

Biological functions of the ISWI chromatin remodeling complex NURF

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The nucleosome remodeling factor (NURF) is one of several ISWI-containing protein complexes that catalyze ATP-dependent nucleosome sliding and facilitate transcription of chromatin *in vitro*. To establish the physiological requirements of NURF, and to distinguish NURF genetically from other ISWI-containing complexes, we isolated mutations in the gene encoding the large NURF subunit, *nurf301*. We confirm that NURF is required for transcription activation *in vivo*. In animals lacking NURF301, heat-shock transcription factor binding to and transcription of the *hsp70* and *hsp26* genes are impaired. Additionally, we show that NURF is required for homeotic gene expression. Consistent with this, *nurf301* mutants recapitulate the phenotypes of *Enhancer of bithorax*, a positive regulator of the Bithorax-Complex previously localized to the same genetic interval. Finally, mutants in NURF subunits exhibit neoplastic transformation of larval blood cells that causes melanotic tumors to form.

[*Keywords:* NURF; chromatin remodeling; ISWI; *Drosophila*; JAK/STAT pathway]

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The establishment of the body plan of an animal requires the correct initiation and maintenance of expression of a number of developmental regulators. In insects, segmental identity is conferred by region-specific expression of overlapping sets of homeotic selector genes. Systematic genetic screens for changes in segmental fate have been used to identify a wide class of positive-acting transregulatory factors required for homeotic gene function, the *trithorax-group* (*trxG*; Kennison 1995). Members of the *trxG* define novel chromatin-modifying activities. One class comprises the ATP-dependent chromatin remodeling factors. These are multisubunit protein complexes that utilize the energy of ATP hydrolysis to alter gene architecture by perturbing the dynamic properties of nucleosomes, the basic units of chromatin. They can be divided into broad families depending on the catalytic subunit utilized. Brahma, one of the defining members of the *trxG*, and its yeast homolog SWI2/SNF2 provide the catalytic subunit of the first group of SWI/SNF chromatin remodelers identified (Tamkun et al. 1992).

Other categories of ATP-dependent chromatin remodeling complexes have been discovered using biochemical approaches. In *Drosophila*, the ISWI ATPase forms the catalytic core of three protein complexes that have been purified from nuclear extracts: ACF (ATP-utilizing chromatin assembly and remodeling factor; Ito et al. 1997),

CHRAC (chromatin accessibility complex; Varga-Weisz et al. 1997) and NURF (nucleosome remodeling factor; Tsukiyama et al. 1995). All catalyze energy-dependent nucleosome sliding *in vitro* (Corona et al. 1999; Hamiche et al. 1999; Langst et al. 1999), but with distinct results by the different complexes. ACF and CHRAC can assemble and slide nucleosomes to establish regular ordered arrays (Ito et al. 1997; Varga-Weisz et al. 1997). In contrast, NURF-induced sliding disrupts nucleosome periodicity (Tsukiyama and Wu 1995; Hamiche et al. 1999). NURF acts in concert with transcription factors to mobilize nucleosomes at promoters, facilitating transcription activation *in vitro* (Tsukiyama and Wu 1995; Mizuguchi et al. 1997). These properties suggest that NURF should be required for transcription *in vivo*.

NURF is a complex of four protein subunits. In addition to NURF140—the ISWI ATPase (Tsukiyama et al. 1995)—NURF contains NURF55, a WD-40 repeat protein found in other protein complexes involved in histone metabolism (Martinez-Balbas et al. 1998); NURF38, an inorganic pyrophosphatase (Gdula et al. 1998); and a large subunit NURF301 (Xiao et al. 2001). NURF301 is distantly related to the ACF1/WCRF1 subunits of the ACF and WCRF chromatin remodeling and assembly complexes (Ito et al. 1999). NURF301 contains the PHD fingers, bromodomain, WSTF/Acf1/cbp146 (WAC), and WSTF/Acf1/KIAA0314/ZK783.4 (WAKZ) motifs found in these proteins, but also possesses an N-terminal HMGI/Y-like domain. Reconstitution experiments reveal that both NURF301 and ISWI contribute to the

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chromatin remodeling activities of NURF. In addition, NURF301 provides an interface through which NURF can interact with a number of transcription factors, in particular the GAGA transcription factor (Xiao et al. 2001).

We sought to characterize the physiological function of NURF. Clues to this have been provided by mutations that affect the catalytic ISWI subunit. Analysis of *iswi* mutant animals indicates that ISWI is required for homeotic gene activation and chromatin condensation in vivo (Deuring et al. 2000). However, as ISWI is present in NURF and at least two other *Drosophila* chromatin remodeling complexes—ACF (Ito et al., 1997) and CHRAC (Varga-Weisz et al. 1997)—functions could not be ascribed to a specific remodeling complex. Given the importance of NURF301 for the in vitro activities of NURF, we focused our studies on NURF301 and isolated mutations in this largest NURF subunit. Here we show that *nurf301* is required for transcription activation in vivo. In *nurf301* mutants homeotic gene expression is impaired. Moreover, we demonstrate that *nurf301* corresponds to an uncharacterized member of the *trxG*, *Enhancer of bithorax* [*E(bx)*]. NURF301 is required for induction of the heat-shock genes and has additional functions during larval blood cell development.

Results

Isolation and characterization of *nurf301* mutants

The gene encoding the large NURF subunit, *nurf301*, was mapped by chromosomal in situ hybridization to the cytological interval 61A. To isolate *nurf301* mutants, we first screened P-element lines that had been localized to this region for those that contained inserts within or immediately upstream of *nurf301*. We identified one homozygous lethal P-element line (*l(3)ry122* we call *nurf301*¹), in which a P-element had inserted in the untranslated leader sequence of *nurf301* (summarized in Fig. 1A). Subsequently, we recovered a series of EMS-induced mutations that failed to complement *nurf301*¹. The three alleles presented (*nurf301*², *nurf301*³, and *nurf301*⁴) encode truncated NURF301 proteins due to the introduction of stop codons at amino acids 546, 750, and 1536, respectively. Homozygous *nurf301* mutants (and heteroallelic combinations) were lethal at late-third larval instar or early pupal stages. We also identified three homozygous viable EP insertion lines (Rorth 1996) in which P-elements had inserted upstream of the *nurf301* transcription start site. These were used as substrates for imprecise excision to recover lethal excision lines that fail to complement *nurf301*¹. The deficiency

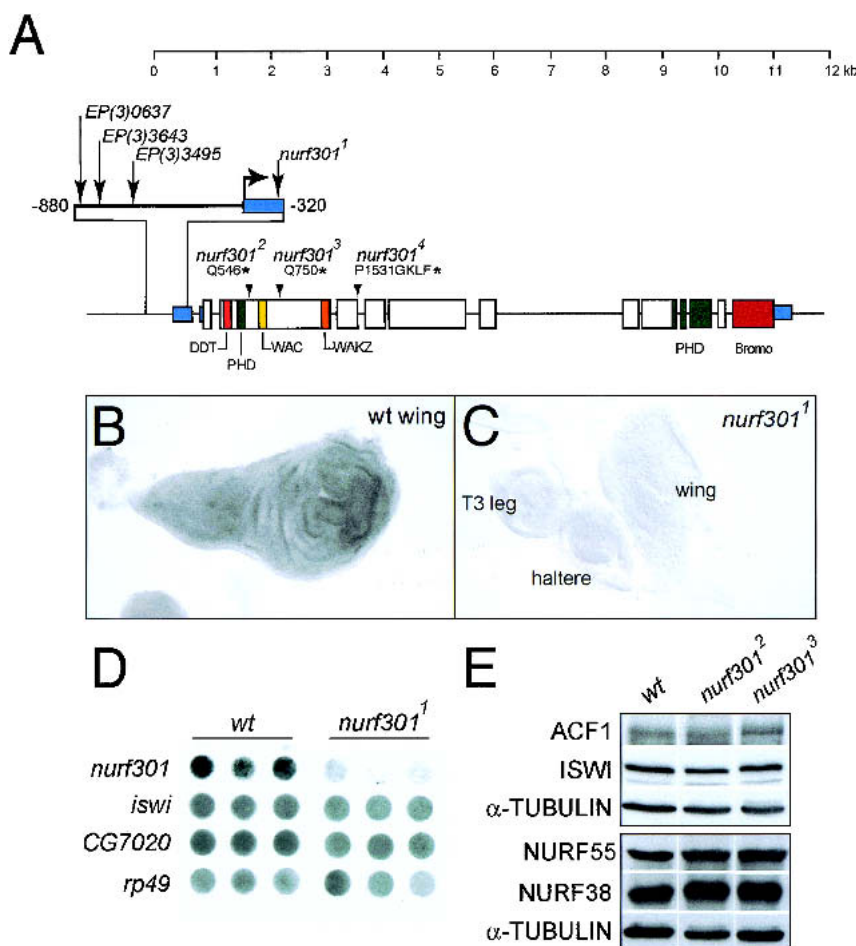


Figure 1. Characterization of *nurf301* mutants. (A) The structure of the *nurf301* locus, the position of P-element insertion lines, and the location of EMS-induced lesions are shown. *nurf301*² and *nurf301*³ encode glutamine to stop codon changes at amino acids 546 and 750, respectively. *nurf301*⁴ contains a splice-donor site mutation that blocks splicing of the fourth intron. The aberrant transcript introduces four additional amino acids (GKLF) and an in-frame stop codon after proline residue 1531. (B,C) In situ hybridization using a 1.3-kb probe spanning the 3' end of *nurf301* (nucleotides 6800–8000) shows that ubiquitous expression of *nurf301* is lost in homozygous mutant *nurf301*¹ larvae (cf. wild-type and mutant third instar discs). (D) Dot-blot analysis of third instar total RNA (in triplicate) shows that *nurf301* RNA is reduced in *nurf301*¹ mutants relative to wild-type. In contrast, *iswi* and CG7020, a putative gene located upstream of *nurf301* (a fragment from the EST clone LP07661 was used as a probe), are unaffected. *rp49* provides a loading control. (E) Protein levels of the NURF subunits. NURF38, NURF55, and ISWI, and the ACF1 subunit of ACF are unchanged in *nurf301*² and *nurf301*³ mutants, relative to α -TUBULIN as shown by Western analysis of extracts from third instar larvae.

