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MINIREVIEW

Chromatin remodeling in mammalian cells by ISWI-type complexes – where, when and why?

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The specific location of nucleosomes on DNA has important inhibitory or activating roles in the regulation of DNA-dependent processes as it affects the DNA accessibility. Nucleosome positions depend on the ATP-coupled activity of chromatin-remodeling complexes that translocate nucleosomes or evict them from the DNA. The mammalian cell harbors numerous different remodeling complexes that possess distinct activities. These can translate a variety of signals into certain patterns of nucleosome positions with specific functions. Although chromatin remodelers have been extensively studied *in vitro*, much less is known about how they operate in their cellular environment. Here, we review the cellular activities of the mammalian imitation switch proteins and discuss mechanisms by which they are targeted to sites where their activity is needed.

Mammalian imitation switch-type chromatin remodeling complexes

Chromatin structure is a key determinant of gene regulation. The wrapping of the DNA around the histone octamer protein core in the nucleosome impedes the access of other protein factors to the DNA sequence information. To mediate access, a diverse chromatin-remodeling machinery exists in the eukaryotic cell nucleus to translocate, remove or assemble nucleosomes as needed. It is centered around numerous different types of ATP-driven molecular machines that can move the nucleosomal DNA with respect to the histone octamer core via an ATP-driven mechanism, as described by Owen-Hughes & Flaus in this issue [1]. One of the best-conserved ATPase families involved in chromatin remodeling is the imitation switch (ISWI) family [2] (Fig. 1). It consists of the two ATPases sucrose nonfermenting 2 homologue (Snf2H) and

Abbreviations
ACF, ATP-utilizing chromatin assembly and remodeling factor; Acf1, ATP-dependent chromatin assembly factor 1; BPTF, bromodomain PHD finger transcription factor; CECR2, cat eye syndrome chromosome region candidate 2; CENP-A, centromere protein A; CERF, CECR2-containing remodeling factor; DDR, DNA damage response; H2A.X, histone 2A variant X; HP1, heterochromatin protein 1; IL, interleukin; ISWI, imitation switch; N-CoR, nuclear receptor corepressor; NoRC, nucleolar remodeling complex; NURF, nucleosome remodeling factor; PCNA, proliferating cell nuclear antigen; RNAP, RNA polymerase; RSF, remodeling and spacing factor; Rsf1, remodeling and spacing factor 1; SANT, Swi3 Ada2 N-CoR TFIIIB; SLIDE, SANT-like ISWI domain; Snf2H, sucrose nonfermenting 2 homologue; Snf2L, sucrose nonfermenting 2 like; StAR, steroidogenic acute regulatory protein; TIP5, TTF-I interacting protein 5; WICH, WSTF–ISWI chromatin remodeling complex; WSTF, Williams syndrome transcription factor.

