Published in final edited form as:

FEBS Lett. 2011 October 20; 585(20): 3197–3207. doi:10.1016/j.febslet.2011.09.003.

The Nucleosome Remodeling Factor

Suehyb G. Alkhatib and Joseph W. Landry*

Department of Human and Molecular Genetics, Virginia Commonwealth University School of Medicine, Richmond, VA 23298, United States

Abstract

An essential component of the chromatin remodeling machinery is NURF (Nucleosome Remodeling Factor), the founding member of the ISWI family of chromatin remodeling complexes. In vertebrates and invertebrates alike, NURF has many important functions in chromatin biology including regulating transcription, establishing boundary elements, and promoting higher order chromatin structure. Since NURF is essential to many aspects of chromatin biology, knowledge of its function is required to fully understand how the genome is regulated. This review will summarize what is currently known of its biological functions, conservation in the most prominent model organisms, biochemical functions as a nucleosome remodeling enzyme, and its possible relevance to human cancer.

Keywords

NURF; Chromatin remodeling; ISWI; Bptf; SNF2L; NURF301

1. Introduction

The fundamental unit of chromatin is the nucleosome, which is composed of four basic histone proteins tightly associated with ~150 bp of DNA contained in 1.75 super helical turns [1]. In many cases, chromatin presents a significant barrier to the interaction of trans-acting factors with DNA. As such, chromatin regulates many biological processes like transcription, DNA replication, DNA repair, and DNA recombination [2]. Epigenetic mechanisms have evolved to regulate the structure of chromatin, and as a result, access to DNA. These mechanisms include the post-translational modification of histones, DNA methylation, incorporation of histone variants, and nucleosome remodeling activities [3]. Nucleosome remodeling and the incorporation of histone variants are largely accomplished through the action of ATP-dependent chromatin remodeling complexes. These complexes are a diverse family grouped into SWI/SNF, ISWI, CHD, or INO80 sub-families, based upon sequence homology of the associated ATPase [4].

Since its discovery, the ISWI family member NURF (Nucleosome Remodeling Factor) chromatin remodeling complex has been documented as a key regulator of development in many prominent model organisms. Evidence suggests that NURF is an ATP-dependent

* Corresponding author. jwlandry2@vcu.edu (J.W. Landry)..

chromatin remodeling complex, specifically targeted to chromatin through interactions with sequence specific transcription factors and modified histones. To examine the evidence for this model, and come to a better understanding of NURF function, this review will: examine key points of its ATP-dependent remodeling reaction with emphasis on its interactions with the nucleosome, summarize known biological functions for NURF, identify potential NURF homologs and conserved functions by sequence conservation in major model organisms, and summarize available evidence for its role in human cancer.

2. The Nucleosome Remodeling Factor

NURF was first identified in *Drosophila melanogaster* as an ATP-dependent biochemical activity that enhanced GAGA factor (GAGAG binding factor)-mediated nuclease accessibility to reconstituted chromatin [5,6]. Biochemical purification of this activity revealed a four subunit complex composed of NURF301, the ATPase ISWI (NURF140), NURF55 and NURF38 polypeptides [7]. Purifications from human cells identified a complex highly homologous to *D. melanogaster* NURF, strongly suggesting that it has been conserved through evolution [8]. *Homo sapiens* NURF contains the NURF301 homolog BPTF, the ISWI homolog SNF2L, and a NURF55 homolog pRBAP46/48; however, a NURF38 homolog has not been identified [8] (Fig. 1A).

2.1. NURF301

D. melanogaster NURF301 has a number of functional domains found in other chromatin associated proteins (Fig. 1B). The N-terminal HMGA (High Mobility Group) domain contains two AT-hook sequences and an acidic patch that interacts with nucleosomes [9]. These interactions are likely due to direct contacts with DNA because the AT hook has known affinity with the minor groove of AT rich DNA [10]. The N-terminal DDT domain (DNA-binding homeobox-containing proteins and the different transcription and chromatin remodeling factors in which they are found) and PHD finger (Plant Homeodomain Zinc Finger) have not been specifically characterized in NURF301; however, a similar DDT domain in ACF1 is essential for interactions with ISWI [11]. The NURF301 WAC and WACZ domains are not well characterized, but similar domains in ACF1 are important for its interactions with DNA [11]. The C-terminal domains include a poly-glutamate region which is intrinsically disordered, two PHD fingers, and a bromodomain [12]. The most C-terminal PHD finger (PHD2) and bromodomain compose a histone recognition module that binds di/trimethyl-K4 on histone H3 (H3K4me2/3) and acetyl-K16 on histone H4 (H4K16ac), respectively [13–15]. Additional domains include nuclear localization signals, poly-proline regions, and LXXLL motifs. The latter have been shown to be important for protein–protein interactions and could be important for interactions with nuclear hormone receptors [16].

2.2. ISWI

Characteristic regions of ISWI are the ATPase domain, common to all remodeling proteins, and the C-terminal HAND, SANT, and SLIDE domains (Fig. 1B). ATPase domains are composed of a number of highly homologous motifs (Ia, Ib, II, III, IV, V and VI) separated by less conserved spacers that vary in length between ATPase family members [17]. The

ISWI ATPase domain interacts with DNA in the nucleosome, ~20 bp away from the dyad axis [18]. Similar contacts have been observed for SWI/SNF chromatin remodeling complexes, suggesting an essential function in the remodeling reaction [19]. The C-terminal HAND, SANT, and SLIDE domains are highly conserved through evolution and are diagnostic of ISWI family members. The HAND, SANT, and SLIDE domains make essential contacts with the histone H4 tail and linker DNA, and are essential to the ISWI remodeling reaction [20,21]. In addition, the ISWI ATPase contains an N-terminal AT hook, and LXXLL motifs which likely interact with the nucleosome and facilitate protein–protein interactions, respectively (see Section 2.1).

2.3. NURF55

D. melanogaster NURF55 contains the highly conserved and widely utilized WD repeat domain (Fig. 1B) [22]. WD repeat-containing proteins are present in almost all organisms and are found in many chromatin associated complexes (for example Sin3, NuRD, CAF-1, PRC2, and pRB) [23]. They are named for the presence of four or more ~40 amino acid repeating units ending with conserved Gly-His (GH) and Trp-Asp (WD) residues [24]. Interestingly, NURF301 and ISWI alone are sufficient for ATP dependent remodeling of reconstituted chromatin suggesting that NURF55 is not essential for NURF activity in vitro [9]. NURF55 is proposed to indirectly interact with chromatin in vivo, likely through chromatin associated complexes [22]. In contrast to NURF55, the *Xenopus laevis* and *H. sapiens* homologs p48 and pRBAP48, respectively, directly interact with histone H4, suggesting that they have direct interactions with chromatin [22,25,26].

2.4. NURF38

NURF38 has a strong inorganic pyrophosphatase activity (Fig. 1B) [27]. Inorganic pyrophosphatases have been highly conserved through evolution because of essential functions in phosphate and nucleotide metabolism. Interestingly, NURF38 is not required for, and functions independently of, chromatin remodeling functions. One proposed function is to hydrolyze inorganic pyrophosphates, a byproduct of the RNA polymerase reaction, to increase the efficiency of transcription [27]. Human NURF does not have an associated pyrophosphatase homolog, making its significance to NURF function unclear.

3. The NURF remodeling reaction

The minimal substrate for the NURF remodeling reaction is a nucleosome with linker DNA [6]. *D. melanogaster* NURF mobilizes nucleosomes in 10 bp bidirectional step movements between stable positioning sequences with little unwrapping of the DNA from the nucleosome surface [28,29]. This is in contrast to activities of the SWI/SNF family, which can generate large loops of DNA from the nucleosome surface and can evict histones, and the INO80 family which are dedicated histone exchange complexes [30–33]. In vitro chromatin remodeling is influenced by linker length, strength of the DNA positioning sequence for the nucleosome, and biochemical properties of the remodeling complex [34,35]. NURF slides nucleosomes into a thermodynamically stable position or to the end of a DNA fragment, rendering them refractory to further remodeling [28,29,34]. DNA binding factors and adjacent nucleosomes can strongly influence the outcome of the remodeling

reaction by providing barriers to the movement of nucleosomes in cis [36]. Thus it is widely assumed that the combination of DNA binding factors, adjacent nucleosomes, and physical properties of the DNA and histones significantly contribute to the outcome of NURF remodeling reactions in vivo.

