

# Mechanisms for ATP-dependent chromatin remodelling: the means to the end

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Chromatin remodelling is the ATP-dependent change in nucleosome organisation driven by Snf2 family ATPases. The biochemistry of this process depends on the behaviours of ATP-dependent motor proteins and their dynamic nucleosome substrates, which brings significant technical and conceptual challenges. Steady progress has been made in characterising the polypeptides of which these enzymes are comprised. Divergence in the sequences of different subfamilies of Snf2-related proteins suggests that the motors are adapted for different functions. Recently, structural insights have suggested that the Snf2 ATPase acts as a context-sensitive DNA translocase. This may have arisen as a means to enable efficient access to DNA in the high density of the eukaryotic nucleus. How the enzymes engage nucleosomes and how the network of noncovalent interactions within the nucleosome respond to the force applied remains unclear, and it remains prudent to recognise the potential for both DNA distortions and dynamics within the underlying histone octamer structure.

## Introduction

Chromatin remodelling is the directed alteration of genome packaging in the eukaryotic cell nucleus. The term is usually used to describe ATP-dependent changes in nucleosome organisation driven by Snf2 family ATPases, although it predates the discovery of those factors and is sometimes also applied to changes such as large-scale nuclear reconfiguration or non-ATP-driven nucleosome rearrangements.

The chromatin-remodelling activity of Snf2 family ATPases was uncovered independently in *Saccharomyces cerevisiae* screens for factors contributing to expression of the HO (HOMothallic) nuclease required for mating type switching [1,2], and sucrose fermentation by invertase encoded at *SUC2* [3]. Both the switching (SWI) and sucrose nonfermenting (SNF) screens revealed the involvement of a gene at locus

YOR290C encoding a large ATPase, since named *SNF2* [4]. Suppressors of *snf2* mutants in turn revealed SWI/SNF-independent  $\text{Sin}^-$  mutants, including point mutants of histone genes and other chromatin components [5–7]. Together with the observed changes to chromatin structure in *snf2* mutants at target loci [8], this suggested that Snf2p affected chromatin structure [9]. The hypothesis was confirmed by mutagenesis *in vivo* and *in vitro* observation of the activity of complexes containing Snf2 family ATPases. Biochemical purifications and bioinformatic sequence comparisons have since revealed that Snf2 family members are ubiquitous and numerous across eukaryotes [10].

Although early identifications concentrated on Snf2 family members as general transcription factors, the *SNF2* locus had previously been identified as

## Abbreviations

EM, electron microscopy; SF2, helicase-like superfamily 2; SHL, superhelical location.

contributing to protection against DNA damage [11]. Subsequent investigations have shown that Snf2 family ATPases are involved in a wide variety of genomic processes, including transcription, replication, repair and recombination. This suggests that ATP-dependent chromatin remodelling is a fundamental functional requirement in the nucleosome-packaged genomes of eukaryotes. However, the occurrence of Snf2 family ATPases in bacteria and archaea illustrates that the DNA-dependent ATP-driven translocase activity of the Snf2 family does not necessarily act on nucleosomes alone.

## Biochemistry of chromatin remodelling

The biochemistry of ATP-dependent remodelling has been an active area of investigation for almost two decades. The challenges for this field arise because the enzymatic activities are provided by large protein complexes whose function often appears redundant and whose members are ubiquitous, and because the chromatin substrate is dynamic and its properties are incompletely understood. Although much of the published work has focused on the mechanism of remodelling on nucleosomes, genetic screens have implicated Snf2 family members in a diverse range of functions.

## Composition and structures of remodelling complexes

All recognised ATP-dependent chromatin remodelling complexes contain a large core polypeptide, which includes a region of homology to the helicase-related Snf2 family ATPase (Fig. 1A). Having such a central molecular motor provides scope for a large number of potential mechanisms to drive the remodelling process. Therefore, a clear understanding of the composition and structure of remodelling complexes is crucial for constraining the many possible mechanistic models that can be imagined for remodelling.

The direct purification of remodelling complexes is technically challenging because many are large multi-protein associations comprised of many small subunits (Table 1). A further complication is that some core Snf2 family polypeptides, such as *S. cerevisiae* Sth1 and Isw1, participate in multiple variant complexes differing by only one or a few subunits [12,13].