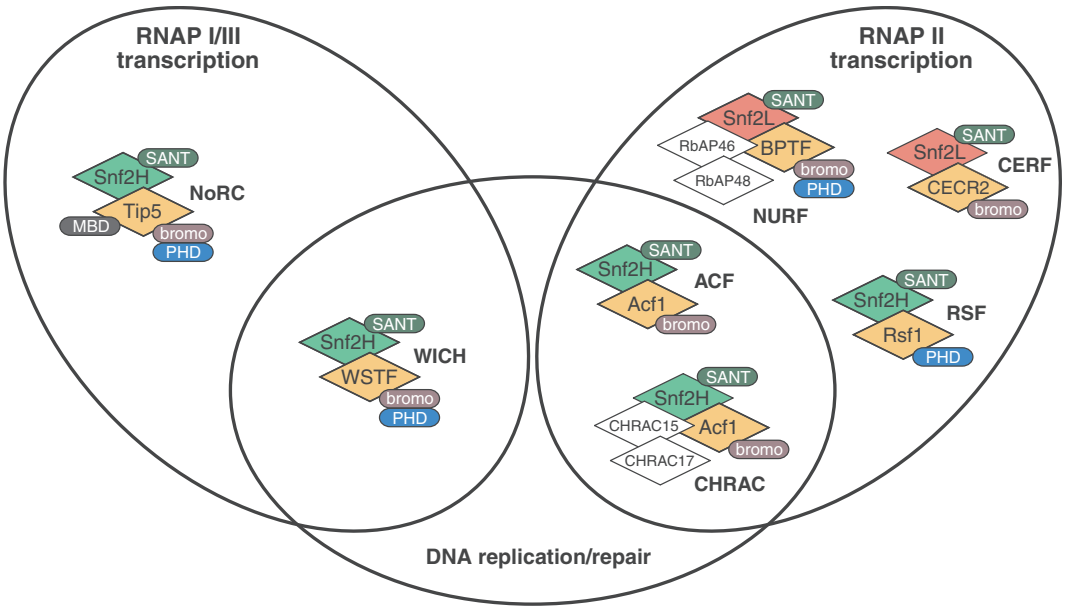

Fig. 1. ISWI complexes and their functions. The human ISWI family of remodeling ATPases comprises the Snf2H and Snf2L proteins that form complexes with different noncatalytic subunits. The functions of these complexes include DNA repair, DNA replication and transcriptional regulation. Snf2L is subject to alternative splicing and is, in some tissues, present as inactive variant Snf2L+13. Snf2H and Snf2L can replace each other, at least in the RSF complex [10].

sucrose nonfermenting 2 like (Snf2L) in humans, which are the orthologues of yeast Isw1 and Isw2 [1]. All ISWI proteins contain a conserved ATPase domain that belongs to the superfamily of DEAD/H (Asp-Glu-Ala-Asp/His)-helicases [3,4], located in the N-terminal half of the proteins. In the C-terminal part, a HAND domain, a Swi3 Ada2 N-CoR TFIIIB (SANT) domain and a juxtaposed SANT-like ISWI (SLIDE) domain are present [5–7]. These domains are important for substrate recognition: they mediate interactions with the nucleosomal DNA as well as the histone core [5–7]. As deletion of either the SANT domain or the SLIDE domain impairs remodeling activity, SANT and SLIDE couple substrate binding to catalysis. Interestingly, structural studies of yeast Isw1a suggest that ISWI remodelers can establish additional contacts to the DNA, allowing them to bind dinucleosomes and to sense the length of the linker DNA [8]. ISWI complexes have various biological functions, including chromatin assembly and nucleosome spacing, replication, transcriptional repression and activation [9–12] (Fig. 1, Table 1). This supports the notion that the accessory proteins present in these remodeling complexes regulate their function [13]. The subunit composition is also critical for targeting of the remodelers: many of the proteins have dedicated domains mediating specific interactions with DNA or with modified histone tails (Table 1).

Table 1. Recognition signals and biological functions of ISWI remodeling complexes.

Subunit	Recognition signals	Complexes	Functions
Acf1	DNA sequence [50]	ACF	RNAP II transcription [28,29]
		CHRAC	Replication/repair [25,26]
BPTF	H3K4me3 [64,65] H4K16ac [66]	NURF	RNAP II transcription [31,32]
CECR2	unknown	CERF	RNAP II transcription [10,33]
Rsf1[a]	CENP-A [43]	RSF	RNAP II transcription [30], centromere structure [43]
TIP5	H4K16ac [67]	NoRC	RNAP I transcription [35–37]
WSTF	H2A.X [27]	WICH	Replication/repair [21,27]
	H2BK12ac, H3K14ac	B–WICH	RNAP I transcription [40,41]
	H4K16ac [77]	WINAC[b]	RNAP II transcription [16,17]

[a] Rsf1 was also found in a complex of ~ 600 kDa with Snf2L and CECR2 [10] but the function of this complex is unknown. [b] WINAC is no ISWI complex but contains Brg1/Brm motor proteins.