Key elements of the nucleosome are required for NURF chromatin remodeling. Nucleosomes composed of histones lacking N-terminal tails are refractory to remodeling by NURF, stressing their importance to the reaction [37]. Mutagenesis has identified the histone H4 tail and its N-terminal proximal residues 16-KRHR-19 as the most important for NURF nucleosome remodeling functions. Site directed mutation of any of these residues, or acetylation of H4K16 or H4K8 by histone acetyltransferases, significantly inhibits the ATPase activity of ISWI [9,38–41]. Acetylation at residues other than H4K16 and H4K8 has little effect on NURF remodeling of mono nucleosomes, but acts synergistically with NURF to enhance transcription of chromatin in vitro [42]. In addition to the N-terminal tail, the H2A C-terminal tail is also essential for efficient chromatin remodeling by ISWI and SHF2H in vitro [43].

Histone variants can also regulate ISWI remodeling. H2A.Z is preferentially incorporated into nucleosomes at distal regulatory elements and nucleosomes at transcription start sites [44]. In vivo pull down assays indicate that human BPTF associates with the histone H2A.Z and in vitro SNF2L preferentially remodels H2A.Z-containing chromatin over H2A-containing chromatin [45]. The preference for H2A.Z chromatin as a substrate is dependent on residues in the extended acidic patch, a structure unique to H2A.Z [46]. These physical and functional interactions suggest that NURF may preferentially localize to, and remodel chromatin containing H2A.Z nucleosomes.

The activities of NURF in vitro have been studied extensively on the nucleosome, but the physiological substrate in vivo is the chromatosome [47]. The chromatosome is composed of the nucleosome core particle and a linker histone. Neither the ATPase activity of NURF, nor its ability to slide nucleosomes in cis, is influenced by linker histones [48]. This result is in contrast to other reports, which show that the SWI/SNF, CHD, and ISWI family members are negatively influenced by linker histones [49]. A direct comparison between these studies is difficult because of distinct differences in the source of the chromatin, the type of linker histones used, and the method of assembly; however, current evidence suggests that NURF can remodel the chromatosome as efficiently as the nucleosome.

In addition to H2A.Z, other regulators of NURF activity include post-translational modifications of ISWI. The post-translational modification of *D. melanogaster* ISWI by poly-ADP-ribosylation reduces its ATPase activity and nucleosome binding both in vivo and in vitro [50]. Poly-ADP-ribosylation of ISWI is thought to contribute to apoptosis through loss of essential chromatin remodeling activities of NURF. In addition to poly-ADP-ribose, *D. melanogaster* ISWI is acetylated on lysine753 in vivo and in vitro by the acetyltransferase GCN5. GCN5 acetylated ISWI is found exclusively in the NURF complex in vivo; however, its biological functions have not been determined [51].

Small molecule inhibition of NURF has not been extensively characterized. The only small biomolecules known to affect NURF function are the inositol polyphosphates. Biochemical assays demonstrate that NURF and ISWI are negatively regulated by IP6, but not by IP5 or IP4. In *Saccharomyces cerevisiae*, known ISWI gene targets are deregulated in mutants defective for IP6, consistent with a regulatory function for the small molecule during in vivo chromatin remodeling [52]. It is not completely understood how NURF is regulated by inositol polyphosphates and the physiological significance of this control remains unclear.

4. NURF and its interaction with chromatin

Many studies show interactions between components of the NURF complex, or the highly related FAC1 protein (Fetal Alz-50-reactive Clone 1), and sequence specific transcription factors (Table 1) [9,53–65]. FAC1 has sequence identity to the N-terminus of the human NURF301 homolog BPTF, strongly suggesting the two are related [66,67]. These interactions are thought to recruit NURF to specific regions of the genome. In many cases the exact nature of the NURF transcription factor interaction has not been characterized. The most comprehensive study of NURF transcription factor interactions has been in *D. melanogaster*. From these studies, the authors show that HSF (Heat Shock Factor), GAGA and the artificial activation domain VP16 interact with multiple surfaces on NURF301 and weakly with ISWI [9]. Similar interactions have been observed between the SMAD transcription factors and the BPTF and SNF2L subunits of the human NURF complex (Landry, J., unpublished data). These results suggest interactions between NURF and transcription factors could occur over multiple surfaces. Whether these interaction surfaces are important in vivo needs to be explored [15].

In addition to interactions with transcription factors, NURF301 has two well characterized domains which bind specific histone post-translational modifications. The PHD finger juxtaposed to the bromodomain (PHD2) interacts with H3K4me2/3, and the adjacent bromodomain binds H4K16ac (Fig. 1A) [13,14,68]. These adjacent domains may serve as a binding module specific for regions of active chromatin.

In addition to interactions with chromatin associated proteins, NURF likely directly interacts with DNA and some evidence suggests that it could be sequence specific. The AT hooks and N-terminal acidic patch on *D. melanogaster* NURF301 are important for NURF interactions with nucleosomes and for its remodeling activity [9]. AT hooks interact with AT rich DNA sequences, suggesting that this domain could foster direct contacts between NURF and DNA [10]. Sequence specific binding has been reported for FAC1, suggesting that NURF could also have this activity. Using a PCR assisted binding site selection assay, the consensus sequence CACAA-CAC was obtained for the N-terminal ~400 amino acids of FAC1. The consensus sequence represses luciferase reporter constructs when located near a SV40 promoter or enhancer, suggesting that it has activity in vivo [69]. Sequence specific binding activity is not unprecedented in remodeling complexes. For example, RSC in yeast has sequence specific binding activities through its Rsc3 subunit [70]. It will be important to investigate whether the sequence specific binding identified for FAC1 has relevance to DNA binding in vivo.

In vivo studies on the human NURF subunit SNF2L have revealed a uniform distribution throughout the nucleus at the same density as chromatin [71]. SNF2L is mobile throughout the nucleus with an average residence time of 1–2 ms, and only a small fraction (~3% in G1/2 phase and ~10% during S phase) is immobile with a residence time of ~150 ms. Depletion of cellular ATP or the expression of an ATPase inactive SNF2L + 13 splice variant (see below for explanation) results in increased mobility for the mobile fraction and decreased mobility for the immobile fraction. From these experiments a sampling model of chromatin remodeling is proposed, in which SNF2L continuously samples a large number of nucleosomes without chromatin remodeling (i.e., low residence time of 1–2 ms). Chromatin remodeling is proposed to occur (i.e., conversion to high residence time of ~150 ms) when a SNF2L-containing remodeling complex encounters chromatin marked by histone modifications, transcription factors or histone variants which have high affinity for the complex [71].

Consequences of NURF recruitment to chromatin have been observed both in vivo and in vitro. GAGA factor recruits *D. melanogaster* NURF to the *HSP70* and *Ftz* promoters, as shown using reconstituted systems [6,72]. At *HSP70* NURF remodeling activities are required for the heat shock-induced binding of HSF, an essential transcription factor for stress induced transcription [73]. NURF-dependent enhancement of transcription is not observed with naked DNA templates, suggesting that it functions to relieve the inhibitory effects of chromatin on transcription [74]. In a similar study, PR (Progesterone Receptor) was shown to target NURF to the MMTV promoter. Using this system NURF targeting and chromatin remodeling was shown to be important for subsequent NF1 binding and synergistic PR binding, two essential requirements for efficient transcription of the MMTV promoter [62].

In addition to having key regulatory roles during induced transcription, *D. melanogaster* NURF is also a general regulator of chromatin structure. One of the more striking phenotypes of NURF301 and ISWI mutants is the dramatic decondensation of the male X chromosome [73,75]. The DCC (Dosage Compensation Complex) and H4K16ac adjust for X chromosome haploinsufficiency in males (females have 2 X chromosomes) by increasing its transcription 2-fold relative to autosomes. Mutation of the NURF components NURF301 and ISWI, or the expression of a dominant negative ATPase defective ISWI mutant, results in a drastically decondensed chromatin structure, increased expression of some genes on the X chromosome, and chromosome-wide loss of histone H1, but interestingly no significant defects in DCC association or histone acetylation [76,77]. NURF effects could be direct, through nucleosome remodeling and/or binding of histone H1, or indirect, through the regulation of unknown genes important for chromatin structure of the male X chromosome [78]. One possible mechanism for maintaining X chromosome structure is through NURF-dependent localization of the ATAC acetyltransferase. ATAC is a Gcn5 and Atac2-containing acetyltransferase complex important for regulated transcription [79,80]. Mutations in ATAC phenocopy the male X chromatin defects observed with NURF301 and ISWI mutants. It has been proposed that NURF-dependent localization of ATAC is required for the acetylation of histones and likely non-histone proteins, to regulate the chromatin structure of the male X chromosome [81].

