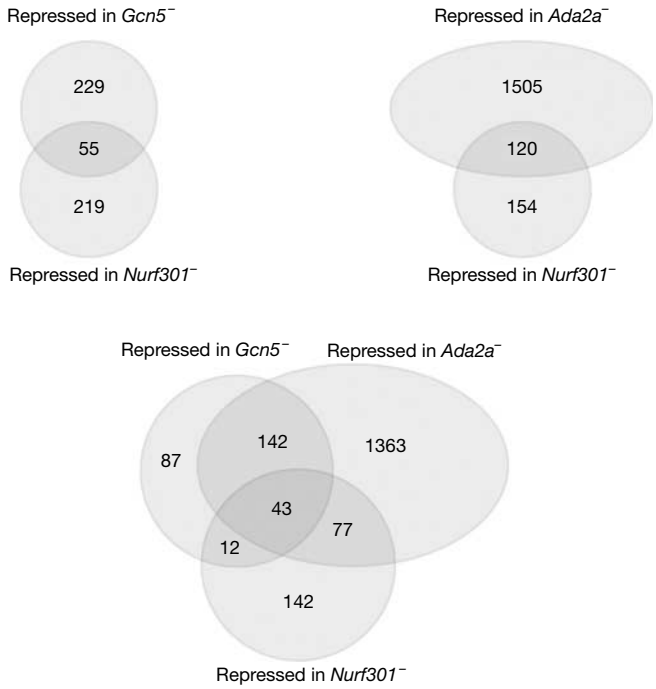

Fig 2 | Gcn5, Ada2a and Nurf301 regulate a common set of genes at the end of the larval phase. Venn diagrams describing the overlap of genes downregulated by at least a factor of three in *Gcn5*, *Ada2a* and *Nurf301* mutants at the end of the third larval instar.

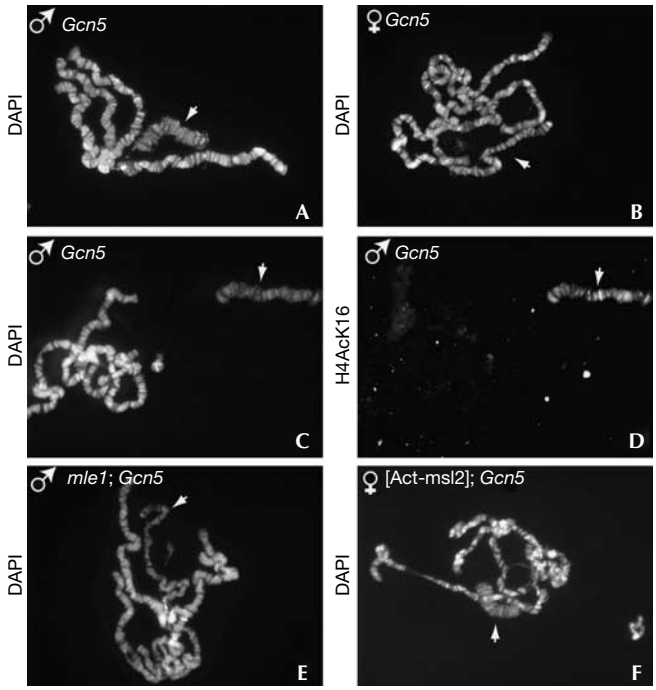

Fig 3 | *Gcn5* mutations induce male-specific X-chromosome decondensation. Polytene chromosomes from salivary glands were prepared from males and females homozygous for the (**A**,**B**) $Gcn5^{f02830}$ or (**C**,**D**) $Gcn5^{E333st}$ mutations and stained with 4,6-diamidino-2-phenylindole (DAPI) or an H4-AcK16 antibody, as indicated. (**E**) DAPI staining of polytene chromosomes from mle^{1}/mle^{1}; $Gcn5^{E333st}/Gcn5^{f02830}$ mutant male larvae. (**F**) DAPI staining of polytene chromosomes from *Act-msl2*/+; $Gcn5^{E333st}/Gcn5^{f02830}$ mutant female larvae. White arrows indicate X chromosomes.

the higher order chromosome structure (Deuring *et al*, 2000; Badenhorst *et al*, 2002; Corona *et al*, 2002). These observations prompted us to search for similar chromosome defects in *Gcn5* and *Ada2a* mutants.