Characterising purified complexes is also challenging because of the difficulty of creating specific *in vitro* activity assays using typical methods such as changes in restriction enzyme or nuclease accessibility, or in

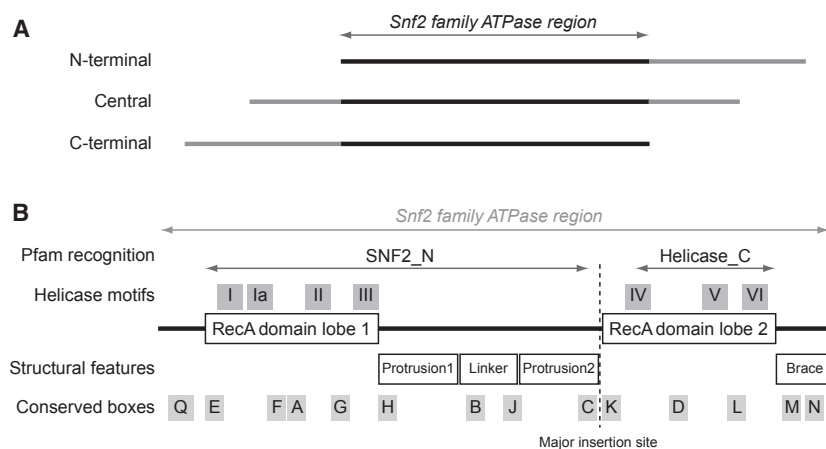
native gel mobility, which have a low resolution and lack standardised parameters. Lack of clarity regarding the relevant biological function of complexes means that defining an appropriate substrate can be confusing, and assembling substrates with specific histone post-translational modifications or defined nucleosomal arrays is technically difficult. In fact, most detailed *in vitro* assays are performed on nucleosomes comprising the '601' DNA sequence artificially selected for unusual stability [14] and core histones with sequence from the frog *Xenopus laevis* that lack any post-translational modifications because they are prepared in *Escherichia coli* [15].

Low-resolution electron microscopy (EM) structural envelopes have been determined for the large *S. cerevisiae* RSC [16–19], human PBAF [20] and *S. cerevisiae* Swi/Snf [21] remodelling complexes. These all reveal a large bowl-like shape with a central depression of appropriate size to hold a nucleosome [22,23]. Evidence for density consistent with the nucleosome is seen in the RSC structure [19]. A dimeric complex of the Iswi subfamily member human SMARCA5/SNF2h on a nucleosome has also been observed [24], but a recent combined crystallographic and EM determination revealed a single asymmetric association of the *S. cerevisiae* Isw1a complex (lacking the ATPase region) with a nucleosome [25].

## Sequence classification of the Snf2 family ATPases

Owing to the large number of genetic or functional investigations and the difficulty of characterising them biochemically, the core Snf2 family polypeptide is typically used as an identifier for chromatin remodelling complexes. These sequences carry a characteristic conserved Snf2 family ATPase region, which can be located at any point within the polypeptide (Fig. 1A). Consistent with this, exchange of the Snf2 family ATPase region has been shown to carry the properties of the remodelling complex with it [26].

An early analysis of the Snf2 family ATPase region, based on only 30 sequences, proposed eight distinct subfamilies (Fig. 2A) [27]. A subsequent comprehensive classification was carried out using over 1300 family members that became available through the extraordinary recent progress in genome sequencing [28]. This confirmed the initial principles identified by Eisen *et al.* [27], and revealed an expanded phylogeny of 23 subfamilies in the main groupings (the 24th and 'distant' SMARCA1 family lies at the edge of the family) with the same effective topology as the original study (Fig. 2B). The classification based on this



**Fig. 1.** Sequence and structural features of the Snf2 family ATPase region. (A) The Snf2 ATPase region is embedded in the full-length polypeptide and can be central, N-terminal or C-terminal. (B) Motifs, conserved boxes and structural elements within the Snf2 ATPase region, as defined by Flaus *et al.* [28], are shown relative to paired RecA domain lobes. The figure is not to scale.

helicase region and the origins of nomenclature for the 17 proteins identified in *S. cerevisiae* and the 32 proteins in the human genomes is shown in Table 2. The 23 subfamilies fall into five major groupings, two of which contain subfamilies most related to the archetypal *S. cerevisiae* Snf2p. These two ‘Snf2-like’ and ‘Swr1-like’ groupings were based on some 400 sequences and include seven and four subfamilies, respectively.