In addition to regulating higher order chromatin structure of the male X chromosome, NURF has been characterized as a regulator of chromatin insulator elements in a number of contexts. In *D. melanogaster*, NURF is essential for the function of insulator elements at homeotic gene clusters including Fab7, SF1, and Fab8 in both S2 cells and animals. Defects in insulator function correlate with increased nucleosome occupancy, suggesting that NURF regulates chromatin structure to facilitate insulator function. NURF is proposed to be recruited to insulators by GAGA factor, a known sequence specific binding protein important for Fab7 and SF1b function, to position nucleosomes and regulate insulator activity [82]. Similar roles for NURF in insulator function were reported using the *Gallus gallus* and *H. sapiens* β -globin locus as a model. These studies show that the β -globin insulator associated factor USF1 co-purifies with a number of chromatin modifying proteins, including subunits of NURF and the hSET1 complexes. Knockdown of BPTF results in increased nucleosome occupancy at the endogenous 5'HS4 insulator element and is required for insulator function using a reporter assay. BPTF localization to the 5' HS4 requires the hSET1 complex suggesting collaboration between the two complexes is required for insulator function [65]. In combination these studies clearly show that NURF has important roles in regulating insulator function, likely as a nucleosome remodeling complex.

There is also some evidence that NURF associates with heterochromatin. Using a biochemical approach, NURF301 was found to co-elute with heterochromatin-associated protein HP1 and HP2. These interactions were confirmed using in vitro pull downs, indicating direct interactions between NURF and HP2 [60]. Assays for NURF301 or ISWI as a dominant suppressor of position effect variegation did not reveal a biological connection between NURF and heterochromatin [73]. Observations supporting a functional role for NURF in facultative heterochromatin may require more sophisticated biological assays.

5. A sequence and phylogenetic analysis of NURF subunits

With the exception of *S. cerevisiae*, NURF301 homologs exist across all eukaryotic species investigated, suggesting that the NURF complex has largely been conserved through evolution (Fig. 2A and B). Many *D. melanogaster* NURF301 domains have been conserved in homologs from other species; these domains include the N-terminal HMGA domain or acidic patch, N-terminal DDT and PHD finger, and in the C-terminal region a polyglutamate region, PHD1 and PHD2 fingers, and a bromodomain. Two C-terminal PHD fingers and a bromodomain in *D. melanogaster* are conserved in *Caenorhabditis elegans*; however, only PHD2 has been conserved in every species investigated except *Arabidopsis thaliana*, alluding to its likely importance for NURF301 function (Fig. 3). While the AT hooks of the HMGA domain are conserved in *D. melanogaster*, *C. elegans*, and *A. thaliana*, only its acidic patch has been largely conserved throughout evolution. The N-terminal DDT, PHD finger, and surrounding residues are the most highly conserved domains, making these sequences diagnostic of the NURF301 protein family (Fig. 4). These regions have been shown to interact with the transcription factors MAZ, hKeap1, and GAGA [9,55,56].

One or more homologs of *D. melanogaster* ISWI exist in each of the eukaryotes investigated (Fig. 5). Each homolog shares extensive sequence similarity within the ATPase and HAND, SANT, and SLIDE domains. *D. melanogaster*, *C. elegans* and *Takifugu rubripes* have a

single homolog, which has been named ISWI. In contrast, *S. cerevisiae*, *Xenopus tropicalis*, *H. sapiens*, *Mus musculus*, *G. gallus*, and *Danio rerio* have two distinct homologs which differ at amino acids in the N and C-terminal tails. These tails are important for the assembly of the ATPase subunit into specific remodeling complexes [83]. Members from each of these groups were first identified in *H. sapiens* and named SNF2L (SNF2-Like) and SNF2H [84,85]. Some chromatin remodeling complexes preferentially assemble with either the SNF2L or SNF2H variant, whereas others do not have a preference [86–89]. NURF has been shown to selectively assemble with SNF2L [8].

6. The NURF family of remodeling complexes

As observed for the SWI/SNF family of remodeling complexes, the ISWI family varies its subunit composition as a means of regulating its in vivo functions [90]. Work in *D. melanogaster* and *C. elegans* indicates that NURF is not a single complex, but rather a family of related complexes (the elements of which largely differ as a consequence of alternative splicing) [13,91]. NURF301 is transcribed as four isoforms in *D. melanogaster* (NURF301A, B, C, or D) and five isoforms in *C. elegans* (NURFA, B, C, D, or E, none of which are a full length NURF301 isoform). Specific functions of these isoforms are unknown, with the exception of *D. melanogaster* NURF301C. Studies on a *D. melanogaster* NURF301 mutant allele that approximates the NURF301C short isoform show that the C-terminal PHD fingers and bromodomain of full length NURF301 are not required for viability. Pull downs from these mutants indicate that the truncated NURF301 mutant protein can assemble into a complex with ISWI, NURF55, and NURF38, suggesting that it has remodeling activities [13]. Similar truncated isoforms likely exist in mammals. A short form of BPTF called FAC1 was identified as a protein recognized by the ALZ50 antibody [67]. Antibodies specifically raised to FAC1 identify a protein of 125 kDa found in human brain, and independent studies have identified a similar protein in *M. musculus* [67] [53]. It is unknown whether FAC1, like the NURF301C isoform in *D. melanogaster*, associates in a complex with SNF2L and pRBAP46/48 to form an active chromatin remodeling complex, or if its functions are completely unrelated to chromatin remodeling.

Like NURF301, ISWI is expressed in different splice forms and in some cases the splice variant has significance to chromatin remodeling activities [61,92]. In humans, but not mice, a novel SNF2L splice variant is expressed which incorporates a 13th exon into the mature transcript (SNF2L + 13 variant), interrupting the ATPase domain [92]. Pull down experiments show that it assembles into the NURF remodeling complex but is catalytically inactive and unable to remodel chromatin. These results are significant because they show for the first time that chromatin remodeling complexes can be regulated by incorporating a catalytically inactive ATPase, thus providing a novel method of regulating activity.

7. Biological functions for NURF as a regulator of development

7.1. *D. melanogaster*

The genomic interval containing *Nurf301* was identified as *E(bx)* (Enhancer of Bithorax) in screens to identify regulators of body patterning in *D. melanogaster* [93]. These results assigned the genomic interval containing *Nurf301* as a trithorax member, a positive trans-

regulator of homeotic genes in *D. melanogaster* [94]. Mutants in *Nurf301* and *Iswi* are lethal at the third instar larval stage [73,75]. *Nurf301* mutants, which likely reflect biological functions for NURF more specifically than *Iswi* mutants, have defects in regulated expression of homeobox, heat shock, JAK/STAT and ecdysone responsive genes [57,58,73]. NURF is likely a direct regulator of ecdysone and STAT responsive genes, as shown by its interactions with the ecdysone receptor and Ken, a repressor of STAT signaling [57,58]. Subsequent work showed that the regulation of ecdysone, homeobox, heat shock, and JAK/STAT pathways do not require the full length NURF301, but rather that the short splice form NURFC is sufficient to regulate the pathways [13].

The role of NURF during adult *D. melanogaster* development has been investigated using the tissue specific expression of dominant negative alleles and synthetic genetic studies. NURF positively regulates both the canonical Wingless and Notch pathways, as shown in genetic screens to identify regulators of wing development [59,64]. These genetic interactions were supported by in vitro or in vivo pull downs suggesting that NURF acts as a co-regulator of Armadillo or Pzg function. More recent studies show that *Pzg* mutants phenocopy many of the *Nurf301* mutant phenotypes, and suggest that interactions between NURF and Pzg are important for many NURF functions during development [95]. A more general role for Pzg in regulating NURF functions could be through its interactions with TRF2 and DREF, two proteins that interact with NURF and act as selective regulators of transcription during development and cell cycle progression [63].