We observed that *Gcn5* mutant males display a specific and highly penetrant decondensation of the X chromosome that is never observed in *Gcn5* mutant females (Fig 3A,B). In addition, previous studies had shown that the parallel alignment of interbands of polytene chromosomes is often disrupted in *Gcn5* mutants, leading to an altered banding pattern (Ciurciu *et al*, 2006).

In *Drosophila* males, the dosage compensation complex (DCC) containing maleless (Mle), male-specific lethal 1 (Msl1), Msl3 and the male-specific Msl2 protein recruits the Mof HAT to the X chromosome, resulting in a specific hyperacetylation of H4-K16 residues, which ensures a twofold increase of X-linked genes (Akhtar, 2003). To test whether the decondensation of the X chromosome reflects an alteration in the DCC, we analysed H4-K16 chromosome acetylation in *Gcn5* mutant males. Despite its bloated appearance, we found that the X chromosome in these males is still highly acetylated on H4-K16 residues (Fig 3C,D). We therefore explored the possibility that the particular structure conferred by the DCC to the male X chromosome makes it more susceptible to loss of function of *Gcn5*. The X-bloated phenotype was suppressed in *mle*, *Gcn5* double mutant males in which dosage compensation is impaired (Fig 3E). Moreover, we could induce X-chromosome decondensation by ectopically expressing Msl2 in *Gcn5* mutant females to induce DCC formation (Fig 3F). These experiments indicate that normal DCC function is necessary and sufficient to alter the structure of the X chromosome in *Gcn5* mutants. It is noteworthy that male X-chromosome decondensation induced by *Iswi* mutations shows a similar requirement for DCC function (Corona *et al*, 2002).

We also found a high frequency of X-bloated chromosomes in preparations from *Ada2a* mutant males but not females (Fig 4). Similar to the situation encountered in *Gcn5* mutant males, X-bloated chromosomes from *Ada2a* mutants were normally hyperacetylated on H4-K16 residues (Ciurciu *et al*, 2006). We did not observe X-chromosome bloating in *Ada2b* mutants (Fig 4). These data strongly suggest that the ATAC complex is required for a correct male X-chromosome structure. It can be noted that mutations affecting several chromatin-associated proteins, including Hp1, Su(var)3-7 and Jil1, also induce male-specific X-chromosome decondensation (Deng *et al*, 2005; Spierer *et al*, 2005). As proposed for these mutations, the effect of loss of function of ATAC probably reflects a higher sensitivity of the male X-chromosome structure conferred by the activity of the dosage compensation machinery, rather than a specific role in the regulation of X-linked genes. In support of this conclusion, genome-wide profiling did not show a disproportionate set of X-linked genes among the genes affected in *Gcn5* or *Ada2a* mutants (supplementary Table 1 online).

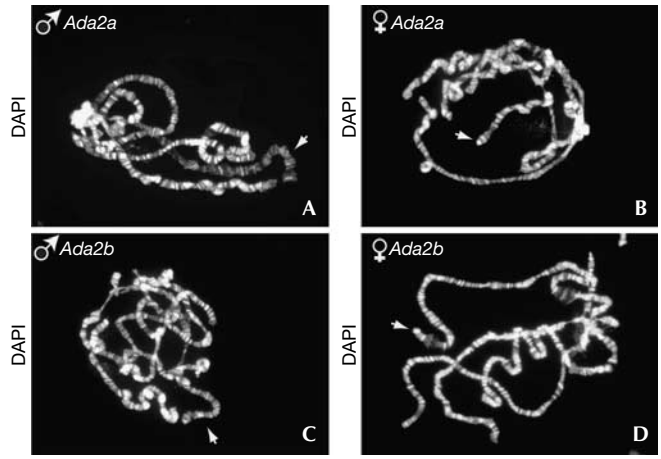

Fig 4 | Mutation of the ATAC subunit Ada2a induces male-specific X-chromosome bloating. Polytene chromosomes from homozygous (**A,B**) *Ada2a*$^{\Delta 189}$ or (**C,D**) *Ada2b*$^{\Delta 842}$ third instar mutant larvae were stained with 4,6-diamidino-2-phenylindole. White arrows indicate X chromosomes.