Df(3L)3643 was characterized by PCR and shown to remove the ATG of *nurf301* and at least two flanking genes downstream of *nurf301*.

We showed by in situ hybridization (Fig. 1B,C) and Northern analysis (Fig. 1D) that the normal expression of *nurf301* is reduced in the P-element insertion line *nurf301¹*. In this mutant, transcription of *iswi* or a predicted gene upstream of *nurf301* (CG7020) is unaffected (Fig. 1D). We confirmed that lethality of this line is caused by the P-element insertion as precise excision of the P-element restores viability. In all the *nurf301* mutants, the protein levels of the three other NURF subunits (ISWI, NURF55, and NURF38) and the ACF1 subunit of ACF and CHRAC are unchanged (data shown for *nurf301²* and *nurf301³* in Fig. 1E). This indicates that the *nurf301* mutations specifically compromise NURF activity without significantly affecting the other ISWI-containing complexes ACF or CHRAC.

Heat-shock gene activation is impaired in nurf301 mutant animals

NURF had originally been purified from nuclear extracts as a factor required to disrupt chromatin assembled in vitro on the promoters of the *Drosophila* heat-shock gene *hsp70*. Using in vitro assays it was shown that NURF cooperates with the GAGA factor to mobilize nucleosomes on the promoter of the heat-shock genes, establishing a nucleosome-free domain over the promoter, thus exposing sites for the heat-shock transcription factor (HSF; Tsukiyama et al. 1994; Tsukiyama and Wu 1995).

We examined if expression of the heat shock genes is affected in *nurf301* mutant animals. RNA dot-blot analysis shows that heat-shock-induced transcription of *hsp26* and *hsp70* is impaired in *nurf301¹* larvae (Fig. 2A). Impaired transcription leads to reduced heat-shock protein accumulation. Although HSP70 is detected in wild-type siblings after 10 min heat-shock, no protein is expressed in mutant animals (Fig. 2B). After 20 min of heat-shock, HSP70 is highly expressed in wild-type larvae. In contrast, *nurf301* mutant animals only express low levels of HSP70 (Fig. 2B).

Consistent with these findings, binding of the heat shock transcription factor HSF is impaired in *nurf301* mutants. In wild-type animals, immunofluorescence of salivary gland polytene chromosomes shows that, with the exception of the *hsp83* locus at 63B, HSF does not generally bind to polytene chromosomes in the absence of heat stress (Fig. 2C). HSF-binding to *hsp83* has been shown to be qualitatively different from the other heat-shock loci (Shopland and Lis 1996). The *hsp83* locus does not appear to be GAGA factor-dependent as no (GA.CT)_n elements are present in the promoter, nor does GAGA factor accumulate at this locus (Tsukiyama et al. 1994). Instead, HSF-binding is proposed to be facilitated by other transacting factors (Shopland and Lis 1996).

Within several minutes of heat-shock, the bulk of HSF forms trimers competent for specific DNA-binding, and binds to the heat-shock loci and a number of other sites

(Westwood et al. 1991). Prominent are the 87A and 87C loci (Fig. 2C, bracketed) which carry two and three copies, respectively, of the *hsp70* gene. In *nurf301* mutant animals, *hsp83* accumulates HSF as in nonshocked conditions. After 2 or 5 min heat-shock, no binding of HSF to *hsp70* loci is detectable (Fig. 2C). HSF-binding to the *hsp70* loci can only be detected after 10 min of heat-shock, and HSF staining is reduced compared to wild-type animals (Fig. 2C). Impaired HSF-binding is accompanied by reduced accumulation of RNA polymerase II at the *hsp70* loci (data not shown).

nurf301 is required for homeotic gene expression

Deuring et al. (2000) had shown that ISWI, the catalytic subunit of NURF, is required for expression of the homeotic gene *engrailed* (*en*). However, ISWI is also a component of two other chromatin remodeling complexes, ACF (Ito et al. 1997) and CHRAC (Varga-Weisz 1997). To resolve which ISWI-containing complex is required for homeotic gene expression we examined expression of *Ultrabithorax* (*Ubx*) and *engrailed* (*en*) in *nurf301* mutant animals. We found that when both copies of *nurf301* are mutated, in homozygous mutant *nurf301¹* larvae, expression of the UBX protein becomes undetectable. The normal expression of UBX in the haltere and third leg discs of wild-type third instar larvae is absent in *nurf301* mutant animals (Fig. 3A,B). We also showed that expression of the homeotic gene *en* requires *nurf301*. The normal expression of EN in the posterior compartment of imaginal discs is abolished in *nurf301²* mutants (Fig. 3C,D). Semiquantitative RT-PCR analysis confirms that *Ubx* and *en* transcript levels are reduced in *nurf301* mutant animals. These results confirm that the defects in homeotic transcription seen in *iswi* mutants are caused by abrogated NURF function.

nurf301 recapitulates E(bx) phenotypes

A positive regulator of the Bithorax-Complex, *E(bx)*, had previously been localized genetically to 61A, the same cytological interval as *nurf301* (Bhosekar and Babu 1987; Lewis cited in Lindsley and Zimm 1992). However, unlike numerous regulators of the BX-C, *E(bx)* had not been cloned. As NURF is required for expression of *Ubx*, we tested whether *nurf301* corresponds to *E(bx)*. Both alleles of *E(bx)* were no longer extant, so we examined if the mutations we had isolated in *nurf301* recapitulated the published morphological properties of *E(bx)* mutants.

We found that *nurf301* mutants, like *E(bx)*, increase the severity of *bithorax* (*bx*) mutant phenotypes. *bx* is a DNA regulatory element required for correct expression of *Ubx* in regions that give rise to the third (T3), but not second thoracic segment (T2) of the adult fly (Lewis 1978; Bender et al. 1983). This expression distinguishes T3 from T2 identity. Loss or reduction of UBX levels in *bx* mutant animals (*Ubx^{6.28}/bx^{34e}* and *Ubx^{6.28}/bx⁸* mutant combinations (shown in Fig. 4A,B) causes a homeotic transformation of the third thoracic segment to the