Special attention has been given to investigating NURF function during germ stem cell (GSC) development in the ovary. Mutation of *Iswi* in the female germ line results in reduced proliferation and self renewal of GSC. These defects are similar to mutations in the BMP signaling pathway, suggestive of a role for ISWI in regulating this pathway [96]. Subsequent work with *Nurf301* mutants confirmed that these phenotypes are likely due to the NURF complex; however, a new role was uncovered for NURF as a regulator of ecdysone signaling in GSC self renewal [97]. It is currently unknown if NURF works exclusively through BMP or ecdysone signaling pathways, or a combination of the two, to regulate GSC self renewal in the *D. melanogaster* ovary.

Studies in the male germline show that NURF is required for self-renewal of GSC and cyst progenitor cells (CPC) by activating JAK/STAT signaling. Germ line clones of *Nurf301* mutants lacking either the full length or short isoforms of NURF301 show a progressive loss of GSC and CPC, likely due to premature differentiation into early daughter cells (i.e., defective self renewal). Defects were observed for mutants of *Iswi* and *Nurf38*, and not for *Acf1* or *Dmi-2*, documenting that the NURF complex, but not ACF, CHRAC or NURD complexes, are required for GSC and CPC stem cell self-renewal [98]. Similar experiments looking at sperm development showed that full length NURF301 is selectively required for late stage spermatogenesis. In flies lacking full length NURF301, there is a pronounced block in sperm development after the first meiotic division. The block in meiosis correlates with reduced levels of cyclin B, a known regulator of cell cycle progression. Analysis of gene transcription profiles shows that NURF regulates the Bruno family of translational inhibitors, which are known inhibitors of cyclin B protein levels [13]. These studies show that both the full length and short NURF301 isoforms are required for GSC and CPC self

renewal, but conversely, only full length NURF301 is required for late stage spermatocyte development.

7.2. *M. musculus* and *H. sapiens*

Bptf knockout mice do not gastrulate due to defects in the differentiation of extra-embryonic tissue lineages. *Bptf*-dependent lineages include the distal visceral endoderm, an essential precursor to one of the first organizers of the mammalian embryo, and the ectoplacental cone, an essential tissue at the interface between the embryo and decidua [53,99]. This could be due in part because of a requirement for Bptf in Nodal signaling through the Smad transcription factors, an essential signaling pathway for the specification of embryonic and extra-embryonic tissue differentiation in the early embryo. Characterization of *Bptf* knockout *M. musculus* embryonic stem cells shows a greater NURF requirement for mesoderm and endoderm differentiation than for ectoderm differentiation [53]. The same *Bptf* knockout embryonic stem cells have significant defects in the expression of homeobox genes, supporting similar observations from *D. melanogaster*, *X. laevis*, and *H. sapiens* [8,14,53,73,98].

As with *D. melanogaster*, studies on NURF function in the adult mammal have been limited because Bptf is essential for embryonic development. Cre-loxp conditional knockout technology revealed that *Bptf* is essential for adult thymocyte development [54]. In vitro and in vivo pull downs show specific interactions between NURF and SRF and AP-1, two transcription factors essential for T cell receptor-dependent gene regulation. In this study defects in chromatin structure and transcription were observed prior to and after β -selection; consistent with a role for NURF having functions very early in thymocyte development prior to the positive selection process.

As is true for *D. melanogaster*, mammalian NURF likely has important functions during differentiation of adult germ line stem cells. During ovarian follicle development the *Snf2l* transcript and protein levels are elevated with hCG stimulation. hCG in concert with progesterone regulates the development of oocytes in mammals. In vivo pull down from granulosa cells show stimulation-dependent interactions between Snf2l and the PR, a key transcription factor which regulates the transcription of gene targets in response to hCG. ChIP studies show stimulation-dependent recruitment of Snf2l to the promoter of a Snf2l-dependent granulosa cell differentiation marker gene StAR, suggesting direct functions in its regulation [61].

7.3. *X. laevis*

Biochemical purifications of ISWI-containing complexes from egg extracts did not identify a NURF complex homolog [100]. These results suggest that the NURF complex does not exist in *X. laevis* oocytes. A morpholino knockdown of NURF301 at the 2 cell stage of development reveals defects in axial development, gut formation and blood cell development, as well as aberrant regulation of homeobox gene expression [14]. A similar morpholino knockdown of ISWI at the one cell stage results in pronounced defects in gastrulation, neural fold closure, and eye development. ISWI knockdown mutants have

defects in many neural specific gene targets including *Bmp4*, which is proposed to be a direct target of ISWI [101].

Some very interesting chromatin remodeling-independent functions for ISWI were discovered using the *X. laevis* model. ISWI was identified biochemically as a microtubule-associated protein whose interaction with microtubules is independent of its ATPase activity. ISWI knockdowns in both *X. laevis* egg extracts and in *D. melanogaster* S2 cells results in dramatic decondensation of microtubules during anaphase, suggesting that ISWI has activities independent of chromatin remodeling in promoting microtubule stability during anaphase [102].

7.4. *C. elegans*

In *C. elegans* both the NURF301 homolog NURF-1 and ISW-1 are essential for the synMuv (Synthetic Multivulva) phenotype. Syn-Muv genes are negative regulators of vulvae cell development and frequently encode novel nuclear components, repressors of transcription, and factors that remodel chromatin. Nurf-1 or Iswi-1 mutations suppressed all synMuv combinations tested, demonstrating that NURF acts as an important positive regulator of the vulval cell fate. SynMuv alleles regulated by NURF include gain of function mutations in the ras signaling pathway, suggesting NURF may have important regulatory functions in this pathway [91].

7.5. *A. thaliana*

Mutants of NURF301 in *A. thaliana* have not been reported; however, a knockout of the ISWI homolog CHR11 has been characterized. Loss of function of CHR11 results in defects during the diploid and haploid phases of plant development. Diploid phase knockdown resulted in reduced plant height and small cotyledonary embryos, whereas knockdown during female gametogenesis resulted in arrested megagametogenesis. These phenotypes correlate with reduced cell expansion possibly causing the reduction in the size of the embryos [103].

8. Roles for NURF in human cancer

Primary human cancers, and cancer cell lines from many tissues including brain, breast, lung, liver, and prostate, frequently duplicate the 17q distal chromosome arm containing the BPTF gene [104–107]. Many genes have been proposed to be responsible for selection of this chromosomal duplication; however, none of the reports considered BPTF as a possible candidate [108–111]. As a result, it is unknown if BPTF gene duplication provides an advantage to cancer cells or is an indirect consequence of 17q21 distal chromosome duplications. One report identified a non-reciprocal translocation (der(X)t(X;17)) in the BPTF gene when human embryonic lung cells were maintained in continuous culture [112]. The chromosomal translocation resulted in increased BPTF mRNA levels and correlated with increased cellular proliferation. Consistent with the literature on 17q distal duplications, the authors report frequent BPTF duplications in neuroblastomas, lung tumors, leukemia and colon cancers.

In a targeted study, *SNF2L* expression was found to be equivalent in tumor tissues and normal tissue controls [113]. The authors observed that a number of human cancer cell lines are sensitive to knockdown of SNF2L but not SNF2H. Knockdown of SNF2L resulted in reduced proliferation, increased DNA damage and increased apoptosis, suggesting that the selective inhibition of SNF2L could be a viable therapy for some cancers. Why these cancer cells are sensitive to SNF2L, but not SNF2H knockdown is unknown and likely a topic for future study. There is evidence that SNF2 protein expression is elevated in some human cancers. In a targeted study of prostate cancer, the SNF2 protein was significantly elevated in prostate neoplasm compared to benign prostatic hyperplasia [114]. However, the antibody used in this study reacts with both SNF2H and SNF2L, making it difficult to determine which of the variants are increased during the progression of prostate cancer.