Acitivities of ISWI chromatin remodelers

Chromatin remodelers have been linked to various biological functions. Although only a limited number of motor ATPases are involved in chromatin remodeling, the combination of these motors with different

accessory subunits generates a large variety of remodeling complexes (Fig. 1). Notably, some of these subunits can associate with different ATPases. For example, remodeling and spacing factor 1 (Rsf1) is found in complexes with both Snf2H and Snf2L [10,14,15], and the Williams syndrome transcription factor (WSTF) associates with Snf2H in the WSTF–ISWI chromatin remodeling complex (WICH) and with Brg1/Brm motor proteins in the WSTF including nucleosome assembly complex (WINAC) [16,17]. This combinatorial complexity is further increased by the occurrence of cell-type-specific splice variants with distinct activities and intracellular localization, as reported for Snf2L [18,19]. Depending on the composition of the complex and the presence of chromatin-associated signals, the targeting and function of the remodelers is modulated (Fig. 2). This gives the cell the opportunity to fine-tune specific remodeling activities. This specificity seems to be more important for remodeler activities such as transcriptional regulation than for chromatin assembly during DNA repair or replication, where many different remodelers and subunits are present simultaneously.

DNA replication

DNA replication is one of the biological processes associated with extensive chromatin remodeling. Chromatin structure is rearranged during DNA duplication and has to be restored afterwards. Histones are deposited on the nascent DNA, and their modification status as well as their positioning pattern has to be adjusted in an appropriate way. Consequently, many remodelers are recruited to replication foci in the S phase of the cell cycle. These include members of all remodeler families, amongst them the Snf2H-containing complexes ATP-utilizing chromatin assembly and remodeling factor (ACF) [20] and WICH [21], as well as the Snf2L ATPase [22]. ACF and WICH play different roles during the replication process. ACF, consisting of Snf2H and ATP-dependent chromatin assembly factor 1 (Acf1), is important for the replication of condensed heterochromatin. Accordingly, depletion of Acf1 causes a delay in later stages of the S phase when pericentromeric heterochromatin is replicated [20]. This delay can be reversed by artificial chromatin decondensation. Thus, ACF seems to be responsible for establishing an open chromatin structure downstream of the replication fork. The WICH complex, composed of WSTF and Snf2H, is important for the assembly of newly synthesized DNA into chromatin. Upon knockdown of WSTF, the nascent chromatin compacts and displays characteristic features of heterochromatin, including accumulation of heterochromatin protein 1 (HP1) [21]. This suggests a function of WICH in opening up chromatin after nucleosome assembly