Iswi is required for ATAC chromosome binding

We explored further the functional relationships between NURF and ATAC. Whole-genome RNA profiling indicated that mutations in *Gcn5* or *Ada2a* do not significantly affect the expression of *Iswi* in late third instar larvae. In addition, immunostaining of polytene chromosomes from either of these mutants did not show changes in the Iswi binding pattern (supplementary Fig S2A online). In turn, quantitative reverse transcription–PCR analysis indicated that the *Iswi* mutation does not significantly alter the level of *Gcn5* and *Ada2a* expression (supplementary Fig S3 online). Strikingly, however, Ada2a binding was strongly reduced in the *Iswi* mutant compared with wild-type polytene chromosomes (Fig 5A,B; supplementary Fig S2B online). Non-specific loss of Ada2a binding could result from the general alteration of chromosomes caused by the *Iswi* mutation (Deuring *et al*, 2000). However, this does not seem to be the case, as the catalytic subunit of Pol II and, more importantly, the SAGA-specific subunit Ada2b remain associated with the *Iswi* mutant polytene chromosome under the same experimental conditions (Fig 5C,D; supplementary Fig S2A,B online).

We reasoned that a specific impairment of Ada2a binding to chromosomes should have an effect on their acetylation by ATAC. Indeed, acetylation of H4-K12 residues is markedly reduced in *Iswi* mutants (Fig 5E,F; supplementary Fig S4 online), whereas acetylation of H3-K9 and H3-K14 residues generated by SAGA does not seem to be affected (Fig 5H,I). A similar and specific decrease of H4-K12 acetylation of polytene chromosomes was observed in nuclei from the salivary glands of *Nurf301* mutants (Fig 5G–J; supplementary Fig S4B online). In agreement with these data, western blot analysis showed a significant decrease of H4-K12 acetylation in salivary glands from mutant *Iswi* and *Nurf301* late third instar larvae (supplementary Fig S5 online). These results strongly indicate that a functional NURF complex is required for the binding of ATAC to chromatin and for subsequent acetylation of H4-K12 residues.

Conclusions

Here we show that loss of function of the ATAC complex markedly affects the morphology of the *Drosophila* male X chromosome. This suggests that HATs, as previously shown for nucleosome remodelling complexes (Varga-Weisz & Becker, 2006), might also fulfil an architectural function to regulate higher order chromosome structures.

Interestingly, mutations of Hp1 and Su(var)3-7 also induce male X-chromosome bloating, suggesting that defects in heterochromatin formation lead to perturbation of higher order chromosome structure (Spierer *et al*, 2005). However, loss of function of ATAC probably perturbs chromosome structure through a distinct pathway: *Gcn5* mutations do not suppress the variegation of heterochromatic markers and do not affect the methylation of H3-K9 residues as well as the recruitment of Hp1 to chromosomes (Carre *et al*, 2005; data not shown).

By contrast, we observed genetic interactions between *Iswi*, *Gcn5* and *Ada2a*, and transcripts downregulated in *Nurf301*, *Gcn5* and *Ada2a* mutants significantly overlap. These data indicate an interplay between ATAC and NURF. Strikingly, *Iswi* mutation impairs the binding of Ada2a to chromosomes, and both *Iswi* and *Nurf301* mutations strongly reduce acetylation of H4-K12 residues. This suggests that NURF is required for the recruitment of ATAC to chromatin and for subsequent acetylation of H4-K12 residues by this complex.

Speculation

A mutation of the histone variant H2Av was shown to impair the acetylation of H4-K12 residues on polytene chromosomes (Labrador & Corces, 2003). However, this mutation, in contrast to the *Gcn5* and *Ada2a* mutations, does not induce male X-chromosome decondensation (C.C., unpublished data). It is therefore unlikely that loss of histone H4 acetylation at K12 residues is responsible *per se* for perturbations of chromosome structure. Gcn5 also acetylates non-histone substrates (Sterner & Berger, 2000). Indeed, Gcn5 was recently shown to acetylate the Rsc4 subunit of the RSC remodelling complex in yeast, potentially modulating RSC binding to its nucleosomal substrate (Vandemark *et al*, 2007). Moreover, *Drosophila* Gcn5 efficiently acetylates Iswi *in vitro*, and its depletion in cultured cells reduces the amount of acetylated Iswi (Ferreira *et al*, 2007). We speculate that NURF provides ATAC with access to chromatin and that acetylation of Iswi by Gcn5 might, in turn, be important for regulating its remodelling activity. Thus, the perturbation of such an interaction might result in changes in the global chromosome structure as well as in downregulation of NURF target genes.