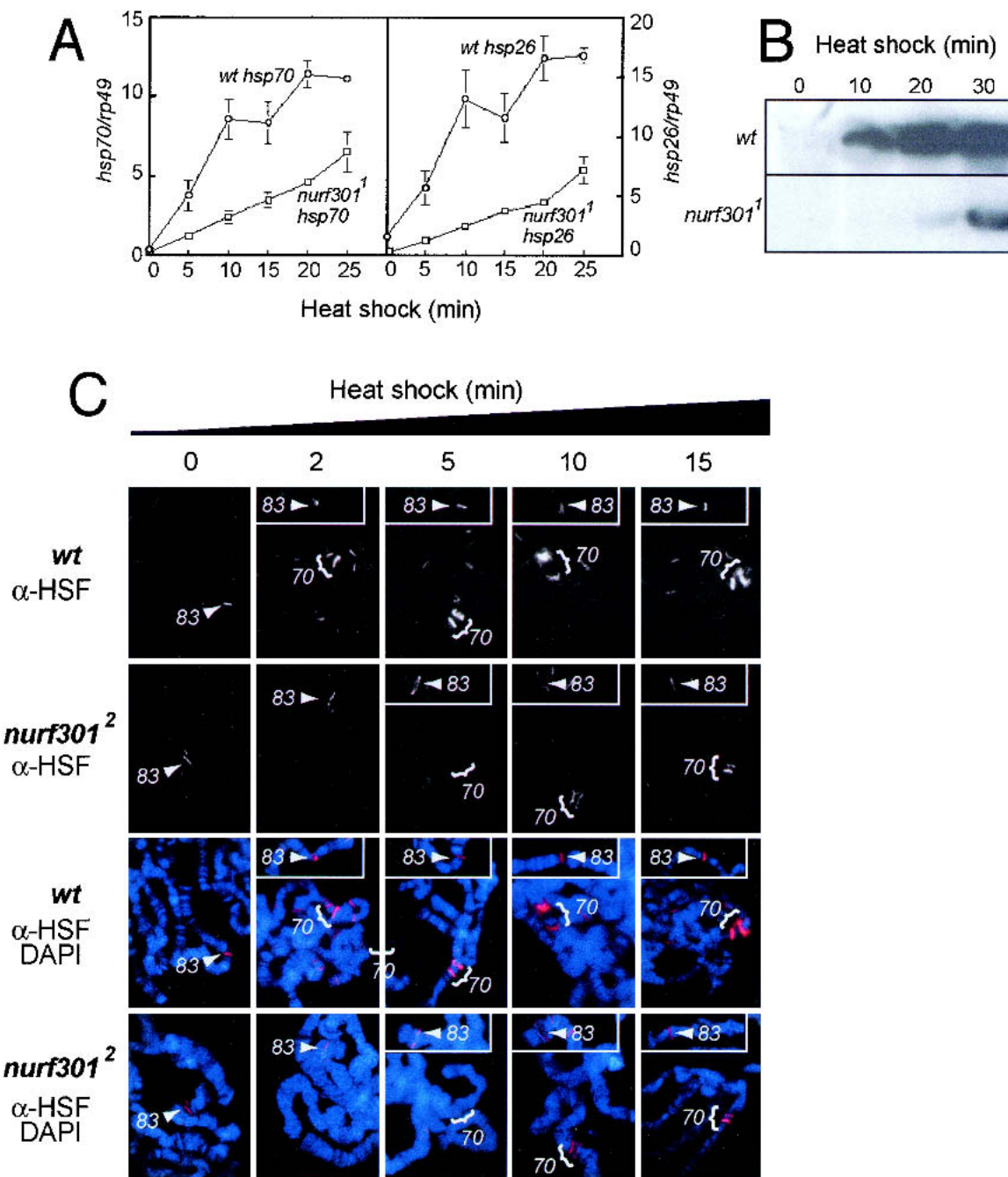


Figure 2. *nurf301* is required for heat-shock gene induction. (A) A quantitation of RNA dot-blots shows delay in *hsp26* and *hsp70* transcription in *nurf301*¹ mutant animals. *hsp26* and *hsp70* transcript levels are normalized to *rp49*. Points and bars represent the mean and standard deviation of three independent determinations. (B) Western blotting shows that HSP70 protein synthesis in homozygous mutant animals is reduced relative to the wild-type. (C) HSF was localized to the *hsp70* loci (brackets) and *hsp83* locus (arrowheads) on polytene chromosomes with antibodies against HSF, following 0-, 2-, 5-, 10- and 15-min heat-shock. HSF-binding to the major heat-shock loci is impaired in *nurf301*² mutants.

anterior second thoracic segment. Thus, the third thoracic segment, which is normally vestigial and naked, is transformed into the second thoracic segment, increasing its size and causing sensory bristles to develop (Fig. 4A). Moreover, the haltere (T3) is transformed toward wing fate (T2), manifested by increases in size and the

development of bristles. The strength of these transformations is increased when one copy of *E(bx)* also is removed (Bhosekar and Babu 1987). Mutation of one copy of *nurf301* similarly enhances *bx* phenotypes. With one copy of either the *nurf301*¹, *nurf301*² or a deficiency that removes *nurf301*—*Df(3L)3643*—the strength of the

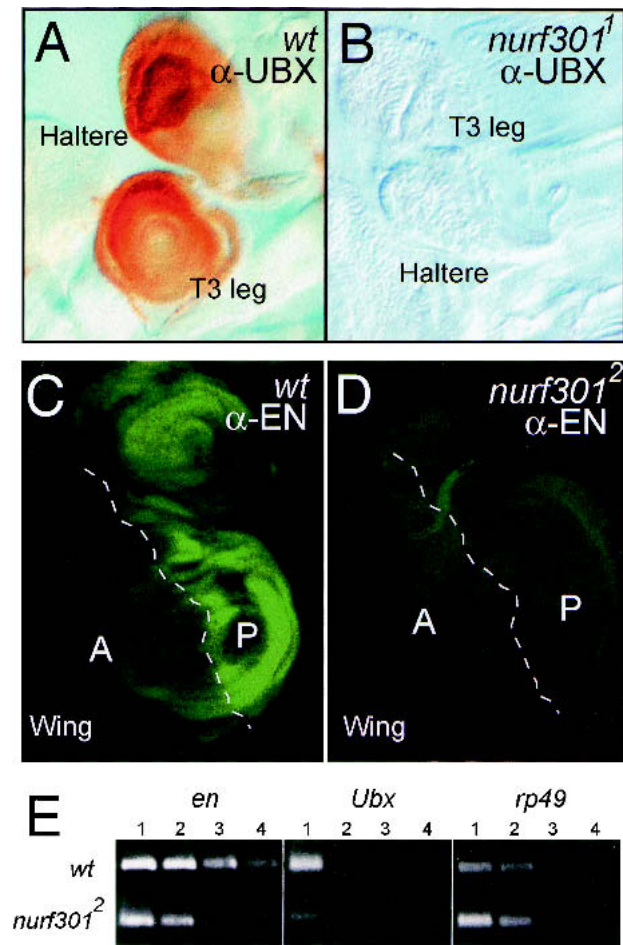


Figure 3. *nurf301* is required for homeotic gene expression. (A,B) UBX protein in imaginal discs of the third thoracic segment (brown staining) is undetectable in *nurf301*¹ homozygotes. (C,D) Antibody staining shows that EN protein (revealed in green), which normally can be detected in the posterior compartment of all imaginal discs, is lost in *nurf301*² mutant larvae. (E) Semiquantitative RT-PCR analysis confirms that *Ubx* and *en* transcript abundance is reduced between 5- and 25-fold in total RNA isolated from *nurf301* mutant animals. Lanes represent fivefold serial dilutions of mutant and wild-type total RNA (lane 1, 200 ng; lane 2, 40 ng; lane 3, 8 ng; and lane 4, 1.6 ng).

transformation is enhanced (Fig. 4A,B). *nurf301* enhances both *bx*^{34e} and *bx*⁸ mutations.

Although NURF is required for expression of the homeotic genes in imaginal discs, neither *E(bx)* nor *nurf301* homozygous mutant larvae display obvious homeotic transformations of the larval cuticle. The absence of mutant larval cuticle phenotypes is likely due to the large maternal dowry of *nurf301* transcript contributed to embryos (data not shown). Larval cuticular patterning is established before these transcripts have dissipated. We attempted to generate embryos lacking the maternal *nurf301* contribution through use of the dominant female sterile technique. Although we could produce germ-line clones using the parental chromosome, we were unable to recover germ-line clones using

*nurf301*¹. Like ISWI (Deuring et al. 2000), NURF301 is required for ovary development.

Aberrant male X chromosome morphology in nurf301 mutant animals

The defects in homeotic transcription seen in *nurf301* mutant animals effectively duplicate those reported for animals lacking the catalytic ISWI subunit (Deuring et al. 2000). However, ISWI is also required to maintain higher order chromosome structure. In *iswi* mutants the male X chromosome is grossly disrupted relative to autosomes as revealed by polytene chromosome preparations (Fig. 5B; Deuring et al. 2000). We found that in *nurf301* mutants the male X chromosome is similarly affected. The male X chromosome, identified by anti-MSL2 staining, is reduced in length and breadth, as seen in *iswi* mutant animals (cf. wild-type and mutant animals in Fig. 5A,D–F). The male X chromosome in homozygous mutant *nurf301*² animals is highly aberrant (Fig. 5D). Disorganized male X chromosome morphology also was observed in *nurf301*²/*nurf301*³ and hemizygous *nurf301*¹/*Df(3L)3643* mutant animals (Fig. 5E,F). Our results demonstrate that the chromosome condensation defect caused by perturbed ISWI function is mediated through the NURF complex. Although the effects on male X chromosome structure suggest that NURF can influence global chromosome structure, we found that NURF function was not required for heterochromatic gene silencing. Reduction in NURF301 levels has no effect on position effect variegation (data not shown). This is consistent with results reported by Cryderman et al. (1999) who showed that *iswi* is not a modifier of PEV.

Mutations in NURF subunits affect hematopoietic development

During the course of this analysis we noticed that *nurf301* mutant animals display a high incidence of melanotic tumors. Melanotic tumors have previously been reported in a number of mutant backgrounds and are generally caused by neoplastic transformation of the larval blood cells. The circulating cells (hemocytes) of the larval blood or hemolymph provide one tier of the innate immune system of insects by encapsulating or engulfing pathogens (for review, see Immler and Hoffman 2001; Luo and Dearolf 2001; Tzou et al. 2002). A number of mutations have been shown to trigger the overproliferation and premature differentiation of hemocytes. Tumors form when these cells aggregate, or invade and encapsulate normal larval tissues (Gateff 1978; Rizki and Rizki 1979).

We observed melanotic tumors both in EMS-induced *nurf301* mutants that truncate NURF301, the P-element induced mutation that reduces *nurf301* transcript levels, and allelic combinations of these mutants (Fig. 6A). Tumor penetrance is extremely high (100% for *nurf301*² at 25°C). Consistent with tumor development, circulating hemocyte cell number was increased dramatically in hemolymph isolated from *nurf301* mutant animals (Fig.