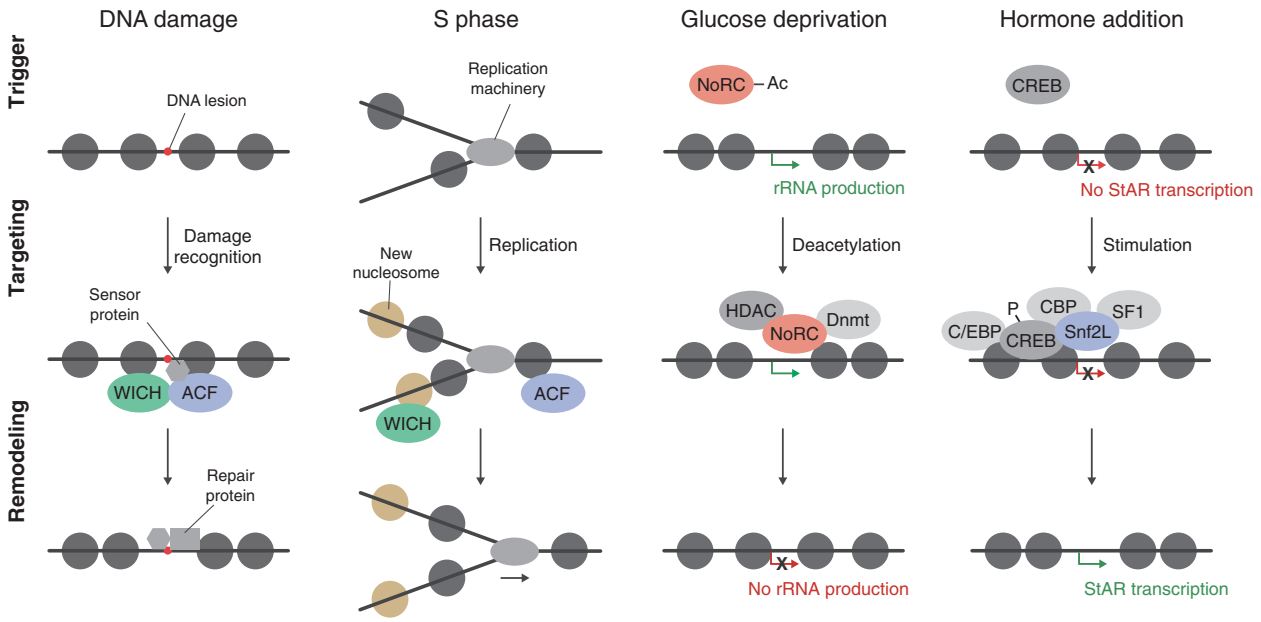

Fig. 2. Stimuli that trigger chromatin remodeling. Chromatin remodeling activity can be triggered by different stimuli. In differentiated cells, such trigger signals are, for example, the appearance of DNA damage, entrance into the S phase of the cell cycle, lack of nutrition or hormone release. Different remodeling complexes respond to different signals, depending on subunit composition and post-translational modification status.

upstream of the replication fork. Taken together, ISWI complexes at replication foci (re)establish the appropriate nucleosomal patterning on both sides of the replication fork and define the chromatin accessibility for other factors involved in the replication process.

DNA repair

Mammalian cells have a dedicated pathway to respond to DNA lesions; this pathway is referred to as the DNA damage response (DDR) [23,24]. The repair process is typically accompanied by local alterations of the chromatin structure. During DDR, lesions are first recognized by certain sensor proteins and afterwards bound by catalytic effector proteins that mediate the repair process. Among the factors recruited to DNA damage sites are many different types of remodelers, including the ISWI complexes ACF/chromatin accessibility complex (CHRAC) [25,26] and WICH [27], as well as the Snf2L ATPase [22,26]. Knockdown of Acf1 or Snf2H leads to increased sensitivity to DNA damage [25], whereas knockdown of WSTF impairs the persistence of DNA repair foci on the timescale of hours [27]. Besides translocating nucleosomes, other functions of ISWI complexes at repair sites have been described. Acf1 is responsible for the recruitment of the Ku70/80 complex to the repair site via direct protein interactions. Furthermore, the WSTF protein, a subunit of the WICH complex, has been shown to phosphorylate Tyr142 of the histone 2A variant X (H2A.X) at the DNA damage site [27], which might serve as a signal for downstream factors. In summary, ISWI complexes seem to have important functions at DNA repair sites that are not restricted to nucleosome repositioning but include recruitment of other factors and post-translational modification of histones.

Transcription regulation

ISWI chromatin remodelers not only play a role during chromatin (dis)assembly, as is the case in DNA repair or replication, but they can also switch nucleosome positions to block or clear promoters. This can directly change the expression level of the corresponding gene. These switching processes are triggered in the cell by different signals (e.g. hormone-dependent stimulation or metabolic changes) (Fig. 2) and a number of exemplary cases are discussed in the following for ACF, remodeling and spacing factor (RSF), nucleosome remodeling factor (NURF), cat eye syndrome chromosome region candidate 2 (CECR2)-containing remodeling factor (CERF), nucleolar remodeling complex (NoRC) and WICH.

The ACF complex regulates cytokine expression in stimulated mouse T lymphocytes [28], namely the expression of interleukin (IL)-2 and IL-3. The ACF complex binds to the corresponding gene loci before and after stimulation, and induces IL-3 expression but reduces IL-2 expression. The Snf2H occupancy slightly increased after stimulation but it is unclear if this is responsible for the observed changes in gene expression. Acf1 was also reported to interact with the nuclear receptor corepressor (N-CoR) to repress vitamin D3 receptor-regulated genes [29]. Upon hormone treatment, Acf1 is released from the respective promoter regions, resulting in the activation of gene expression. The RSF complex, consisting of Snf2H and Rsf1 [14,15], was shown to affect the expression levels of several cancer-related genes relevant for drug resistance in ovarian cancer cells [30]. The Snf2L-containing complexes have been shown to be critical for development by regulating the expression of certain master regulatory transcription factors. NURF is composed of Snf2L and bromodomain PHD finger transcription factor (BPTF), and regulates the engrailed gene, which has an important role in brain development [31]. In addition, NURF has been shown to associate with Smad transcription factors and is required for proper differentiation of mouse embryonic stem cells [32]. The CERF complex, containing Snf2L and CECR2, is connected to neurulation [10], and regulates different mesenchymal/ectodermal transcription factors associated with exencephaly in mice [33]. Similarly to the Snf2H-containing ACF complex, Snf2L has been shown to respond to hormone stimulation [34]. Upon treatment with luteinizing hormone (LH), Snf2L associates with the promoter of the steroidogenic acute regulatory protein (StAR) in ovarian granulosa cells, resulting in increased StAR expression levels that are required for terminal differentiation.