METHODS

Fly strains. The Act-[msl2] transgenic line and *mle*^{1} mutants were provided by B. Baker and the *Iswi* and *Nurf301* mutant stocks were provided by P. Badenhorst. The *Gcn5*^{E333st} null mutant stock has been previously characterized (Carre *et al*, 2005). Homozygous *Gcn5*^{f02830} null mutants (Excelexis collection) were fully rescued by a *Gcn5* genomic construct. Homozygous *Ada2a*$^{\Delta 189}$ and *Ada2b*$^{\Delta 842}$ null mutants were obtained by genetic crossing as described (Pankotai *et al*, 2005). The enhancement of the Iswi^{K159R} dominant-negative phenotype in eyes was examined by crossing an *eye*-Gal4, UAS-Iswi^{K159R}/*Tm3*, *Ser* stock (Papoulas *et al*, 2001) with UAS-IR[Gcn5] RNAi (Roignant *et al*, 2003),

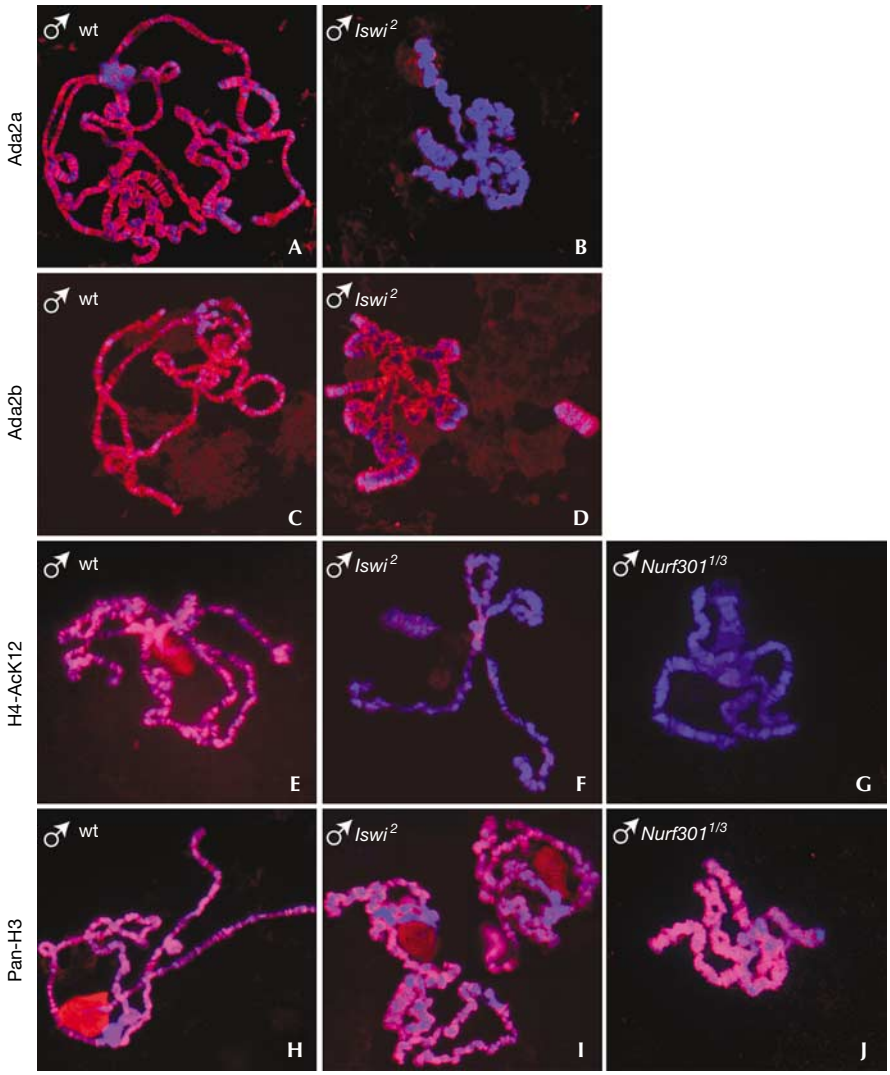

Fig 5 | Binding of Ada2a to chromosomes and H4-AcK12 acetylation are impaired by mutations in components of the Iswi-containing NURF remodelling complex. Polytene chromosomes from wild-type (wt), homozygous *Iswi*^{2} and heteroallelic *Nurf301*^{1}/*Nurf301*^{3} mutant males were co-stained with 4,6-diamidino-2-phenylindole (blue) and Ada2a, Ada2b, H4-AcK12 or H3-AcK9/14 (red) antibodies, as indicated.