9. Concluding remarks

The literature clearly documents that the NURF complex has critical functions in many aspects of chromatin molecular biology. Most importantly, NURF has critical functions regulating higher order chromatin structure and gene expression output of many prominent signaling pathways to the nucleus. While this ground-work has established much in the way of understanding NURF function, many important questions remain to be answered. Some of these questions include

1. What are the direct targets of NURF chromatin remodeling in vivo?
Comprehensively identifying direct NURF targets from multiple cell types and model organisms is an essential step towards understanding its in vivo functions. Use of high throughput methods to map changes in chromatin structure with *Bptf* NURF301 knockout and determine NURF localization to the genome will begin to identify these sites.
2. How is NURF directed to specific regions of the genome? NURF is likely recruited to specific regions of the genome by interactions with transcription factors, histone modifications, and possibly specific DNA sequences. Identifying all of these elements will make a significant contribution towards identifying NURF dependent genes a priori.
3. What is the outcome of the NURF remodeling reaction in vivo? Germane to this line of inquiry is understanding how physiologically relevant elements of chromatin, like transcription factors, linker histones and histone modifications influence the outcome of the in vivo remodeling reaction.
4. Is the ATP-dependent remodeling activity of NURF its only function in vivo? Recent reports show that the Brg1 remodeling enzymes have gene regulatory functions independent of their ATPase activity [115]. It will be essential to our study of the complex to investigate if NURF, like Brg1-containing complexes, has similar ATP-independent functions.
5. Are remodeling reactions by NURF stable or transitory in vivo? Knowing how dynamic the events of chromatin remodeling are in vivo will help us to understand their short and long term effects on chromatin structure.

6. Is NURF301/BPTF exclusive to the NURF complex? It will be essential to know if the largest and essential subunit of NURF functions exclusively as a component of the NURF complex in both mammals and other model organisms.
7. Will regulating NURF activities have any therapeutic benefit to humans? It has been well documented that NURF functions as a critical component of signaling pathways relevant to human disease (TGF β and JAK/STAT, as two examples). Exploring NURF as a therapeutic target to regulate these pathways for the treatment of human diseases will be an important avenue of research.

The challenge for the future will be to address these outstanding questions and learn much about this essential chromatin remodeling complex with the objective of advancing both basic and translational science.

Acknowledgements

The author would like to apologize for not including all references due to space constraints. A special thanks to Kelly Jordan-Sciotto, Hua Xiao, Toshi Tsukiyama, Joyce Lloyd, Kevin Hogan, Heidi M Sankala, and members of the Landry Lab for suggestions to improve the manuscript. The author's laboratory is funded by startup funds from the Virginia Commonwealth University School of Medicine and the Massey Cancer Center.

References

- [1]. Luger K, et al. Crystal structure of the nucleosome core particle at 2.8 Å resolution. *Nature*. 1997; 389(6648):251–260. [PubMed: 9305837]
- [2]. Jiang C, Pugh BF. Nucleosome positioning and gene regulation: advances through genomics. *Nat. Rev. Genet.* 2009; 10(3):161–172. [PubMed: 19204718]
- [3]. Berger SL, et al. An operational definition of epigenetics. *Genes Dev.* 2009; 23(7):781–783. [PubMed: 19339683]
- [4]. Clapier CR, Cairns BR. The biology of chromatin remodeling complexes. *Annu. Rev. Biochem.* 2009; 78:273–304. [PubMed: 19355820]
- [5]. Tsukiyama T, Becker PB, Wu C. ATP-dependent nucleosome disruption at a heat-shock promoter mediated by binding of GAGA transcription factor. *Nature*. 1994; 367(6463):525–532. [PubMed: 8107823]
- [6]. Tsukiyama T, Wu C. Purification and properties of an ATP-dependent nucleosome remodeling factor. *Cell*. 1995; 83(6):1011–1020. [PubMed: 8521501]
- [7]. Tsukiyama T, et al. ISWI, a member of the SWI2/SNF2 ATPase family, encodes the 140 kDa subunit of the nucleosome remodeling factor. *Cell*. 1995; 83(6):1021–1026. [PubMed: 8521502]
- [8]. Barak O, et al. Isolation of human NURF: a regulator of Engrailed gene expression. *EMBO J.* 2003; 22(22):6089–6100. [PubMed: 14609955]
- [9]. Xiao H, et al. Dual functions of largest NURF subunit NURF301 in nucleosome sliding and transcription factor interactions. *Mol. Cell*. 2001; 8(3):531–543. [PubMed: 11583616]
- [10]. Reeves R, Nissen MS. The A.T-DNA-binding domain of mammalian high mobility group I chromosomal proteins. A novel peptide motif for recognizing DNA structure. *J. Biol. Chem.* 1990; 265(15):8573–8582. [PubMed: 1692833]
- [11]. Fyodorov DV, Kadonaga JT. Binding of Acf1 to DNA involves a WAC motif and is important for ACF-mediated chromatin assembly. *Mol. Cell. Biol.* 2002; 22(18):6344–6353. [PubMed: 12192034]
- [12]. Uversky VN, Oldfield CJ, Dunker AK. Showing your ID: intrinsic disorder as an ID for recognition, regulation and cell signaling. *J. Mol. Recognit.* 2005; 18(5):343–384. [PubMed: 16094605]

- [13]. Kwon SY, et al. Alternative splicing of NURF301 generates distinct NURF chromatin remodeling complexes with altered modified histone binding specificities. *PLoS Genet.* 2009; 5(7):e1000574. [PubMed: 19629165]
- [14]. Wysocka J, et al. A PHD finger of NURF couples histone H3 lysine 4 trimethylation with chromatin remodelling. *Nature.* 2006; 442(7098):86–90. [PubMed: 16728976]
- [15]. Ruthenburg AJ, et al. Recognition of a mononucleosomal histone modification pattern by BPTF via multivalent interactions. *Cell.* 2011; 145(5):692–706. [PubMed: 21596426]
- [16]. Savkur RS, Burris TP. The coactivator LXXLL nuclear receptor recognition motif. *J. Peptide Res.: Off. J. Am. Peptide Soc.* 2004; 63(3):207–212.
- [17]. Eisen JA, Sweder KS, Hanawalt PC. Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucleic Acids Res.* 1995; 23(14):2715–2723. [PubMed: 7651832]
- [18]. Dang W, Bartholomew B. Domain architecture of the catalytic subunit in the ISW2-nucleosome complex. *Mol. Cell. Biol.* 2007; 27(23):8306–8317. [PubMed: 17908792]
- [19]. Dechassa ML, et al. Architecture of the SWI/SNF-nucleosome complex. *Mol. Cell. Biol.* 2008; 28(19):6010–6021. [PubMed: 18644858]
- [20]. Aasland R, Stewart AF, Gibson T. The SANT domain: a putative DNA-binding domain in the SWI-SNF and ADA complexes, the transcriptional co-repressor N-CoR and TFIIB. *Trends Biochem. Sci.* 1996; 21(3):87–88. [PubMed: 8882580]
- [21]. Grune T, et al. Crystal structure and functional analysis of a nucleosome recognition module of the remodeling factor ISWI. *Mol. Cell.* 2003; 12(2):449–460. [PubMed: 14536084]
- [22]. Martinez-Balbas MA, et al. Drosophila NURF-55, a WD repeat protein involved in histone metabolism. *Proc. Natl. Acad. Sci. USA.* 1998; 95(1):132–137. [PubMed: 9419341]
- [23]. Hennig L, Bouveret R, Grussem W. MSI1-like proteins: an escort service for chromatin assembly and remodeling complexes. *Trends Cell Biol.* 2005; 15(6):295–302. [PubMed: 15953547]
- [24]. Smith TF, et al. The WD repeat: a common architecture for diverse functions. *Trends Biochem. Sci.* 1999; 24(5):181–185. [PubMed: 10322433]
- [25]. Verreault A, et al. Nucleosome assembly by a complex of CAF-1 and acetylated histones H3/H4. *Cell.* 1996; 87(1):95–104. [PubMed: 8858152]
- [26]. Verreault A, et al. Nucleosomal DNA regulates the core-histone-binding subunit of the human Hat1 acetyltransferase. *Curr. Biol.* 1998; 8(2):96–108. [PubMed: 9427644]
- [27]. Gdula DA, et al. Inorganic pyrophosphatase is a component of the Drosophila nucleosome remodeling factor complex. *Genes Dev.* 1998; 12(20):3206–3216. [PubMed: 9784495]
- [28]. Schwanbeck R, Xiao H, Wu C. Spatial contacts and nucleosome step movements induced by the NURF chromatin remodeling complex. *J. Biol. Chem.* 2004; 279(38):39933–39941. [PubMed: 15262970]
- [29]. Hamiche A, et al. ATP-dependent histone octamer sliding mediated by the chromatin remodeling complex NURF. *Cell.* 1999; 97(7):833–842. [PubMed: 10399912]
- [30]. Mizuguchi G, et al. ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science.* 2004; 303(5656):343–348. [PubMed: 14645854]
- [31]. Papamichos-Chronakis M, et al. Global regulation of H2A.Z localization by the INO80 chromatin-remodeling enzyme is essential for genome integrity. *Cell.* 2011; 144(2):200–213. [PubMed: 21241891]
- [32]. Dechassa ML, et al. SWI/SNF has intrinsic nucleosome disassembly activity that is dependent on adjacent nucleosomes. *Mol. Cell.* 2010; 38(4):590–602. [PubMed: 20513433]
- [33]. Gutierrez JL, et al. Activation domains drive nucleosome eviction by SWI/SNF. *EMBO J.* 2007; 26(3):730–740. [PubMed: 17235287]
- [34]. Flaus A, Owen-Hughes T. Dynamic properties of nucleosomes during thermal and ATP-driven mobilization. *Mol. Cell. Biol.* 2003; 23(21):7767–7779. [PubMed: 14560021]
- [35]. Rippe K, et al. DNA sequence- and conformation-directed positioning of nucleosomes by chromatin-remodeling complexes. *Proc. Natl. Acad. Sci. USA.* 2007; 104(40):15635–15640. [PubMed: 17893337]