Besides regulation of RNA polymerase (RNAP) II transcription, ISWI complexes also have an impact on gene products transcribed by RNAP I and RNAP III. The NoRC complex, composed of Snf2H and TIP5, was originally characterized as a repressor of rRNA transcription by RNAP I [35,36]. This repression seems to be partly mediated by chromatin remodeling [37] and partly by recruitment of the histone deacetylase 1 (HDAC1) and the DNA methyltransferases Dnmt1 and Dnmt3b. Recently, NoRC was also implicated in the silencing of centric and pericentric repeats [38]. Its TIP5 subunit is reversibly acetylated in response to the intracellular energy status (i.e. TIP5 is deacetylated upon glucose depletion) [39], resulting in abolished NoRC activity. Thus, rRNA transcription is coupled

to the metabolic state of the cell via NoRC. The WSTF–Snf2H complex WICH has been shown to interact with several nuclear proteins, including nuclear myosin I, to form the complex B–WICH that is involved in the regulation of rRNA transcription by polymerase I [40] and in 5S rRNA/7SL RNA transcription by RNAP III [41]. B–WICH has been hypothesized to act as the counterpart of NoRC at the rDNA promoter to drive active rRNA transcription [40,42]. Taken together, ISWI-type remodeling complexes have different roles in transcriptional regulation, including activation and repression of protein-coding genes, as well as regulation of rRNA transcription. The corresponding switching events are triggered by different stimuli and do not seem to occur continuously in the cellular context.

Chromosome structure

Some ISWI complexes have functions connected to the regulation of chromosome structure. The RSF complex (Rsf1/Snf2H) has been shown to maintain proper centromere structure by stabilizing the centromere protein A (CENP-A) histone variant at the centromeres [43]. RSF binds CENP-A chromatin in mid-G1 phase and Rsf1 depletion causes loss of centromeric CENP-A. Furthermore, RSF is required for normal mitotic progression. Snf2H is also present in a complex that loads cohesin onto mitotic chromosomes [44]. Targeting of this complex seems to be dependent on histone modifications, such as acetylation of H3/H4 or trimethylation of H3K4, as well as DNA methylation. In *Xenopus*, the ISWI protein was found to be required for chromosome segregation [45], arguing for a conserved function of ISWIs in mitosis. Interestingly, ATPase activity is dispensable for the mitotic function of ISWI in *Xenopus*, which could mean that nucleosome translocations are not the only task of human ISWI remodelers at mitotic chromosomes.

ISWI chromatin remodelers in living cells

Mobility and chromatin interactions

While sophisticated *in vitro* experiments have provided a wealth of information on the mechanisms and features of chromatin-remodeling complexes [1,7,46], much less is known about their activity in the cell. Recently, we analyzed the dynamics and interaction behavior of Snf2H, Snf2L, inactive Snf2L + 13 as well as Acf1 in living cells using a fluorescence fluctuation microscopy approach that combined fluorescence bleaching and correlation spectroscopy experiments [22,26]. All proteins studied were highly dynamic in the nucleus during the G1/2 phase and bound only transiently to chromatin with upper limits for the residence times of < 150 ms for Snf2H/L and < 500 ms for Snf2L + 13 and Acf1. This is at the low end of values reported for other chromatin-interacting proteins but similar to some transcription factors [47,48]. By comparing Snf2L with the inactive Snf2L + 13 splice variant in conjunction with ATP-depletion experiments we concluded that only a small fraction of a given ISWI-type ATPase is involved in active translocation of nucleosomes during the G1/2 phase. In contrast, up to 40–70% of the remodeler pool was more tightly bound at replication foci during the S phase or at DNA repair sites. These molecules displayed increased residence times that are sufficient for the catalysis of several nucleosome translocation reactions assuming the velocities measured *in vitro* [49]. As inactive Snf2L + 13 was immobilized to the same extent as active Snf2L, target-site binding seems to be largely independent of ATPase activity. This suggests a mechanism in which an increase in the binding affinity of a given remodeler to its nucleosomal substrate favors productive nucleosome translocations, which was proposed previously based on *in vitro* studies [50]. Independently of the mechanism by which the end product of a translocation reaction is determined, the patterns obtained by the analysis of nucleosome position changes during the activation of human $CD4^+$ T cells [51] are indicative of the presence of three major classes of remodeler activities [52], namely (a) the establishment of a regular nucleosome spacing in the vicinity of a strong positioning signal acting as a boundary, (b) the enrichment/depletion of nucleosomes through amplification of intrinsic DNA-sequence-encoded signals and (c) the removal of nucleosomes from high-affinity binding sites.