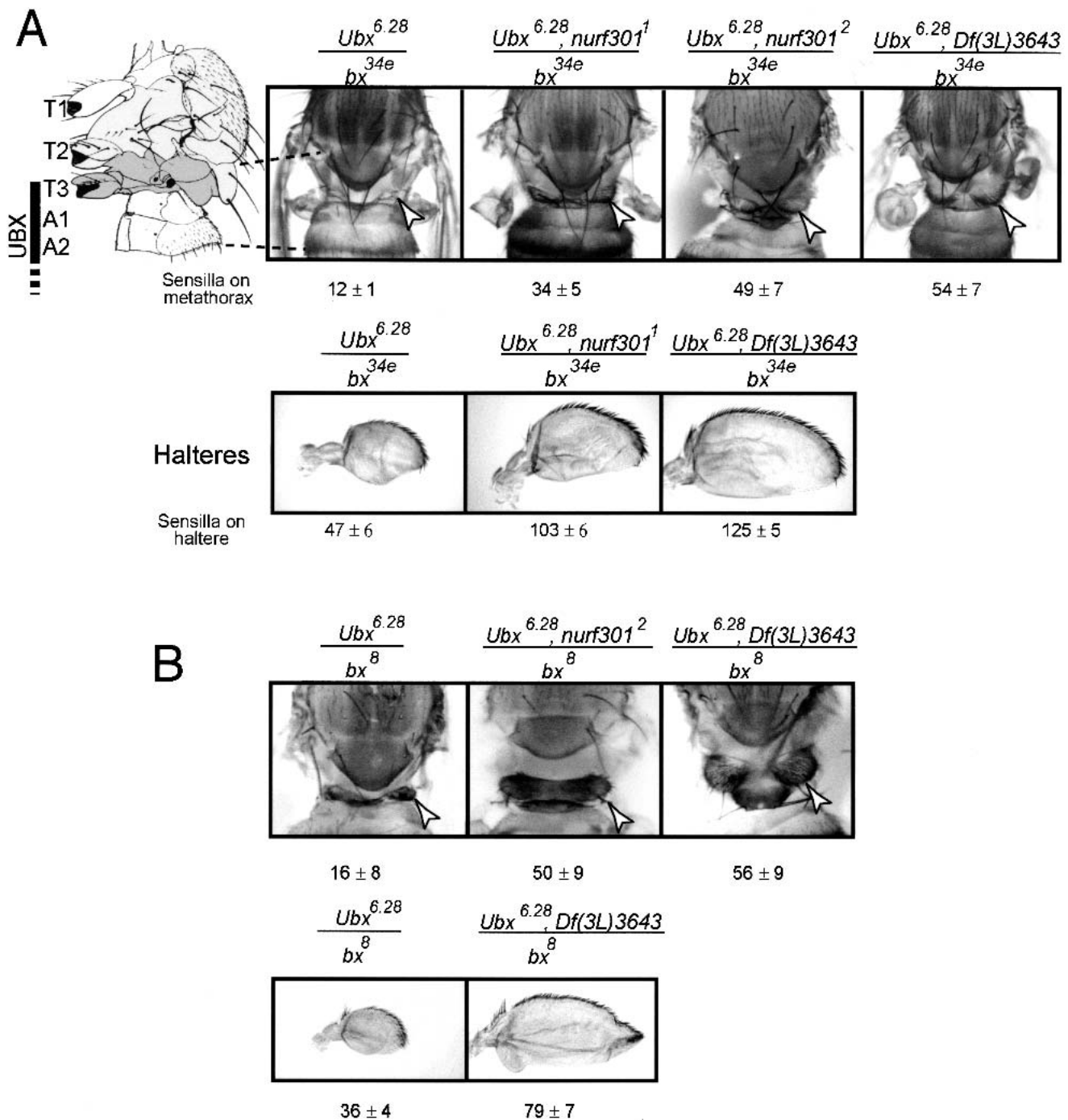


Figure 4. *nurf301* corresponds to *E(bx)*. In the presence of one copy of either *nurf301*¹, *nurf301*², or the *nurf301* deficiency *Df(3L)3643*, the strength of the anterior transformation seen in *bithorax* mutant combinations is increased. Transformation is scored both by increases in the size and bristle number on the metathoracic segment and the severity of the haltere (T3) to wing (T2) transformation. A shows *bx*^{34e}/*Ubx*^{6.28} combinations and B shows *bx*⁸/*Ubx*^{6.28} combinations. Mean haltere and T3 bristle numbers ± standard deviation for 20 flies of each genotype are indicated. Arrowheads denote the metathorax (T3). Photographs of nota are composites of three images taken at different focal planes of the animals.

6B). A large percentage of animals lacking ISWI, the catalytic subunit of NURF, also displayed melanotic tumors confirming that disrupted NURF function induces tumor formation (data not shown). In *iswi* mutant animals the number of circulating hemocytes is also increased

(Fig. 6B). In both *nurf301* and *iswi* mutant hemolymph, small aggregates of hemocytes are often observed. All hemocyte cell types are present, from small round cells (prohemocytes) to crystal cells and lamellocytes.

In *Drosophila*, larval blood cell transformation and

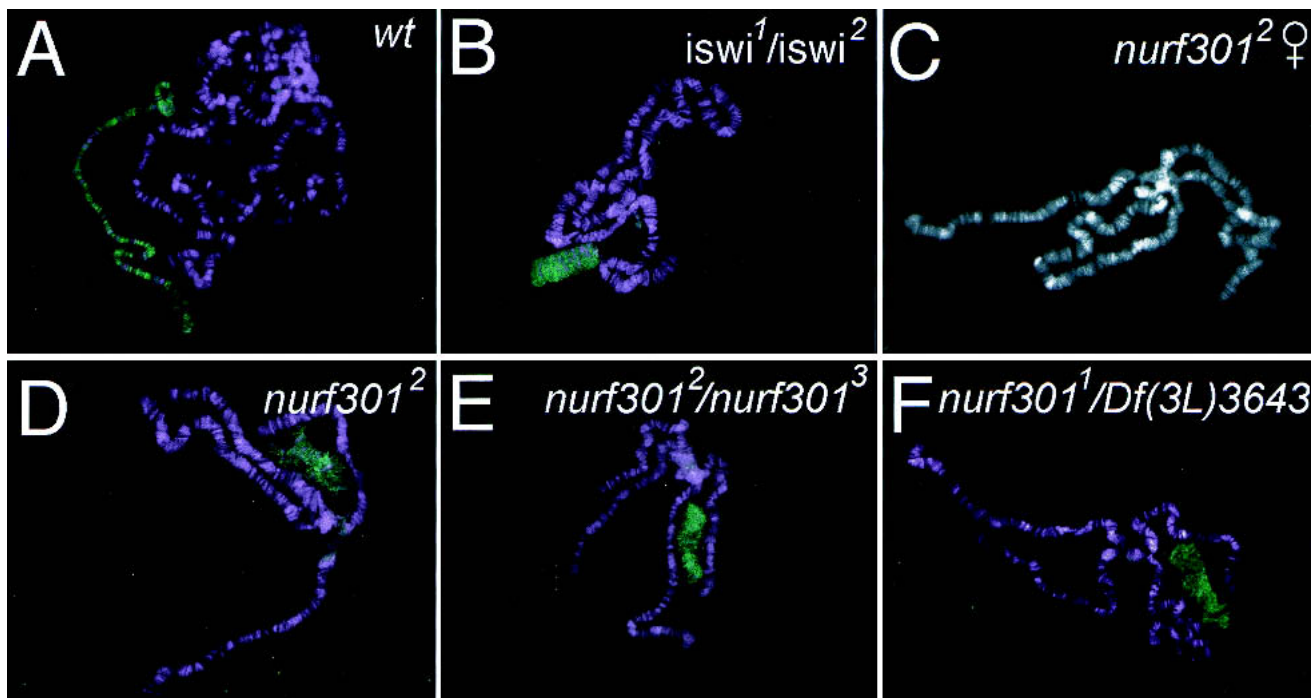


Figure 5. Aberrant male X chromosome in *nurf301* mutant animals. Polytene chromosomes prepared from male *wt* (A), *iswi* (B), and *nurf301* (D–F) mutant animals were stained with antibodies against MSL2 (shown in green) to reveal the male X chromosome. As seen in *iswi*¹/*iswi*² mutant animals (Deuring et al. 2000), male X chromosome morphology is perturbed in *nurf301* mutants. (C) The X chromosome morphology in female *nurf301* mutants is unaffected.

melanotic tumor formation can be induced by inappropriate activation of either of two distinct signaling cascades: the TOLL or the JAK/STAT pathway. Inappropriate activation and nuclear-localization of the *Drosophila* NF- κ B homolog DORSAL, caused either by constitutive activation of the TOLL receptor or removal of the inhibitor, the *Drosophila* I κ B CACTUS, leads to melanotic tumors in third instar larvae (Qui et al. 1998). In the second pathway, gain-of-function mutations in HOPSCOTCH (HOP), the *Drosophila* Janus Kinase (JAK), induce melanotic tumors (Hanratty and Dearolf 1993; Harrison et al. 1995; Hou et al. 1996). HOP gain-of-function mutants cause tumor development by triggering constitutive activation and DNA-binding by the *Drosophila* STAT transcription factor, STAT92E.

To resolve whether the melanotic tumors seen in the *nurf301* mutants were caused by misregulation of either the TOLL or HOP/STAT92E pathways, we determined if *nurf301* mutants enhance tumor phenotypes seen in constitutively active TOLL or HOP mutant lines. We found that tumor incidence in animals carrying one copy of a gain-of-function HOP mutation—*hop*^{Tum-1}—is increased by simultaneous reduction in NURF301 levels (Fig. 7A). In contrast, removal of one copy of NURF301 fails to enhance the TOLL gain-of-function allele *TI*^{10b} (data not shown). The results suggest that NURF acts as a negative regulator within the *Drosophila* JAK/STAT signaling pathway.