Gcn5^{E333st}*/Tm3 Sb, Gcn5*^{f02830}*/Tm3 Sb, Ada2a*$^{\Delta 189}$*/Tm3 Sb* or *Ada2b*$^{\Delta 842}$*/Tm3 Sb* stocks.

Polytene chromosome staining. Immunostaining of polytene chromosomes was performed as described (Zink & Paro, 1995). Antibodies against H4-AcK16 (Turner *et al*, 1992), H4-AcK12 and H3-AcK9/K14 (Upstate Laboratory, Millipore, Saint-Quentin-en-Yvenlines, France) were used at 1:200 dilution. The Iswi antibody (provided by J. Tamkun) was used at 1:150 dilution. Pol II, Ada2a and Ada2b antibodies were used as described (Muratoglu *et al*, 2003).

Whole-genome expression analysis. RNA was isolated from homozygous and heterozygous *Gcn5*^{E333st} and *Ada2a*$^{\Delta 189}$ third instar larvae before pupariation and was labelled and hybridized to Affymetrix *Drosophila* genome arrays, as detailed in the supplementary information online. A full set of microarray data (experiment E-MEXP-1208) is available from the ArrayExpress database (http:www.ebi.ac.uk/arrayexpress/).

Supplementary information is available at *EMBO reports* online (http://www.emboreports.org).

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REFERENCES

Akhtar A (2003) Dosage compensation: an intertwined world of RNA and chromatin remodelling. *Curr Opin Genet Dev* **13:** 161–169

Badenhorst P, Voas M, Rebay I, Wu C (2002) Biological functions of the ISWI chromatin remodeling complex NURF. *Genes Dev* **16:** 3186–3198

Badenhorst P, Xiao H, Cherbas L, Kwon SY, Voas M, Rebay I, Cherbas P, Wu C (2005) The *Drosophila* nucleosome remodeling factor NURF is required for Ecdysteroid signaling and metamorphosis. *Genes Dev* **19:** 2540–2545

Brownell JE, Zhou J, Ranalli T, Kobayashi R, Edmondson DG, Roth SY, Allis CD (1996) *Tetrahymena* histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. *Cell* **84:** 843–851

Cairns BR (2005) Chromatin remodeling complexes: strength in diversity, precision through specialization. *Curr Opin Genet Dev* **15:** 185–190

Carre C, Szymczak D, Pidoux J, Antoniewski C (2005) The histone H3 acetylase dGcn5 is a key player in *Drosophila melanogaster* metamorphosis. *Mol Cell Biol* **25:** 8228–8238

Ciurciu A, Komonyi O, Pankotai T, Boros IM (2006) The *Drosophila* histone acetyltransferase Gcn5 and transcriptional adaptor Ada2a are involved in nucleosomal histone H4 acetylation. *Mol Cell Biol* **26:** 9413–9423

Clapier CR, Nightingale KP, Becker PB (2002) A critical epitope for substrate recognition by the nucleosome remodeling ATPase ISWI. *Nucleic Acids Res* **30:** 649–655

Corona DF, Clapier CR, Becker PB, Tamkun JW (2002) Modulation of ISWI function by site-specific histone acetylation. *EMBO Rep* **3:** 242–247

Deng H, Zhang W, Bao X, Martin JN, Girton J, Johansen J, Johansen KM (2005) The JIL-1 kinase regulates the structure of *Drosophila* polytene chromosomes. *Chromosoma* **114:** 173–182

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

Elfring LK, Deuring R, McCallum CM, Peterson CL, Tamkun JW (1994) Identification and characterization of *Drosophila* relatives of the yeast transcriptional activator SNF2/SWI2. *Mol Cell Biol* **14:** 2225–2234

Featherstone M (2002) Coactivators in transcription initiation: here are your orders. *Curr Opin Genet Dev* **12:** 149–155