Target search mechanism

As discussed previously, the combination of relatively high micromolar nuclear protein concentrations and short residence times (at the 100-ms timescale) in the nucleosome-bound state leads to an efficient mechanism to sample the genome for nucleosomes that need to be translocated [22,26]. This has been referred to as a 'continuous sampling mechanism' (Fig. 3A). From the experimentally determined mobility and concentration parameters, average sampling times of tens of seconds for Snf2H-containing remodelers were calculated for probing 99% of all genomic nucleosomes. Thus, after setting an appropriate signal, a nucleosome

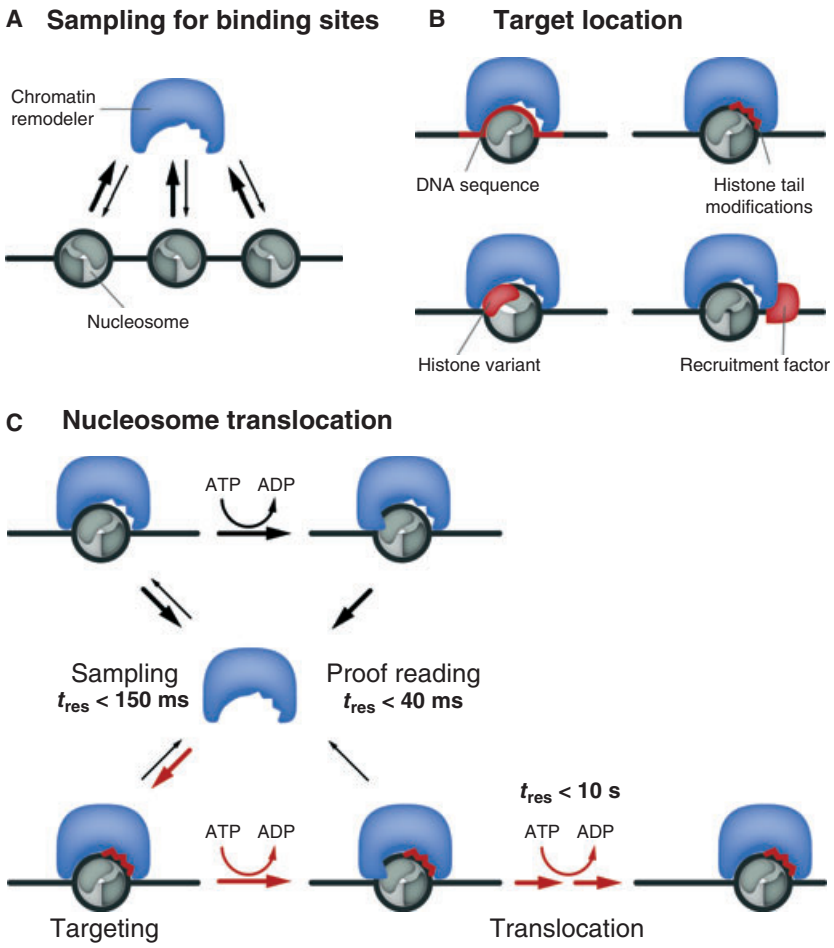

Fig. 3. Target location and translocation mechanisms for ISWI remodelers. (A) ISWI chromatin remodelers are very mobile in the nucleus and sample nucleosomes in transient binding reactions. (B) Interaction with the DNA, histone variants, post-translationally modified histone tails or other chromatin-associated proteins can be critical for their targeting. (C) Discrimination between correct and incorrect nucleosomes might occur according to a kinetic proofreading scheme that involves the ATP-dependent generation of a high-energy intermediate (the productive nucleosome translocation pathway is highlighted by the red arrows) [53]. Average residence times (t_{res}) were taken from [22]. From the comparison of ISWI mobility in the presence and absence of ATP, as well as Snf2L versus its inactive splice variant Snf2L+13, the upper limit of t_{res} < 40 ms associated with a putative proofreading step was estimated. It is noted that direct experimental evidence is still lacking that would demonstrate the presence of a proofreading mechanism for the translocation of nucleosomes by chromatin remodelers.

translocation reaction can be initiated very quickly (Fig. 3B). This fast-target location was confirmed in experiments where the kinetics of chromatin remodeler recruitment to DNA-damage sites was studied [26]. In these experiments, proliferating cell nuclear antigen (PCNA) was found to be even more mobile than Snf2H with significantly weaker chromatin binding. This difference might reflect the additional time required for the sampling reaction of ISWI chromatin remodelers that possibly includes ATP hydrolysis and formation of a high-energy initiation intermediate according to a kinetic proofreading scheme [26,53] (Fig. 3C). In such a mechanism a certain time is required to decide if a productive translocation is initiated or if the