Molecular signatures of both JAK and TOLL activation have been defined. It is known that HOP gain-of-func-

tion mutants induce expression of a complement-like protein TEP1 (Lagueux et al. 2000). Overactivation of the TOLL pathway also induces TEP1 synthesis but primarily induces expression of antimicrobial peptides, including DROSOMYCIN (DRS) and DIPTERICIN (DPT). We found that loss of *nurf301* induces *tep1* but fails to induce *drs* or *dpt* (Fig. 7B), demonstrating that NURF301 principally affects the HOP/STAT92E pathway. We tested whether *nurf301* interacts genetically with other known components of the HOP/STAT92E pathway. Certain mutations in *unpaired* (*upd*, also known as *outstretched*), which encodes a ligand for the HOP receptor, display a characteristic wings-out phenotype, due to decreased activation of HOP and consequently decreased STAT92E function. When NURF301 levels are simultaneously decreased in these mutant backgrounds, animals are mostly restored to the wild-type (Fig. 7D). These genetic interactions confirm that NURF301 acts as a negative regulator of the HOP/STAT92E pathway, at a point downstream of HOP. Hence, disruption of NURF could affect either STAT92E or the targets of STAT92E. We found that in *nurf301* mutants, levels of the STAT92E transcription factor are not elevated (Fig. 7C), suggesting that NURF acts to repress the activity of STAT92E or the expression of some STAT92E target genes.

Discussion

Here we provide a physiological correlate for the demonstrated biochemical properties of the nucleosome re-

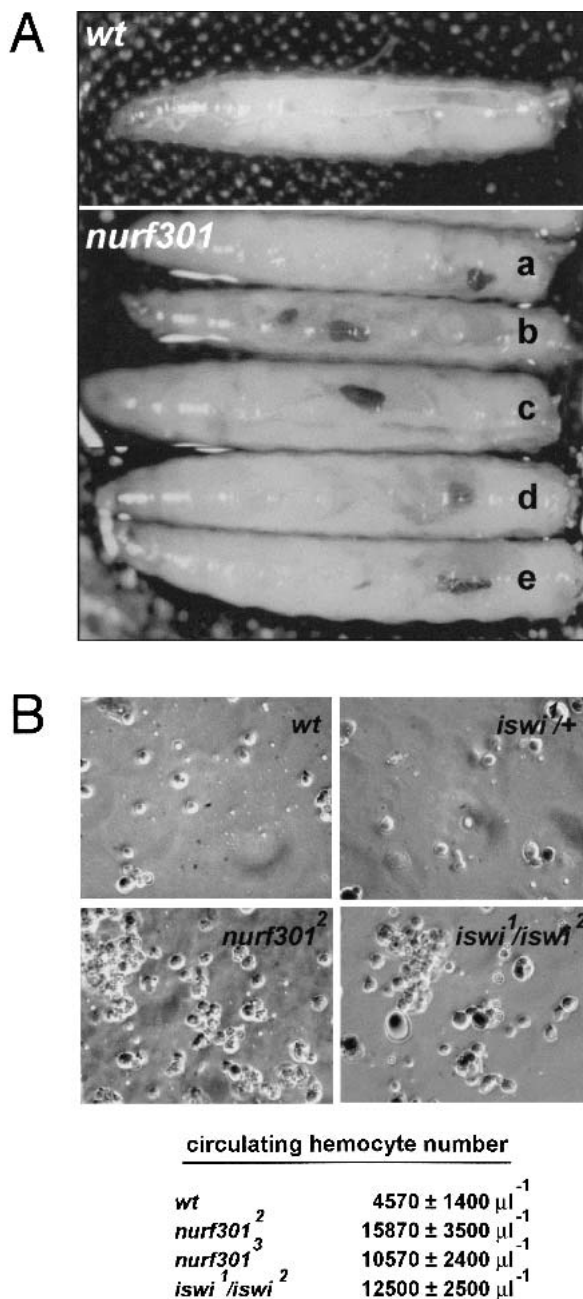


Figure 6. Melanotic tumors in NURF mutant animals. Melanotic tumors occur in homozygous mutant *nurf301* third instar larvae. (A) Tumors are observed in all combinations of *nurf301* mutant alleles. Genotypes displayed are *nurf301*²/*nurf301*² (a), *nurf301*²/*nurf301*³ (b), *nurf301*²/*nurf301*⁴ (c), *nurf301*¹/*nurf301*² (d), and *nurf301*¹/*nurf301*³ (e). (B) The circulating hemocyte cell number in hemolymph isolated from *nurf301* and *iswi* mutant third instar larvae is increased considerably with respect to wild-type animals.

modeling factor (NURF). Our results show that the largest subunit of NURF, NURF301, is required for transcription in vivo. These data confirm an earlier report of Tamkun and co-workers (Deuring et al. 2000) that showed that ISWI, the catalytic subunit of NURF, is re-

quired for transcription activation in vivo. However, because ISWI is also a subunit of the ACF (Ito et al. 1999) and CHRAC (Varga-Weisz, et al. 1997) chromatin remodeling complexes, it had not previously been possible to ascribe *iswi* phenotypes to a specific remodeling factor. As NURF301 is not present in ACF and CHRAC, by analyzing *nurf301* mutations we have been able to resolve specific functions of NURF. We show that for the tested phenotypes, mutations in *nurf301* and *iswi* are indistinguishable. We also demonstrate that NURF functions during larval blood cell development. In the absence of either the NURF301 or ISWI subunits, circulating hemocytes undergo a neoplastic transformation that results in the overproliferation of blood cell types. Melanotic tumors are formed when these cells invade and encapsulate normal larval tissues. Our data suggest this is caused by misregulation of the *Drosophila* JAK/STAT pathway.

NURF is required for transcription in vivo

Our results demonstrate that NURF is also required in vivo for transcription of the heat shock and homeotic genes. This agrees with the demonstrated biochemical properties of NURF. NURF can catalyze nucleosome sliding in vitro, in this manner disrupting chromatin structure (Hamiche et al. 1999). Nucleosome sliding on promoters offers a simple way to clear chromatin from promoter elements and has been shown to potentiate the binding of transcription factors and the recruitment of transcription initiation machinery in vitro (Mizuguchi et al. 1997; Kang et al. 2002). Deployment of NURF at target promoters should be critical for gene activation.

An important question is how NURF is recruited to target sites in vivo. Four genes we show to be dependent on *nurf301* for expression—*Ubx*, *en*, *hsp26*, and *hsp70*—all contain multiple binding sites for the GAGA factor, which is genetically required for their correct expression (Soeller et al. 1993; Farkas et al. 1994; Bhat et al. 1996). On the *Drosophila hsp70* and *hsp26* promoters (GA.CT)_n cognate elements to which the GAGA factor binds are required for HSF-binding. When these sequences are deleted, HSF-binding to transgenes in polytene chromosomes is impaired (Shopland et al. 1995; Shopland and Lis 1996), consistent with the defects we see in *nurf301* mutant animals. It is therefore compelling that recent biochemical studies show that NURF and the GAGA factor bind to each other in crude extracts, and that purified NURF301 and GAGA factor interact directly in vitro (Xiao et al. 2001). The principal interacting domains map to an N-terminal region of NURF301 and a stretch flanking the Zn finger DNA-binding motif of GAGA factor (Xiao et al. 2001). These data suggest that NURF is recruited by the GAGA factor through specific, direct interactions with the NURF301 subunit, to catalyze local sliding of nucleosomes at *bx*, *en*, *hsp26*, and *hsp70* promoters, increasing accessibility to sequence-specific transcription factors and RNA polymerase II. Curiously, though, reduction of *nurf301* levels fails to enhance phenotypes of mutations in *Trithorax-like*, the