- [36]. Kang JG, Hamiche A, Wu C. GAL4 directs nucleosome sliding induced by NURF. *EMBO J.* 2002; 21(6):1406–1413. [PubMed: 11889046]
- [37]. Georgel PT, Tsukiyama T, Wu C. Role of histone tails in nucleosome remodeling by *Drosophila* NURF. *EMBO J.* 1997; 16(15):4717–4726. [PubMed: 9303316]
- [38]. Shogren-Knaak M, et al. Histone H4-K16 acetylation controls chromatin structure and protein interactions. *Science.* 2006; 311(5762):844–847. [PubMed: 16469925]
- [39]. Clapier CR, Nightingale KP, Becker PB. A critical epitope for substrate recognition by the nucleosome remodeling ATPase ISWI. *Nucleic Acids Res.* 2002; 30(3):649–655. [PubMed: 11809876]
- [40]. Clapier CR, et al. Critical role for the histone H4 N terminus in nucleosome remodeling by ISWI. *Mol. Cell. Biol.* 2001; 21(3):875–883. [PubMed: 11154274]
- [41]. Corona DF, et al. Modulation of ISWI function by site-specific histone acetylation. *EMBO Rep.* 2002; 3(3):242–247. [PubMed: 11882543]
- [42]. Mizuguchi G, et al. ATP-dependent nucleosome remodeling and histone hyperacetylation synergistically facilitate transcription of chromatin. *J. Biol. Chem.* 2001; 276(18):14773–14783. [PubMed: 11279013]
- [43]. Vogler C, et al. Histone H2A C-terminus regulates chromatin dynamics, remodeling, and histone H1 binding. *PLoS Genet.* 2010; 6(12):e1001234. [PubMed: 21170357]
- [44]. Marques M, et al. Reconciling the positive and negative roles of histone H2A.Z in gene transcription. *Epigenet.: Off. J. DNA Methylation Soc.* 2010; 5(4):267–272.
- [45]. Goldman JA, Garlick JD, Kingston RE. Chromatin remodeling by imitation switch (ISWI) class ATP-dependent remodelers is stimulated by histone variant H2A.Z. *J. Biol. Chem.* 2010; 285(7):4645–4651. [PubMed: 19940112]
- [46]. Suto RK, et al. Crystal structure of a nucleosome core particle containing the variant histone H2A.Z. *Nat. Struct. Biol.* 2000; 7(12):1121–1124. [PubMed: 11101893]
- [47]. Maier VK, Chioda M, Becker PB. ATP-dependent chromatin remodeling. *Biol. Chem.* 2008; 389(4):345–352. [PubMed: 18208359]
- [48]. Clausell J, et al. Histone H1 subtypes differentially modulate chromatin condensation without preventing ATP-dependent remodeling by SWI/SNF or NURF. *PLoS ONE.* 2009; 4(10):e0007243. [PubMed: 19794910]
- [49]. Horn PJ, et al. Phosphorylation of linker histones regulates ATP-dependent chromatin remodeling enzymes. *Nat. Struct. Biol.* 2002; 9(4):263–267. [PubMed: 11887184]
- [50]. Sala A, et al. The nucleosome-remodeling ATPase ISWI is regulated by poly-ADP-ribosylation. *PLoS Biol.* 2008; 6(10):e252. [PubMed: 18922045]
- [51]. Ferreira R, et al. Site-specific acetylation of ISWI by GCN5. *BMC Mol. Biol.* 2007; 8:73. [PubMed: 17760996]
- [52]. Shen X, et al. Modulation of ATP-dependent chromatin-remodeling complexes by inositol polyphosphates. *Science.* 2003; 299(5603):112–114. [PubMed: 12434013]
- [53]. Landry J, et al. Essential role of chromatin remodeling protein *Bptf* in early mouse embryos and embryonic stem cells. *PLoS Genet.* 2008; 4(10):e1000241. [PubMed: 18974875]
- [54]. Landry JW, et al. Chromatin remodeling complex NURF regulates thymocyte maturation. *Genes Dev.* 2011; 25(3):275–286. [PubMed: 21289071]
- [55]. Jordan-Sciutto KL, et al. Fetal Alz-50 clone 1 (FAC1) protein interacts with the Myc-associated zinc finger protein (ZF87/MAZ) and alters its transcriptional activity. *Biochemistry.* 2000; 39(12):3206–3215. [PubMed: 10727212]
- [56]. Strachan GD, et al. Fetal Alz-50 clone 1 interacts with the human orthologue of the Kelch-like Ech-associated protein. *Biochemistry.* 2004; 43(38):12113–12122. [PubMed: 15379550]
- [57]. Badenhorst P, et al. The *Drosophila* nucleosome remodeling factor (NURF) is required for Ecdysteroid signaling and metamorphosis. *Genes Dev.* 2005; 19(21):2540–2545. [PubMed: 16264191]
- [58]. Kwon SY, et al. The nucleosome remodeling factor (NURF) regulates genes involved in *Drosophila* innate immunity. *Dev. Biol.* 2008; 316(2):538–547. [PubMed: 18334252]