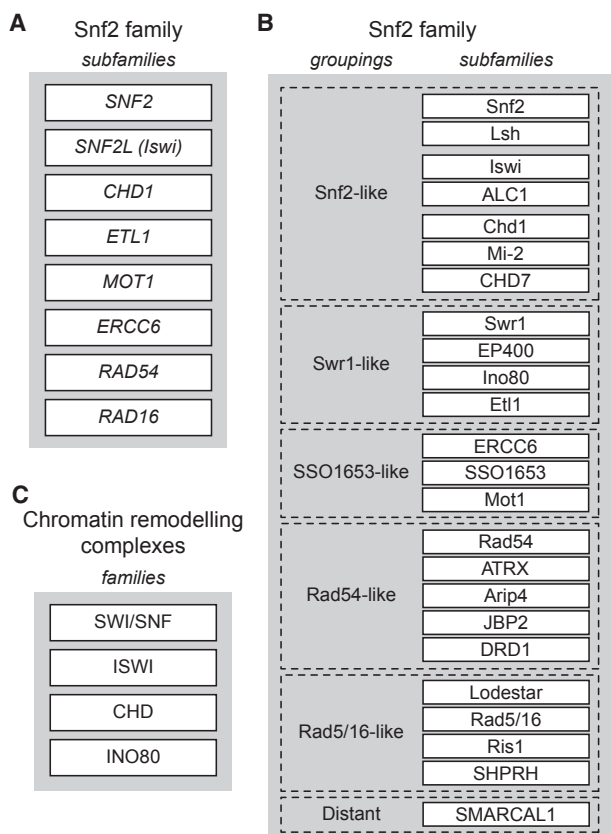
More recently, an alternative classification has been adopted, collecting remodelling complexes into four nominal, but separate, families, typically SWI/SNF, ISWI, CHD and INO80 (Fig. 2C) [10,29]. This classification, based on empirical assignments of the ATPase

and flanking regions, is a simplification, obscuring diversity within the CHD grouping and making it difficult to categorise enzymes such as Alc1 [30], Lsh [31] and ATRX [32], which are likely to have chromatin-related functions. It also ignores relationships with the broader range of Snf2-related ATPases. Flanking domains are presently poorly categorised and standard domain-finding tools leave large areas unassigned in many sequences, making this a difficult basis for classification.

The availability of a large number of sequences enables sequence-based definition of the Snf2 family from the multiple alignment. Early studies had identified the ATPase as a member of helicase-like super-

**Table 1.** *Saccharomyces cerevisiae* Snf2 family abundance and complex involvement. Subfamily groupings from [28]. Molecules per cell from TAP-tag western blotting quantifications in [56]. Relative parts per million (ppm) from five *S. cerevisiae* proteome-wide studies tabulated in pax-db.org [57]. Abundance data sets show significant overall variances [58]. Complex subunits from indicated references.

| Snf2 family member | Grouping     | Abundance molec./cell (western) | Abundance ppm (PAX DB) | Complex     | Subunits / complex | Refs         |
|--------------------|--------------|---------------------------------|------------------------|-------------|--------------------|--------------|
| Snf2               | Snf2-like    | 217                             | 9                      | SWI/SNF     | 11                 | [21,103,104] |
| Sth1               | Snf2-like    | 1990                            | 79                     | RSC         | 15                 | [12,19,105]  |
| lsw1               | Snf2-like    | 1500                            | 83                     | lsw1a/ sw1b | 2/3                | [13]         |
| lsw2               | Snf2-like    | 1520                            | 23                     | lsw2        | 4                  | [90,106]     |
| Chd1               | Snf2-like    | 1620                            | 60                     | Chd1        | 1                  | [107,108]    |
| Irc5               | Snf2-like    | –                               | 1                      | Lsh         | ?                  |              |
| Swr1               | Swr1-like    | 656                             | 5                      | Swr1        | ~12                | [109–112]    |
| Fun30              | Swr1-like    | 6800                            | 55                     | Fun30       | 1                  | [78,113]     |
| Ino80              | Swr1-like    | 6280                            | 65                     | Ino80       | ~12                | [114,115]    |
| Rad54              | Rad54-like   | –                               | –                      | Rad54       | 1–2                | [116,117]    |
| Rdh54              | Rad54-like   | 1270                            | 15                     | Rdh54       | ?                  | [117–119]    |
| Rad5               | Rad5/16-like | 1520                            | 8                      | Rad5        | 3                  | [120,121]    |
| Rad16              | Rad5/16-like | 358                             | 2                      | Rad16       | 2–3                | [122,123]    |
| Irc20              | Rad5/16-like | 143                             | 2                      | ?           | ?                  | [124]        |
| Uls1               | Rad5/16-like | –                               | 1                      | Ris1/Uls1   | ?                  | [117,125]    |
| Rad26              | Sso1653-like | –                               | 1                      | Rad26       | ?                  | [126,127]    |
| Mot1               | Sso1653-like | 6260                            | 109                    | Mot1        | 2                  | [128–130]    |