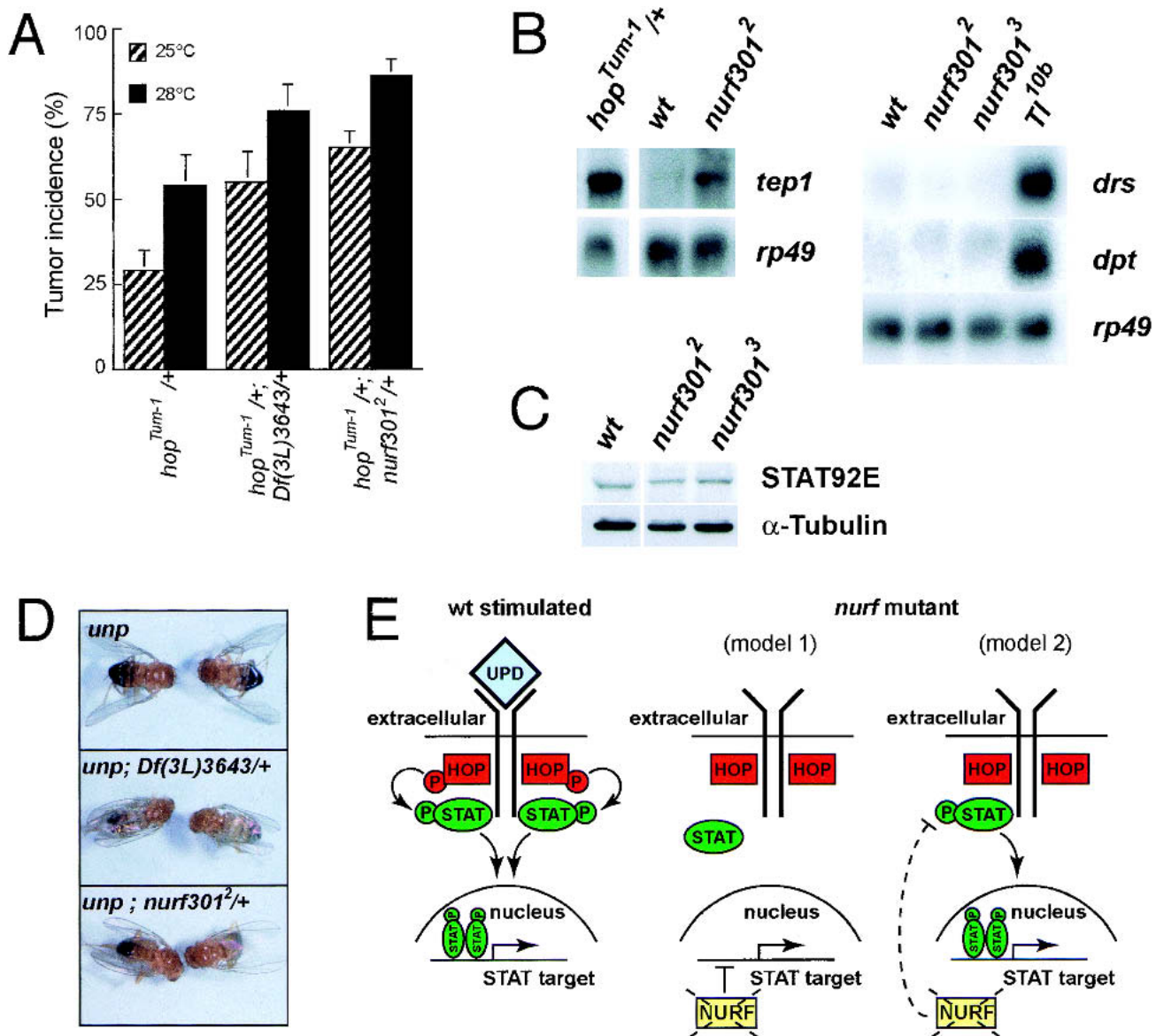


Figure 7. NURF is a negative regulator of the JAK/STAT signaling pathway. (A) Reduction of *nurf301* levels enhances gain-of-function *hop^{Tum-1}* mutant phenotypes. Vials of flies were raised at either 25°C or 28°C. The percentage of adult female flies of each genotype in a vial that display tumors was scored as tumor incidence. Data are the mean and standard deviation of at least six vials of flies. (B) *nurf301* mutant animals express elevated levels of *tep1* but neither the antimicrobial peptide gene *drosomycin* (*drs*) nor *diptericin* (*dpt*) is induced. (C) Levels of the STAT92E transcription factor are not elevated in *nurf301* mutant animals. (D) Reduction of NURF301 levels suppresses the phenotypes of mutations in a ligand for the JAK receptor UNPAIRED (*outstretched*). (E) Models for how NURF may down-regulate the HOP/STAT92E signaling pathway. NURF could either repress targets of STAT92E or be required for expression of a negative regulator of STAT92E activation. In either case, in the absence of NURF, STAT92E target genes will be transcribed, mimicking STAT92E activation.

gene that encodes the GAGA factor (P. Badenhorst, unpubl.).

Conceptual models of the function of chromatin remodeling machines reinforce the view that these complexes are required exclusively during gene activation to expose or "open-up" chromatin. However, nucleosome sliding could equally be harnessed to repress genes. Phenotypic analysis of the orthologous yeast ISW2 complex suggests that ISWI-containing complexes may function

both to activate and repress genes. The yeast ISW complexes appear to be recruited to sites within the genome through direct interaction with DNA-binding proteins to activate and repress genes by repositioning nucleosomes (Goldmark et al. 2000; Kent et al. 2001). *Drosophila* ISWI has been shown to be associated with transcriptionally silent regions of chromatin in salivary gland nuclei, suggesting that it may be involved in repression in this tissue (Deuring et al. 2000). Our analysis of NURF function

during larval blood cell development suggests that NURF can repress targets of the JAK/STAT signaling pathway. We show that in *nurf301* mutants the expression of the complement-like protein TEP1 is induced. It remains to be established whether *tep1* is a direct target of NURF.

NURF and tumorigenesis

Misregulation of TEP1 is seen in mutants that overactivate the *Drosophila* JAK/STAT (HOP/STAT92E) signaling cascade and induce the formation of melanotic tumors. Increased signaling through the HOP/STAT92E pathway leads to the overproliferation and aberrant differentiation of larval blood cells that subsequently invade and encapsulate normal host tissue (Hanratty et al. 1993; Harrison et al. 1995). We have found that animals lacking NURF301, or the catalytic ISWI subunit, exhibit an identical neoplastic transformation of larval blood cells. In the absence of NURF, the proliferation and differentiation of hemocytes, and the accumulation of lamellocytes, is triggered.

Our data suggests that NURF normally represses targets of the HOP/STAT92E pathway. Genetic epistasis places *nurf301* downstream of HOP. We show that loss of NURF resembles gain-of-function mutations in *hop*, and that targets of the HOP/STAT92E cascade are up-regulated in *nurf301* mutants. Normally, HOP activation leads to the expression and posttranslational modification of STAT92E (Chen et al. 2002). We found that although *nurf301* mutants activate the HOP/STAT92E pathway, the levels of the STAT92E transcription factor are unchanged in NURF mutant animals. We suggest that NURF acts downstream of STAT92E. In resting cells, in the absence of HOP/STAT92E signaling, NURF could normally repress STAT92E target genes. When NURF is removed, repression is no longer maintained, and targets are transcribed mimicking the effects of HOP activation. However, STAT activity is also influenced by a number of inhibitory, STAT-binding proteins. Among these are the suppressor of cytokine signaling (SOCS; Callus and Mathey-Prevot 2002) and protein inhibitor of activated STAT (PIAS) family of inhibitors (Betz et al. 2001; Hari et al. 2001). It is possible that NURF is required for expression of one such inhibitor, and that loss of NURF activates the HOP/STAT92E cascade by removing a STAT inhibitor.

The involvement of NURF in larval blood cell development agrees well with recent literature implicating a number of chromatin-modifying or chromatin-associated complexes in hemocyte development and melanotic tumor formation. Mutations in *modulo*, which encodes an interacting partner of the coactivator CBP, cause melanotic tumors (Bantignies et al. 2001). Of particular significance, screens for mutations that cause hematopoietic defects identified *domino* (*dom*), which encodes a member of the SWI2/SNF2 family of DNA-dependent ATPase that is distantly related to ISWI (Ruhf et al. 2001). Mutations in *domino* cause overproliferation of hemocytes, like NURF mutants. However, unlike NURF

mutants, hemocytes fail to enter the hemolymph and remain trapped in enlarged lymph glands that become melanized. It will be interesting to assess the relative contributions of the ISWI and DOM complexes in the regulatory hierarchy of larval blood development.

NURF maintains normal sex chromosome morphology

A striking feature of male animals that lack either NURF301 or the catalytic subunit ISWI is the distorted, bloated morphology of the male X chromosome (Deuring et al. 2000; this study). This implicates NURF in the maintenance of male X chromosome morphology. In flies, X chromosome dosage compensation is achieved by up-regulating transcription from the male X chromosome. One characteristic of the male X chromosome is the specific acetylation of histone H4 at Lys 16 (H4-K16), which is believed to favor a looser chromatin structure that allows increased transcription. These patterns of acetylation are established by the male-specific expression of components of the MSL complex that are tethered on the male X chromosome and subsequently recruit the histone acetyl transferase MOF (for review, see Park and Kuroda 2001).

Genetic studies demonstrate that H4-K16 acetylation antagonizes ISWI function on the X chromosome (Corona et al. 2002). Biochemical characterization of the ISWI-containing ACF and CHRAC complexes has revealed that they can assemble and slide nucleosomes to establish regular ordered arrays (Ito et al. 1997; Varga-Weisz et al. 1997). Regular nucleosome arrays are presumed to provide better substrates for chromatin compaction and, thus, it was speculated that ACF and CHRAC might be the complexes that help compact the male X chromosome (Corona et al. 2002). However, here we show that NURF is the ISWI complex required for normal male X chromosome morphology. Unlike ACF or CHRAC, NURF disrupts regular, ordered arrays of nucleosomes (Tsukiyama et al. 1995). While it is possible that NURF is required for global aspects of higher order chromosome morphology that are needed to maintain normal male X chromosome structure, other local or transcription-based mechanisms could also account for the *nurf301* and *iswi* phenotypes. The dosage compensation machinery is recruited to the male X chromosome at specific, high affinity sites or entry points and subsequently spreads into flanking chromatin (Kageyama et al. 2001). NURF may regulate chromatin accessibility at one or a number of these initiation sites. In the absence of NURF, entry of the dosage compensation machinery at such sites may be changed. Alternatively, NURF may control transcription of components of the sex-determination and dosage compensation pathway. Irrespective, the observed antagonistic relationship between ISWI function and H4-K16 acetylation (Corona et al. 2002) suggests that the action of NURF on the X chromosome is correspondingly influenced by H4 lysine acetylation. This influence on NURF could be direct, as suggested by effects of acetylated H4 tail peptide on ISWI ATPase activity in vitro (Corona et al. 2002).

Physiological activities of the ISWI chromatin remodeling machines

ISWI is the catalytic subunit of at least three protein complexes that have demonstrated in vitro chromatin-remodeling activity—NURF, ACF, and CHRAC. Here we have shown that mutation of a NURF-specific component reproduces the published properties of mutations in *iswi*. Both *iswi* and *nurf301* are required for homeotic gene expression, proper larval blood cell development, and normal male X chromosome morphology. The specific in vivo functions of the ACF and CHRAC complexes remains to be established.