- [59]. Song H, Spichiger-Hausermann C, Basler K. The ISWI-containing NURF complex regulates the output of the canonical Wingless pathway. *EMBO Rep.* 2009; 10(10):1140–1146. [PubMed: 19713963]
- [60]. Stephens GE, et al. Heterochromatin protein 2 interacts with Nap-1 and NURF: a link between heterochromatin-induced gene silencing and the chromatin remodeling machinery in *Drosophila*. *Biochemistry.* 2006; 45(50):14990–14999. [PubMed: 17154536]
- [61]. Lazzaro MA, et al. The imitation switch protein SNF2L regulates steroidogenic acute regulatory protein expression during terminal differentiation of ovarian granulosa cells. *Mol. Endocrinol.* 2006; 20(10):2406–2417. [PubMed: 16740656]
- [62]. Di Croce L, et al. Two-step synergism between the progesterone receptor and the DNA-binding domain of nuclear factor 1 on MMTV minichromosomes. *Mol. Cell.* 1999; 4(1):45–54. [PubMed: 10445026]
- [63]. Hochheimer A, et al. TRF2 associates with DREF and directs promoter-selective gene expression in *Drosophila*. *Nature.* 2002; 420(6914):439–445. [PubMed: 12459787]
- [64]. Kugler SJ, Nagel AC. A novel PzG-NURF complex regulates Notch target gene activity. *Mol. Biol. Cell.* 2010; 21(19):3443–3448. [PubMed: 20685964]
- [65]. Li X, et al. Chromatin boundaries require functional collaboration between the hSET1 and NURF complexes. *Blood.* 2011; 118(5):1386–1394. [PubMed: 21653943]
- [66]. Jones MH, Hamana N, Shimane M. Identification and characterization of BPTF, a novel bromodomain transcription factor. *Genomics.* 2000; 63(1):35–39. [PubMed: 10662542]
- [67]. Bowser R, Giambrone A, Davies P. FAC1, a novel gene identified with the monoclonal antibody Alz50, is developmentally regulated in human brain. *Dev. Neurosci.* 1995; 17(1):20–37. [PubMed: 7621746]
- [68]. Li H, et al. Molecular basis for site-specific read-out of histone H3K4me3 by the BPTF PHD finger of NURF. *Nature.* 2006; 442(7098):91–95. [PubMed: 16728978]
- [69]. Jordan-Sciutto KL, et al. Fetal Alz-50 clone 1, a novel zinc finger protein, binds a specific DNA sequence and acts as a transcriptional regulator. *J. Biol. Chem.* 1999; 274(49):35262–35268. [PubMed: 10575013]
- [70]. Badis G, et al. A library of yeast transcription factor motifs reveals a widespread function for Rsc3 in targeting nucleosome exclusion at promoters. *Mol. Cell.* 2008; 32(6):878–887. [PubMed: 19111667]
- [71]. Erdel F, et al. Human ISWI chromatin-remodeling complexes sample nucleosomes via transient binding reactions and become immobilized at active sites. *Proc. Natl. Acad. Sci. USA.* 2010; 107(46):19873–19878. [PubMed: 20974961]
- [72]. Okada M, Hirose S. Chromatin remodeling mediated by *Drosophila* GAGA factor and ISWI activates fushi tarazu gene transcription in vitro. *Mol. Cell. Biol.* 1998; 18(5):2455–2461. [PubMed: 9566866]
- [73]. Badenhorst P, et al. Biological functions of the ISWI chromatin remodeling complex NURF. *Genes Dev.* 2002; 16(24):3186–3198. [PubMed: 12502740]
- [74]. Mizuguchi G, et al. Role of nucleosome remodeling factor (NURF) in transcriptional activation of chromatin. *Mol. Cell.* 1997; 1(1):141–150. [PubMed: 9659911]
- [75]. Deuring R, et al. The ISWI chromatin-remodeling protein is required for gene expression and the maintenance of higher order chromatin structure in vivo. *Mol. Cell.* 2000; 5(2):355–365. [PubMed: 10882076]
- [76]. Bai X, et al. Regional control of chromatin organization by noncoding roX RNAs and the NURF remodeling complex in *Drosophila melanogaster*. *Genetics.* 2007; 176(3):1491–1499. [PubMed: 17507677]
- [77]. Bhadra MP, et al. Gene expression analysis of the function of the male-specific lethal complex in *Drosophila*. *Genetics.* 2005; 169(4):2061–2074. [PubMed: 15716510]
- [78]. Corona DF, et al. ISWI regulates higher-order chromatin structure and histone H1 assembly in vivo. *PLoS Biol.* 2007; 5(9):e232. [PubMed: 17760505]
- [79]. Suganuma T, et al. The ATAC acetyltransferase complex coordinates MAP kinases to regulate JNK target genes. *Cell.* 2010; 142(5):726–736. [PubMed: 20813260]

- [80]. Suganuma T, et al. ATAC is a double histone acetyltransferase complex that stimulates nucleosome sliding. *Nat. Struct. Mol. Biol.* 2008; 15(4):364–372. [PubMed: 18327268]
- [81]. Carre C, et al. The *Drosophila* NURF remodelling and the ATAC histone acetylase complexes functionally interact and are required for global chromosome organization. *EMBO Rep.* 2008; 9(2):187–192. [PubMed: 18084186]
- [82]. Li M, Belozerov VE, Cai HN. Modulation of chromatin boundary activities by nucleosome-remodeling activities in *Drosophila melanogaster*. *Mol. Cell. Biol.* 2010; 30(4):1067–1076. [PubMed: 19995906]
- [83]. Fan HY, et al. Swapping function of two chromatin remodeling complexes. *Mol. Cell.* 2005; 17(6):805–815. [PubMed: 15780937]
- [84]. Aihara T, et al. Cloning and mapping of SMARCA5 encoding hSNF2H, a novel human homologue of *Drosophila* ISWI. *Cytogenet. Cell Genet.* 1998; 81(3–4):191–193. [PubMed: 9730600]
- [85]. Okabe I, et al. Cloning of human and bovine homologs of SNF2/SWI2: a global activator of transcription in yeast *S. cerevisiae*. *Nucleic Acids Res.* 1992; 20(17):4649–4655. [PubMed: 1408766]
- [86]. Bozhenok L, Wade PA, Varga-Weisz P. WSTF-ISWI chromatin remodeling complex targets heterochromatic replication foci. *EMBO J.* 2002; 21(9):2231–2241. [PubMed: 11980720]
- [87]. Banting GS, et al. CECR2, a protein involved in neurulation, forms a novel chromatin remodeling complex with SNF2L. *Hum. Mol. Genet.* 2005; 14(4):513–524. [PubMed: 15640247]
- [88]. LeRoy G, et al. Purification and characterization of a human factor that assembles and remodels chromatin. *J. Biol. Chem.* 2000; 275(20):14787–14790. [PubMed: 10747848]
- [89]. Poot RA, et al. HuCHRAC, a human ISWI chromatin remodelling complex contains hACF1 and two novel histone-fold proteins. *EMBO J.* 2000; 19(13):3377–3387. [PubMed: 10880450]
- [90]. Hargreaves DC, Crabtree GR. ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. *Cell Res.* 2011; 21(3):396–420. [PubMed: 21358755]
- [91]. Andersen EC, Lu X, Horvitz HR. *C. elegans* ISWI and NURF301 antagonize an Rb-like pathway in the determination of multiple cell fates. *Development.* 2006; 133(14):2695–2704. [PubMed: 16774993]
- [92]. Barak O, et al. A tissue-specific, naturally occurring human SNF2L variant inactivates chromatin remodeling. *J. Biol. Chem.* 2004; 279(43):45130–45138. [PubMed: 15310751]
- [93]. Bhosekar S, Babu P. Enhancer of bithorax: a mutation of *Drosophila melanogaster*. *J. Genet.* 1987; 66(3)
- [94]. Schuettengruber B, et al. Genome regulation by polycomb and trithorax proteins. *Cell.* 2007; 128(4):735–745. [PubMed: 17320510]
- [95]. Kugler SJ, et al. The Putzig-NURF nucleosome remodeling complex is required for ecdysone receptor signaling and innate immunity in *Drosophila melanogaster*. *Genetics.* 2011; 188(1):127–139. [PubMed: 21385730]
- [96]. Xi R, Xie T. Stem cell self-renewal controlled by chromatin remodeling factors. *Science.* 2005; 310(5753):1487–1489. [PubMed: 16322456]
- [97]. Ables ET, Drummond-Barbosa D. The steroid hormone ecdysone functions with intrinsic chromatin remodeling factors to control female germline stem cells in *Drosophila*. *Cell Stem Cell.* 2010; 7(5):581–592. [PubMed: 21040900]
- [98]. Cherry CM, Matunis EL. Epigenetic regulation of stem cell maintenance in the *Drosophila* testis via the nucleosome-remodeling factor NURF. *Cell Stem Cell.* 2010; 6(6):557–567. [PubMed: 20569693]
- [99]. Goller T, et al. Transcriptional regulator BPTF/FAC1 is essential for trophoblast differentiation during early mouse development. *Mol. Cell. Biol.* 2008; 28(22):6819–6827. [PubMed: 18794365]
- [100]. Guschin D, et al. Multiple ISWI ATPase complexes from *Xenopus laevis*. Functional conservation of an ACF/CHRAC homolog. *J. Biol. Chem.* 2000; 275(45):35248–35255. [PubMed: 10942776]