**Fig. 2.** Snf2 family classification schemes. (A) Original subfamilies were defined by sequence comparison, using ATPase and flanking sequences [27]. (B) Schematic of subfamilies defined by expanded phylogenomic comparison, using the Snf2 family ATPase region [28]. Subfamilies take the name and nomenclature from the first biochemically identified archetype. (C) Example of an empirical classification of chromatin-remodelling enzymes into separate families [10].

family 2 (SF2) through the presence of seven helicase motifs (Fig. 1B) [33], and this formed the basis for biochemical investigations demonstrating the underlying DNA translocase activity [34]. The Snf2 family ATPases are distinguished from other SF2 members by an extended span of sequence between the two RecA domains [35].

Together with historical quirks in seed alignments, this extended spacing led to the Snf2 ATPase being recognised by common motif-finding algorithms such as bipartite Snf2\_N and HelicaseC regions. Some Snf2 family members, such as those in the Swr1-like grouping (Fig. 2B), contain very large sequence regions at a specific major insertion site that generate an expansion of such scale that it confounds simple alignment algorithms [28] and they have been termed ‘split ATPases’ [36].

### Sequence-structure relationships in Snf2 family ATPase region

The characteristic sequence features within Snf2 ATPases (Fig. 1B) can be interpreted through the Zebrafish Rad54 structure, which is currently the most relevant atomic resolution model for the Snf2 family (Table 3) [37]. At its core the structure is composed of the same pair of RecA domain lobes as all helicase superfamily members (Fig. 3A). The extended span of sequence between the RecA domains contributes to two alpha helical ‘protrusions’ from the spherical RecA domains. The sequence of these protrusions is not itself conserved across the Snf2 family, but amino acid residues around their bases, which stabilise or ‘glue’ the protrusions to the RecA domains, comprise conserved boxes H, C, J and K (Fig. 1B). Box B within the flexible ‘linker’, which passes across the groove between the RecA domains, contains a pair of absolutely conserved arginine residues that are essential for function [38]. The major insertion region, which accommodates such variations in length, is situated behind the second RecA domain, but would be adjacent to DNA towards the back of the ATPase (Fig. 3). An additional region of alpha helical structure, containing conserved boxes, extends from the C-terminus of the second RecA domain, forming a ‘brace’ that stretches towards the modelled DNA and includes highly conserved charged residues in boxes M and N (Fig. 3).

A structure of an Snf2 family ATPase enzyme (SSO1653) from the archaeal *Sulfolobus sulfotaricus* has been determined in complex with double-stranded DNA (Table 3) [39]. The first RecA lobe appears to be engaged in a relevant position based on other helicase complex structures, although the second RecA lobe is probably in a nonfunctional orientation [40]. Using the DNA and the first SSO1653 RecA lobe enables the Rad54 structure to be oriented so that DNA can be modelled on it, and confirms that Snf2 family enzymes are likely to function by the same enzymatic mechanism as other SF2 helicase-like DNA translocases (Fig. 3B).

### Snf2 family proteins as allosterically regulated ATPases

Surprisingly, modelling the DNA-bound structure did not reveal an obvious mechanistic role for the characteristic protrusions, brace or linker region of the Snf2 family. Clues are provided by the recent structural investigation of the *S. cerevisiae* Chd1 protein, which can remodel nucleosomes without additional subunits (Table 3, Fig. 4A) [41]. Although the Chd1 diffraction

**Table 2.** Nomenclature of Snf2 family members in *Saccharomyces cerevisiae* and humans. Nomenclature of Snf2-related proteins using subfamily and grouping classifications from [28] based on alignments of the helicase-like region. Subfamilies are named from the first reported archetype in any organism. *S. cerevisiae* nomenclature is from the Saccharomyces Genome Database and human nomenclature is from ENSEMBL using official Human Genome Naming Commission symbols. Some members are known by a SMARCA (SWI/SNF-related, Matrix-associated, Actin-dependent Regulator Chromatin group A) acronym. Where common alternatives are used alongside SMARCA nomenclature, the official Human Genome Naming Commission name is shown first.