Clues to the function of ACF and CHRAC may be derived from studies of the human Williams Syndrome Transcription Factor–ISWI complex (WSTF–ISWI). The in vitro activities of the WSTF–ISWI complex are essentially identical to ACF. WSTF–ISWI is targeted to pericentric heterochromatin during replication and is believed to allow heterochromatin reassembly in the wake of the replication fork (Bozhenok et al. 2002). It is tempting to speculate that ACF and CHRAC may have similar functions in *Drosophila* and could be implicated in the establishment of repressive chromatin structures after replication. As mutations that selectively compromise individual remodeling complexes become available, whole genome expression analysis will allow the relative contributions of specific complexes to gene activation and silencing events in vivo to be dissected.

Materials and methods

Genetics and fly strains

*nurf301*¹ corresponds to *l(3)ry122* (Berg and Spradling 1991) and was mapped to the *nurf301* untranslated leader sequence, 332 bp upstream of the ATG. The homozygous viable EP insertions EP(3)0637, EP(3)3495, and EP(3)3643 (Rørth 1996) were mapped 880, 720, and 840 bp, respectively, upstream of the ATG of *nurf301*. The deficiency *Df(3L)3643* was generated by imprecise P-element excision from EP(3)3643. EMS-induced mutations in *nurf301* were generated by feeding isogenized *w*¹¹⁸ males 25mM EMS in 10mM Tris, at pH 7.5 and 1% sucrose for 12 h at room temperature. Mutagenized males were batch-crossed to *TM3/TM6B* virgins, and F1 male progeny then crossed individually to one of two deficiency lines that uncover *nurf301*, *Df(3L)XZB970* or *Df(3L)rH321*. F2 progeny were scored for lethal noncomplementation of the deficiency. Alleles of *nurf301* were identified by lethal noncomplementation of the P-element allele, *nurf301*¹.

EMS-induced nucleotide changes were determined by amplifying overlapping DNA fragments covering *nurf301* using *nurf301*-specific primers and an Expand Long Range PCR kit (Boehringer, Mannheim) and DNA isolated from homozygous mutant animals as template. DNAs were sequenced and compared with similarly isolated *w*¹¹⁸ sequence. Altered *nurf301* splicing in *nurf301*⁴ mutants was demonstrated by isolating RNA from *nurf301*⁴ homozygous mutant larvae using an RNaseasy column (Qiagen) and performing RT–PCR using primers flanking the altered splice-site. First-strand cDNA synthesis was performed using Ready-to-go beads (Amersham). For biochemical experiments, *nurf301* mutations were kept over a GFP-marked balancer chromosome [*w*; *TM3*, *P(w⁺ActGFP)*

JMR2, *Ser*¹]. This allowed large numbers of homozygous *nurf301* mutant larvae to be selected by the lack of the GFP marker. Animals for expression analysis were harvested at mid-third larval instar—well prior to the lethal phase. Variegating telomeric transgene inserts are as described in Cryderman et al. (1999). All other fly strains are described in FlyBase (<http://fly-base.bio.indiana.edu>)

Microscopy

Adult cuticles were prepared for microscopy by passage through isopropanol and mounted in Euparal (Asco Laboratories). Mutant and wild-type larval imaginal discs were fixed and immuno-histochemistry performed in parallel as described (Dominguez and Hafen 1997). RNA in situ hybridization was performed as described (Dominguez and Hafen 1997). For polytene chromosome analysis, animals were maintained at 18°C until treatment. For heat-shock experiments, salivary glands were dissected from late-third instar larvae, incubated in prewarmed 36.5°C TBI buffer (Bonner 1981) for the indicated times and chromosomes prepared and stained as described (Gorman et al. 1995). Rabbit anti-HSF antibody was used at a dilution of 1:1000. For X chromosome staining, polytene chromosomes were prepared as described (Deuring et al. 2000) and stained with rabbit anti-MSL2 antibodies (diluted 1:200). DNA was stained using DAPI and is revealed in purple in the merge.

Expression analysis

For heat-shock gene expression analysis, mutant and wild-type larvae were separated, equilibrated for 12 h, then immersed in 1.5-mL microfuge tubes in a 36.5°C water-bath for the listed times. Samples were snap frozen in liquid nitrogen and stored at –70°C until processing. Total RNA was prepared as described (Andres and Thummel 1994) followed by further purification over an RNaseasy column (Qiagen). Dot blots were performed as described (Sambrook and Russell 2001). Probes against *rp49*, *hsp26*, and *hsp70* are as described in Marchler and Wu (2001). Probes against *dipteracin*, *drosomycin*, and *tep1* were PCR fragments amplified from genomic DNA using the following primer sets: *drs* (5'-ATGATGCAGATCAAGTACTTGTTCGCC-3' and 5'-TTAGCATCCTTCGCACCAGCACTTCAG-3'), *dpt* (5'-TTCTTCAATTGAGAACAACCTGAGATGC-3' and 5'-GAAGTCTGCCTCAATGTTCCGGGTAA-3') *tep1* (5'-CTACATTTGATACTGCAGAAAATATAGAAC-3' and 5'-CCGAGCTCCGTAGATATTTATATTCGCAT-3'). Hybridization was in SUREhyb hybridization buffer (Ambion) using DNA probes labeled using a STRIPease kit (Ambion). For analysis of *Ubx* and *en* mRNA levels, total RNA was isolated as described above, subjected to DNase I cleavage (Amplification grade DNase I, Invitrogen), and transcript abundance assayed using an Access RT–PCR kit (Promega). Primer sets and cycle conditions used were: *rp49* (5'-TCTTGAGAACGCAGGCGACCGTTGGGGT TG-3' and 5'-ATCCGCCACCAGTCCGGATCGATATGCTA AG-3', 20 cycles), *en* (5'-CCTCGAATATTGCACGCCCCG GTCAGAAGG-3' and 5'-CAACGACGAGAAGCGTCCACG CACCGCGTT-3', 25 cycles) and *Ubx* (5'-TCTGATTCACA CAATACGGCGGCATATCA-3' and 5'-AATCCCACATACA CCCTACGGATCTAAGGA-3', 25 cycles).