remodeler dissociates. At least some of the binding events of a remodeler could include ATP-dependent discrimination processes, leading to a reduced mobility compared with other nuclear factors such as PCNA [26]. Such a model would also explain the slightly increased mobility measured for Snf2H/L in the absence of ATP and for the increased mobility of inactive Snf2L + 13 compared with active Snf2L [22]. It is noted that the features of the continuous sampling mechanism are in excellent agreement with findings on cyclically occurring epigenetic processes that initiate and terminate remodeling of chromatin by SWI/SNF and nucleosome remodeling and deacetylase (NuRD) complexes [48,54,55].

Targeting signals

The mechanisms discussed above involve the reversible targeting of ISWI chromatin remodeling complexes by chromatin signals that mark sites of activity. As discussed in the following (Fig. 3B), these can be classified into (a) DNA sequence features that influence remodeling, (b) post-translational modifications of histones or DNA that are read out by corresponding binding domains in the remodeling complex (see above), (c) histone variants that substitute for core histones so that distinct nucleosome substrates are created and (d) other proteins that associate with chromatin and recruit remodelers to the corresponding loci. The most prominent signals that are recognized by

ISWI subunits are summarized in Table 1, and the corresponding recognition mechanisms apply also for remodeling complexes from other families [56]. Besides these distinct signals, the local chromatin structure might be an additional determinant of remodeler recruitment and regulation because ISWI complexes can sense the distance between nucleosomes [8,57].

DNA sequence features

DNA sequence and conformation have been shown to be relevant for remodeling activity [50,58–61]. In particular, nucleosome positioning by the ACF complex can be directed by a defined DNA sequence element that displays high intrinsic curvature [50]. Furthermore, noncatalytic subunits of ISWI complexes might have some DNA sequence-dependent variations of their DNA-binding affinity because they contain DNA-binding motifs such as WSTF/Acf1/Cbp146 (WAC) motifs or AT hooks [35,62–64]. Thus, both the ATPase motor protein and the associated subunits are likely to provide some DNA sequence-dependent modulation of the chromatin interaction affinity. However, the relevance of these observations for the specific targeting of ISWI complexes in the cell remains to be established.