Ferreira R, Eberharter A, Bonaldi T, Chioda M, Imhof A, Becker PB (2007) Site-specific acetylation of ISWI by GCN5. *BMC Mol Biol* **8:** 73

Guelman S, Suganuma T, Florens L, Weake V, Swanson SK, Washburn MP, Abmayr SM, Workman JL (2006) The essential gene *wda* encodes a WD40 repeat subunit of *Drosophila* SAGA required for histone H3 acetylation. *Mol Cell Biol* **26:** 7178–7189

Ito T, Bulger M, Pazin MJ, Kobayashi R, Kadonaga JT (1997) ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. *Cell* **90:** 145–155

Kusch T, Guelman S, Abmayr SM, Workman JL (2003) Two *Drosophila* Ada2 homologues function in different multiprotein complexes. *Mol Cell Biol* **23:** 3305–3319

Labrador M, Corces VG (2003) Phosphorylation of histone H3 during transcriptional activation depends on promoter structure. *Genes Dev* **17:** 43–48

Muratoglu S *et al* (2003) Two different *Drosophila* ADA2 homologues are present in distinct GCN5 histone acetyltransferase-containing complexes. *Mol Cell Biol* **23:** 306–321

Pankotai T, Komonyi O, Bodai L, Ujfaludi Z, Muratoglu S, Ciurciu A, Tora L, Szabad J, Boros I (2005) The homologous *Drosophila* transcriptional adaptors ADA2a and ADA2b are both required for normal development but have different functions. *Mol Cell Biol* **25:** 8215–8227

Papoulas O, Daubresse G, Armstrong JA, Jin J, Scott MP, Tamkun JW (2001) The HMG-domain protein BAP111 is important for the function of the BRM chromatin-remodeling complex *in vivo*. *Proc Natl Acad Sci USA* **98:** 5728–5733

Peterson CL, Laniel MA (2004) Histones and histone modifications. *Curr Biol* **14:** R546–R551

Qi D, Larsson J, Mannervik M (2004) *Drosophila* Ada2b is required for viability and normal histone H3 acetylation. *Mol Cell Biol* **24:** 8080–8089

Roignant JY, Carre C, Mugat B, Szymczak D, Lepesant JA, Antoniewski C (2003) Absence of transitive and systemic pathways allows cell-specific and isoform-specific RNAi in *Drosophila*. *RNA* **9:** 299–308

Spierer A, Seum C, Delattre M, Spierer P (2005) Loss of the modifiers of variegation Su(var)3-7 or HP1 impacts male X polytene chromosome morphology and dosage compensation. *J Cell Sci* **118:** 5047–5057

Sterner DE, Berger SL (2000) Acetylation of histones and transcription-related factors. *Microbiol Mol Biol Rev* **64:** 435–459

Tsukiyama T, Wu C (1995) Purification and properties of an ATP-dependent nucleosome remodeling factor. *Cell* **83:** 1011–1020

Turner BM, Birley AJ, Lavender J (1992) Histone H4 isoforms acetylated at specific lysine residues define individual chromosomes and chromatin domains in *Drosophila* polytene nuclei. *Cell* **69:** 375–384

Vandemark AP, Kasten MM, Ferris E, Heroux A, Hill CP, Cairns BR (2007) Autoregulation of the rsc4 tandem bromodomain by gcn5 acetylation. *Mol Cell* **27:** 817–828

Varga-Weisz PD, Becker PB (2006) Regulation of higher-order chromatin structures by nucleosome-remodelling factors. *Curr Opin Genet Dev* **16:** 151–156

Varga-Weisz PD, Wilm M, Bonte E, Dumas K, Mann M, Becker PB (1997) Chromatin-remodelling factor CHRAC contains the ATPases ISWI and topoisomerase II. *Nature* **388:** 598–602

Xiao H, Sandaltzopoulos R, Wang HM, Hamiche A, Ranallo R, Lee KM, Fu D, Wu C (2001) Dual functions of largest NURF subunit NURF301 in nucleosome sliding and transcription factor interactions. *Mol Cell* **8:** 531–543

Zink D, Paro R (1995) *Drosophila* Polycomb-group regulated chromatin inhibits the accessibility of a *trans*-activator to its target DNA. *EMBO J* **14:** 5660–5671