- [101]. Dirscherl SS, Henry JJ, Krebs JE. Neural and eye-specific defects associated with loss of the imitation switch (ISWI) chromatin remodeler in *Xenopus laevis*. *Mech. Dev.* 2005; 122(11): 1157–1170. [PubMed: 16169710]
- [102]. Yokoyama H, et al. ISWI is a RanGTP-dependent MAP required for chromosome segregation. *J. Cell Biol.* 2009; 187(6):813–829. [PubMed: 20008562]
- [103]. Huanca-Mamani W, et al. CHR11, a chromatin-remodeling factor essential for nuclear proliferation during female gametogenesis in *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. USA.* 2005; 102(47):17231–17236. [PubMed: 16286646]
- [104]. Choi JS, et al. Comparative genomic hybridization array analysis and real-time PCR reveals genomic copy number alteration for lung adenocarcinomas. *Lung.* 2006; 184(6):355–362. [PubMed: 17086460]
- [105]. Sun J, et al. Association between sequence variants at 17q12 and 17q24.3 and prostate cancer risk in European and African Americans. *Prostate.* 2008; 68(7):691–697. [PubMed: 18361410]
- [106]. Raidl M, et al. Multiple chromosomal abnormalities in human liver (pre)neoplasia. *J. Hepatol.* 2004; 40(4):660–668. [PubMed: 15030983]
- [107]. Bown N, et al. Gain of chromosome arm 17q and adverse outcome in patients with neuroblastoma. *N. Engl. J. Med.* 1999; 340(25):1954–1961. [PubMed: 10379019]
- [108]. Vandesompele J, et al. Identification of 2 putative critical segments of 17q gain in neuroblastoma through integrative genomics. *Int. J. Cancer.* 2008; 122(5):1177–1182. [PubMed: 17973261]
- [109]. Monni O, et al. Comprehensive copy number and gene expression profiling of the 17q23 amplicon in human breast cancer. *Proc. Natl. Acad. Sci. USA.* 2001; 98(10):5711–5716. [PubMed: 11331760]
- [110]. Wu GJ, et al. 17q23 amplifications in breast cancer involve the PAT1, RAD51C, PS6K, and SIGMA1B genes. *Cancer Res.* 2000; 60(19):5371–5375. [PubMed: 11034073]
- [111]. Beser AR, et al. HER-2, TOP2A and chromosome 17 alterations in breast cancer. *Pathol. Oncol. Res.* 2007; 13(3):180–185. [PubMed: 17922046]
- [112]. Bugarim Y, et al. A novel translocation breakpoint within the BPTF gene is associated with a pre-malignant phenotype. *PLoS ONE.* 2010; 5(3):e9657. [PubMed: 20300178]
- [113]. Ye Y, et al. Inhibition of expression of the chromatin remodeling gene, SNF2L, selectively leads to DNA damage, growth inhibition, and cancer cell death. *Mol. Cancer Res.* 2009; 7(12):1984–1999. [PubMed: 19996304]
- [114]. Mohamed MA, et al. Epigenetic events, remodelling enzymes and their relationship to chromatin organization in prostatic intraepithelial neoplasia and prostatic adenocarcinoma. *BJU Int.* 2007; 99(4):908–915. [PubMed: 17378849]
- [115]. Jani A, et al. A novel genetic strategy reveals unexpected roles of the Swi-Snf-like chromatin-remodeling BAF complex in thymocyte development. *J. Exp. Med.* 2008; 205(12):2813–2825. [PubMed: 18955569]
- [116]. Papadopoulos JS, Agarwala R. COBALT: constraint-based alignment tool for multiple protein sequences. *Bioinformatics.* 2007; 23(9):1073–1079. [PubMed: 17332019]
- [117]. Thompson JD, Higgins DG, Gibson TJ. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* 1994; 22(22):4673–4680. [PubMed: 7984417]

Fig. 1.

Diagram of the NURF remodeling complex and its associated subunits. (A) Cartoon showing the subunit composition of *D. melanogaster* and *H. sapiens* NURF complexes. *D. melanogaster* NURF contains 4 subunits: NURF301, the largest and essential subunit; the ISWI ATPase; NURF55, a WD repeat protein; and NURF38, a pyrophosphatase. *H. sapiens* NURF has homologs strongly related to 3 of these subunits. BPTF is closely related to NURF301, SNF2L to ISWI, and pRBAP46/48 to NURF55. Interestingly, *H. sapiens* NURF does not contain a homolog of the NURF38 pyrophosphatase. (B) Domain analysis of *D. melanogaster* NURF subunits. Domains are color coded (cf. Fig. 2).

Fig. 2.

NURF301 homologs are found in many widely studied model organisms. (A) Alignment of NURF301 homologs showing the positions of conserved domains. Color codes for domains are the same as in Fig. 1. Sequences used for analysis; *H. sapiens* NP_872579.2, *M. musculus* NP_789820.2, *T. rubripes* ENSTRUG00000008386, *X. tropicalis* XP_002942992.1, *G. gallus* ENSGALP00000005731, *D. rerio* XP_001920272.1, *D. melanogaster* NP_728507.1, *C. elegans* NP_001022117.1, *A. thaliana* AT5G12400.1. (B) Phylogenetic analysis of NURF301 homologs showing relatedness between species. Comparisons were made using the default settings for the Cobalt alignment program [116].

Fig. 3.

Strong sequence conservation at C-terminal PHD fingers and bromodomain of NURF301 homologs. (A) Hydrophobic residues important for H3K4me2/3 binding were identified from Ref. [68] and are designated by black arrows. These residues are highly conserved in all species except *A. thaliana*, making it quite likely that binding to H3K4me2/3 is conserved in most species. (B) Alignment and comparison of PHD1 and PHD2 domains of *D. melanogaster*, and *C. elegans* NURF301 and C-terminal PHD finger of *A. thaliana*. (C) The C-terminal PHD finger of *A. thaliana* is significantly different from PHD1 and PHD2 as shown by nearest neighbor analysis using default settings for the Cobalt alignment program [116]. (D) Comparison of bromodomains from NURF301 homologs showing strong sequence conservation through evolution. All alignments were made using default settings for the Clustal W program [117].

Fig. 5. ISWI homologs are found in all widely studied model organisms. (A) The domain structure of *D. melanogaster* ISWI. Because the sequence of *D. melanogaster* ISWI and its homologs is so highly conserved, a single homolog is presented showing the position of conserved domains. Color codes for domains are the same as in Fig. 1. (B) Phylogenetic analysis of ISWI homologs showing relatedness between species. Species with two ISWI homologs can be segregated into SNF2H and SNF2L variants. Comparisons were made using the default settings for the Cobalt alignment program [116]. Sequences used for analysis; *A. thaliana* NP_850847.1 and NP_187291.2, *S. cerevisiae* NP_009804.1 and NP_014948.1, *G. gallus* XP_420329.2 and XP_001234486.1, *D. melanogaster* NP_523719.1, *C. elegans* NP_498468.2, *X. tropicalis* XP_002931866.1 and NP_001007993.1, *T. rubripes* ENSTRUP00000036063, *H. sapiens* NP_003060.2 and NP_003592.2, *M. musculus* NP_444353.3 and NP_444354.2, *D. rerio* NP_001075098.1 and NP_001093467.1.

Table 1

Summary of published NURF interactions with transcription factors.

Organism	Interaction partner	Biological function	Molecular function	Reference
<i>D. melanogaster</i>	GAGA ^{a,b}	Heat shock, embryo patterning	Regulator of chromatin structure at insulators and promoters	[9]
<i>D. melanogaster</i>	HSF ^a	Stress response	Regulator of heat shock response	[9]
<i>M. musculus/H. sapiens</i>	Smad2 ^a	Gastrulation	Nodal signaling	[53]
<i>M. musculus/H. sapiens</i>	SRF ^{a,b}	Thymocyte development	T cell receptor signaling	[54]
<i>M. musculus</i>	AP-1 ^b	Thymocyte development	T cell receptor signaling	[54]
<i>H. sapiens</i>	MAZ ^a	Tissue development	Cell type specific regulation of GC containing promoters	[55]
<i>H. sapiens</i>	hKeap1 ^a	Stress response	Redox signaling	[56]
<i>D. melanogaster</i>	EcR ^a	Larval metamorphosis ^c	Ecdysteroid signaling	[57]
<i>D. melanogaster</i>	Ken ^a	Innate immune system development	Cytokine signaling	[58]
<i>D. melanogaster</i>	Armado ^a	Wing development	Wingless signaling	[59]
<i>D. melanogaster</i>	HP2 ^{a,b}	Heterochromatin	Gene silencing	[60]
<i>H. sapiens/D. melanogaster</i>	PRa,b	MMTV transcription/female reproduction	Expression of MMTV viral genome/regulator of hCGH response	[61,62]
<i>D. melanogaster</i>	TRF2 and TAF4b ^b	Cell proliferation	Cell type specific regulation of genes encoding DNA replication factors	[63]
<i>D. melanogaster</i>	Pzg ^b	Wing development ^d	Notch, ecdysone, JAK/STAT signaling	[64]
<i>G. gallus/H. sapiens</i>	Usf1 ^b	Globin gene regulation	Insulator function	[65]

^aInteraction by in vitro pull down.^bInteraction by in vivo pull down.^cInteraction implied during female germline stem cell self-renewal [97].^dInteraction implied during larval metamorphosis and motility [95].