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References

- Andres, A.J. and Thummel, C.S. 1994. Methods for quantitative analysis of transcription in larvae and prepupae. *Methods Cell Biol.* **44**: 565–573.
- Bantignies, F., Goodman, R.H., and Smolik, S.M. 2001. The interaction between the coactivator dCBP and Modulo, a chromatin-associated factor, affects segmentation and melanotic tumor formation in *Drosophila*. *Proc. Natl. Acad. Sci.* **99**: 2895–2900.
- Bender, W., Akam, M.E., Karch, F., Beachy, P.A., Peifer, M., Spierer, P., Lewis, E.B., and Hogness, D.S. 1983. Molecular genetics of the Bithorax Complex in *Drosophila melanogaster*. *Science* **221**: 23–29.
- Berg, C.A. and Spradling, A.C. 1991. Studies on the rate and site-specificity of P-element transposition. *Genetics* **127**: 515–524.
- Betz, A., Lampen, N., Martinek, S., Young, M.W., and Darnell, J.E. 2001. A *Drosophila* PIAS homolog negatively regulates *stat92E*. *Proc. Natl. Acad. Sci.* **98**: 9563–9568.
- Bhat, K.M., Farkas, G., Karch, F., Gyurkovics, H., Gausz, J., and Schedl, P. 1996. The GAGA factor is required in the early *Drosophila* embryo not only for transcriptional regulation but also for nuclear division. *Development* **122**: 1113–1124.
- Bhosekar, S. and Babu, P. 1987. *Enhancer of bithorax* a mutation of *Drosophila melanogaster*. *J. Genet.* **66**: 187–194.
- Bonner, J.J. 1981. Induction of *Drosophila* heat-shock puffs in isolated polytene nuclei. *Dev. Biol.* **86**: 409–418.
- Bozhenok, L., Wade, P.A., and Varga-Weisz, P. 2002. WSTF-ISWI chromatin remodeling complex targets heterochromatic replication foci. *EMBO J.* **21**: 2231–2241.
- Callus, B.A. and Mathey-Prevot, B. 2002. SOCS36E, a novel *Drosophila* SOCS protein, suppresses JAK/STAT and EGF-R signalling in the imaginal wing disc. *Oncogene* **21**: 4812–4821.
- Chen, H.-W., Chen, X., Oh, S.-W., Marinissen, M.J., Gutkind, J.S., and Hou, S.X. 2002. *mom* identifies a receptor for the *Drosophila* JAK/STAT signal transduction pathway and encodes a protein distantly related to mammalian cytokine receptor family. *Genes & Dev.* **16**: 388–398.
- Corona, D.F., Clapier, C.R., Becker, P.B., and Tamkun, J.W. 2002. Modulation of ISWI function by site-specific histone acetylation. *EMBO Rep.* **3**: 242–247.
- Corona, D.F.V., Langst, G., Clapier, C.R., Bonte, E.J., Ferrari, S., Tamkun, J.W., and Becker P.B. 1999. ISWI is an ATP-dependent nucleosome remodeling factor. *Mol. Cell.* **3**: 239–245.
- Cryderman, D.E., Morris, E.J., Biessmann, H., Elgin, S.C., and Wallrath, L.L. 1999. Silencing at *Drosophila* telomeres: Nuclear organization and chromatin structure play critical roles. *EMBO J.* **18**: 3724–3735.
- Deuring, R., Fanti, L., Armstrong, J.A., Sarte, M., Papoulas, O., Prestel, M., Daubresse, G., Verardo, M., Moseley, S.L., Berloco, M., 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.
- Dominguez, M. and Hafen, E. 1997. Hedgehog directly controls initiation and propagation of retinal differentiation in the *Drosophila* eye. *Genes & Dev.* **11**: 3254–3264.
- Farkas, G., Gausz, J., Galloni, M., Reuter, G., Gyurkovics, H., and Karch, F. 1994. The Trithorax-like gene encodes the *Drosophila* GAGA factor. *Nature* **371**: 806–808.
- Gateff, E. 1978. Malignant neoplasms of genetic origin in IITL-*Drosophila melanogaster*. *Science* **200**: 1448–1459.
- Gdula, D.A., Sandaltzopoulos, R., Tsukiyama, T., Ossipow, V., and Wu, C. 1998. Inorganic pyrophosphatase is a component of the *Drosophila* nucleosome remodeling factor complex. *Genes & Dev.* **12**: 3206–3216.
- Goldmark, J.P., Fazzio, T.G., Estep, P.W., Church, G.M., and Tsukiyama, T. 2000. The Isw2 chromatin remodeling complex represses early meiotic genes upon recruitment by Ume6p. *Cell* **103**: 423–433.
- Gorman, M., Franke, A., and Baker, B.S. 1995. Molecular characterization of the male-specific lethal-3 gene and investigations of the regulation of dosage compensation in *Drosophila*. *Development* **121**: 463–475.
- Hamiche, A., Sandaltzopoulos, R., Gdula, D.A., and Wu, C. 1999. ATP-dependent histone octamer sliding mediated by the chromatin remodeling complex NURF. *Cell* **97**: 833–842.
- Hanratty, W.P. and Dearolf, C.R. 1993. The *Drosophila Tumorous-lethal* hematopoietic oncogene is a dominant mutation in the *hopscotch* locus. *Mol. Gen. Genet.* **238**: 33–37.
- Hari, K.L., Cook, K.R., and Karpen, G.H. 2001. The *Drosophila Su(var)2-10* locus regulates chromosome structure and function and encodes a member of the PIAS protein family. *Genes & Dev.* **15**: 1334–1348.
- Harrison, D.A., Binari, R., Nahreini, T.S., Gilman, M., and Perimon, N. 1995. Activation of a *Drosophila* Janus kinase (JAK) causes hematopoietic neoplasia and developmental defects. *EMBO J.* **14**: 2857–2865.
- Hou, X.S., Melnick, M.B., and Perrimon, N. 1996. *marelle* acts downstream of the *Drosophila* HOP/JAK kinase and encodes a protein similar to the mammalian STATs. *Cell* **84**: 411–419.
- Ito, T., Bulger, M., Pazin, M.J., Kobayashi, R., and Kadonaga, J.T. 1997. ACF, an ISWI-containing and ATP-utilizing chromatin assembly and remodeling factor. *Cell* **90**: 145–155.
- Ito, T., Levenstein, M.E., Fyodorov, D.V., Kutach, A.K., Kobayashi, R., and Kadonaga, J.T. 1999. ACF consists of two subunits, Acl1 and ISWI, that function cooperatively in the ATP-dependent catalysis of chromatin assembly. *Genes & Dev.* **13**: 1529–1539.
- Kageyama, Y., Mengus, G., Gilfillan, G., Kennedy, H.G., Stuckenholz, C., Kelley, R.L., Becker, P.B., and Kuroda, M.I. 2001. Association and spreading of the *Drosophila* dosage compensation complex from a discrete *roX1* chromatin entry site. *EMBO J.* **20**: 2236–2245.
- Kang, J.G., Hamiche, A., and Wu, C. 2002. GAL4 directs nucleosome sliding induced by NURF. *EMBO J.* **21**: 1406–1413.
- Kennison, J.A. 1995. The Polycomb and trithorax group proteins of *Drosophila*: Transregulators of homeotic gene function. *Annu. Rev. Genet.* **29**: 289–303.
- Kent, N.A., Karabetsou, N., Politis, P.K., and Mellor, J. 2001. In vivo chromatin remodeling by yeast ISWI homologs Isw1p and Isw2p. *Genes & Dev.* **15**: 619–626.
- Lagueux, M., Perrodou, E., Levashina, E.A., Capovilla, M., and Hoffmann, J.A. 2000. Constitutive expression of a comple-

- ment-like protein in toll and JAK gain-of-function mutants of *Drosophila*. *Proc. Natl. Acad. Sci.* **97**: 11427–11432.
- Langst, G., Bonte, E.J., Corona, D.F.V., and Becker, P.B. 1999. Nucleosome movement by CHRAC and ISWI without disruption or transdisplacement of the histone octamer. *Cell* **97**: 843–852.
- Lewis, E.B. 1978. A gene complex controlling segmentation in *Drosophila*. *Nature* **276**: 565–570.
- Lindsley, D.L. and Zimm, G.G. 1992. *The genome of Drosophila melanogaster*. Academic Press, Inc., San Diego, CA.
- Luo, H. and Dearolf, C.R. 2001. The JAK/STAT pathway and *Drosophila* development. *BioEssays* **23**: 1138–1147.
- Marchler, G. and Wu, C. 2001. Modulation of *Drosophila* heat shock transcription factor activity by the molecular chaperone DROJ1. *EMBO J.* **20**: 499–509.
- Martinez-Balbas, M.A., Tsukiyama, T., Gdula, D., and Wu, C. 1998. *Drosophila* NURF-55, a WD repeat protein involved in histone metabolism. *Proc. Natl. Acad. Sci.* **95**: 132–137.
- Mizuguchi, G., Tsukiyama, T., Wisniewski, J., and Wu, C. 1997. Role of nucleosome remodeling factor NURF in transcriptional activation of chromatin. *Mol. Cell.* **1**: 141–150.
- Park, Y. and Kuroda, M.I. 2001. Epigenetic aspects of X-chromosome dosage compensation. *Science* **293**: 1083–1085.
- Qui, P., Pan, P.C., and Govind, S. 1998. A role for the *Drosophila* Toll/Cactus pathway in larval hematopoiesis. *Development* **125**: 1909–1920.
- Rizki, R.M. and Rizki, T.M. 1979. Cell interactions in the differentiation of a melanotic tumor in *Drosophila*. *Differentiation* **12**: 167–178.
- Rorth, P. 1996. A modular misexpression screen in *Drosophila* detecting tissue-specific phenotypes. *Proc. Natl. Acad. Sci.* **93**: 12418–12422.
- Ruhf, M.-L., Braun, A., Papoulas, O., Tamkun, J.W., Randsholt, N., and Meister, M. 2001. The *domino* gene of *Drosophila* encodes novel members of the SWI2/SNF2 family of DNA-dependent ATPases, which contribute to the silencing of homeotic genes. *Development* **128**: 1429–1441.
- Sambrook, J. and Russell, D.W. 2001. *Molecular cloning: A laboratory manual*, 3rd ed., pp. 7.49–7.50. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.
- Soeller, W.C., Oh, C.E., and Kornberg, T.B. 1993. Isolation of cDNAs encoding the *Drosophila* GAGA transcription factor. *Mol. Cell Biol.* **13**: 7961–7970.
- Shopland, L.S., Hirayoshi, K., Fernandes, M., and Lis, J.T. 1995. HSF access to the heat-shock elements in vivo depends critically on promoter architecture defined by GAGA factor, TFIID and RNA-polymerase II-binding sites. *Genes & Dev.* **9**: 2756–2769.
- Shopland, L.S. and Lis, J.T. 1996. HSF recruitment and loss at most *Drosophila* heat shock loci is coordinated and depends on proximal promoter sequences. *Chromosoma* **105**: 158–171.
- Tamkun, J.W., Deuring, R., and Scott, M.P. 1992. A regulator of *Drosophila* homeotic genes structurally related to the yeast transcriptional activator SNF2 SWI2. *Cell* **68**: 561–572.
- Tsukiyama, T., Becker, P.B., and Wu, C. 1994. ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. *Nature* **367**: 525–532.
- Tsukiyama, T., Daniel, C., Tamkun, J., and Wu, C. 1995. ISWI, a member of the SWI2 SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. *Cell* **83**: 1021–1026.
- Tsukiyama, T. and Wu, C. 1995. Purification and properties of an ATP-dependent nucleosome remodeling factor. *Cell* **83**: 1011–1020.
- Tzou, P., De Gregorio, E., and Lemaitre, B. 2002. How *Drosophila* combats microbial infection: A model to study innate immunity and host–pathogen interactions. *Curr. Opin. Microbiol.* **5**: 102–110.
- Varga-Weisz, P.D., Wilm, M., Bonte, E., Dumas, K., Mann, M., and Becker, P.B. 1997. Chromatin-remodelling factor CHRAC contains the ATPases ISWI and topoisomerase II. *Nature* **388**: 598–602.
- Westwood, J.T., Clos, J., and Wu, C. 1991. Stress-induced oligomerization and chromosomal relocalization of heat-shock factor. *Nature* **353**: 822–823.
- Xiao, H., Sandaltzopoulos, R., Wang, H.M., Hamiche, A., Ranallo, R., Lee, K.M., Fu, D.G., and Wu, C. 2001. Dual functions of largest NURF subunit NURF301 in nucleosome sliding and transcription factor interactions. *Mol. Cell.* **8**: 531–543.