Histone modifications and DNA methylation

Several regulatory subunits of ISWI remodeling complexes possess dedicated domains that specifically interact with modified histone tails: bromodomains are known to preferentially bind acetylated H3 tails, while PHD fingers recognize trimethylated H3K4, H3K9 and H3K36 [65]. Consequently, NURF-dependent chromatin remodeling is coupled with H3K4me3 recognition via the PHD finger of its BPTF subunit, and this interaction is involved in maintaining homeobox (Hox) gene-expression patterns during differentiation [66,67]. Furthermore, H4K16ac nucleosomes are critical for the regulation of NURF [68]. NoRC is recruited to H4K16ac nucleosomes at the rDNA promoter via the bromodomain of TIP5 [69]. Whether this interaction is directly related to elevated levels of chromatin remodeling is unclear, as H4K16ac nucleosomes are translocated less efficiently by ISWI *in vitro*. This is probably because of the weakened interaction between the ISWI/Snf2H motor and the H4 tail [70,71]. Binding of the Snf2H/cohesin complex to mitotic chromosomes seems to be regulated by histone modifications, namely acetylation of H3/H4 and trimethylation of H3K4, as well as by DNA methylation [44]. In addition, phosphorylation of H3S10 at mitotic chromosomes has been reported to interfere with ISWI binding in *Xenopus* [72]. The link between ISWI complexes and specific histone modifications has not yet been studied systematically, and it is anticipated that a number of other histone-modification signals exist that modulate their interaction with nucleosomes.

Histone variants

Besides the canonical core histones, there are several histone variants that are incorporated into chromatin in a regulated manner. These variants might serve as translocation signals for ISWI remodelers. The histone 2A variant Z (H2A.Z), which is often found in nucleosomes at transcriptional control regions, was shown to increase the activity of Snf2H, Snf2L and most of their complexes [73]. Nucleosomes containing H2A.X, which is important for the maintenance of genome integrity, bind more strongly to the WICH complex than nucleosomes with canonical H2A [27]. Furthermore, H2A.X is a substrate for the WSTF kinase that is part of the WICH complex. Notably, histone variants within the H2A family show high divergence in their C-terminal regions. Since interaction with the C-terminal tail regulates the remodeling reaction, as shown for recombinant human Snf2H or *Drosophila* ISWI and ACF [74], differences in translocation rates or binding affinities for the corresponding histone variants are expected.

Chromatin-associated proteins

Proteins that bind to chromatin are potential targeting signals for remodelers. A previous study reported that the WSTF subunit of the Snf2H-containing WICH complex interacts directly with PCNA [21]. Our finding that only a very minor fraction of mobile complexes containing both Snf2H and PCNA was present in U2OS cells suggests that the initial Snf2H recruitment occurs to a large extent independently of preformed PCNA–WICH complexes [26]. However, the PCNA–chromatin complex could act as a binding platform for several factors with high turnover rates involving also WSTF-mediated interactions between PCNA and Snf2H [75,76]. Other previously reported chromatin remodeler binding partners at DNA-repair sites, in addition to the above-mentioned H2A.X and its phosphorylated form, histone 2A variant X phosphorylated at serine 139 (γH2A.X), are the Ku70/80 proteins [25,77] that mark DNA double-strand breaks for the DNA repair machinery. Interestingly, the Acf1 protein has been shown to interact with the chromo shadow domain of HP1 in *Drosophila* [78], suggesting the

possibility that the ACF complex stabilizes HP1 at the DNA damage sites, or vice versa.

Conclusions

ISWI chromatin remodelers are involved in important genome-associated processes and are instrumental for DNA replication, DNA repair, transcriptional regulation and maintenance of chromosome structure. These activities seem to be highly regulated on different levels. Via specific subunit composition and post-translational modifications, distinct targeting of their activity is achieved in response to different stimuli, such as the metabolic state of the cell, a specific cell cycle phase, hormone treatment or the presence of DNA damage (Fig. 2). In order to detect the presence of such trigger signals, remodelers sample the whole nucleus to find potential places where their activity is needed (Fig. 3). The signals that control their recruitment to these sites include DNA sequence features, modified histone tails, histone variants or interactions with various other chromatin-associated proteins. However, many details of the underlying mechanisms remain to be elucidated. Biochemical *in vitro* data and mobility data from live cell experiments are consistent with a 'release model' [50], which predicts that the probability for nucleosome translocation is increased if the remodeler is bound to its substrate with higher affinity. Furthermore, a kinetic proofreading scheme for ISWI remodelers was proposed, as discussed elsewhere in this issue [53], which fits to the mobility data obtained in living cells. Such a mechanism would lead to a tight regulation of nucleosome translocation activity, ensuring precise identification of nucleosomes that should be translocated. It will be interesting to see if this view is confirmed by future studies on remodelers from the ISWI or other families. In particular, novel experimental readouts for detecting remodeling activity need to be established to address further questions concerning remodeler regulation and function in their natural habitat: the living cell.

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