| Snf2 subfamily | Grouping      | <i>S. cerevisiae</i> genes | <i>S. cerevisiae</i> nomenclature       | Human genes                          | Human nomenclature   |
|----------------|---------------|----------------------------|---|--------------------------------------|--|
| Snf2           | Snf2-like     | <i>SNF2, STH1</i>          | Sucrose Non Fermenting, Snf Two Homolog | <i>SMARCA4/BRG1, SMARCA2/hBRM</i>    | Brm-Related Gene, human BRahMa-like  |
| lswi           | Snf2-like     | <i>ISW1, ISW2</i>          | Imitation SWitch                        | <i>SMARCA1/SNF2L, SMARCA5/hSNF2H</i> | SNF2-Like, human SNF2 Homologue  |
| Lsh            | Snf2-like     | <i>IRC5</i>                | Increased Recombination Centres         | <i>HELLS/SMARCA6</i>                 | HELicase, Lymphoid-Specific  |
| ALC1           | Snf2-like     | –                          |   | <i>ALC1/CHD1L</i>                    | Amplified in Liver Cancer  |
| Chd1           | Snf2-like     | <i>Chd1</i>                | Chromo Domain containing                | <i>CHD1</i>                          | CHD1-like  |
| Mi-2           | Snf2-like     | –                          |   | <i>CHD3, CHD4, CHD5</i>              | CHD1-like  |
| CHD7           | Snf2-like     | –                          |   | <i>CHD6, CHD7, CHD8, CHD9</i>        | Chd1-like  |
| Swr1           | Swr1-like     | <i>SWR1</i>                | Sick With Rat8                          | <i>SRCAP</i>                         | Snf2-related CREBBP activator protein  |
| EP400          | Swr1-like     | –                          |   | <i>EP400</i>                         | E1A binding protein p400   |
| Ino80          | Swr1-like     | <i>INO80</i>               | INOsitol biosynthesis                   | <i>INO80</i>                         | INO80 homologue  |
| Etl1           | Swr1-like     | <i>FUN30</i>               | Function UNKNOWN                        | <i>SMARCAD1</i>                      | SMARCA containing DEAD/H box   |
| Rad54          | Rad54-like    | <i>RAD54</i>               | RADiation sensitive                     | <i>RAD54L, RAD54B</i>                | RAD54-Like, RAD54 homologue B  |
| ATRX           | Rad54-like    | –                          |   | <i>ATRX</i>                          | Alpha Thalassemia/mental Retardation syndrome X-linked   |
| Arip4          | Rad54-like    | –                          |   | <i>RAD54L2</i>                       | RAD54-Like 2   |
| Rad5/16        | Rad5/16-like  | <i>Rad5, Rad16</i>         | RADiation sensitive                     | <i>HLTF/SMARCA3</i>                  | Helicase-Like Transcription Factor   |
| Ris1           | Rad5/16-like  | <i>ULS1</i>                | Ubiquitin Ligase for SUMO conjugates    | –                                    |  |
| Lodestar       | Rad5/16-like  | –                          |   | <i>TTF2</i>                          | Transcription Termination Factor, RNA polymerase II  |
| SHPRH          | Rad5/16-like  | <i>IRC20</i>               | Increased Recombination Centres         | <i>SHPRH</i>                         | SNF2 Histone linker PHD RING Helicase  |
| Mot1           | Sso1653-like  | <i>MOT1</i>                | Modifier Of Transcription               | <i>BTAF1</i>                         | B-TFIID Transcription Associated Factor  |
| ERCC6          | Sso1653-like  | <i>RAD26</i>               | RADiation sensitive                     | <i>ERCC6, ERCC6L, C9orf102</i>       | Excision Repair Cross-Complementing rodent repair deficiency, complementation group 6, chromosome 9 open reading frame 102 |
| SMARCAL1       | Smarcal1-like | –                          |   | <i>SMARCAL1, ZRANB3</i>              | SMARCA Like, Zinc finger, RAN-Binding domain containing 3  |

**Table 3.** Structures related to the Snf2 family ATPase region. Helicase-like superfamily 2 (SF2) structures in Protein Data Bank (PDB) were related to the Snf2 family by homology of the ATPase region. Similarity is shown as an expectation value for an hmmsearch hit with the Snf2 family model [28] to the PDB database. Identity and similarity for global alignment to Snf2 residues 767–1222 were performed using the EMBOSS stretcher. The PDB code is for the most relevant structure where multiple related accessions have been deposited.

| Protein | Organism   | PDB  | Resolution (Å) | Snf2 family homology | Identity/Similarity to Snf2p | Ref   |
|---------|--|------|----------------|----------------------|------------------------------|-------|
| Chd1    | <i>Saccharomyces cerevisiae</i><br>Budding yeast | 3mwy | 3.7            | $e^{-144}$           | 45%/65%                      | [41]  |
| Rad54   | <i>Danio rerio</i> Zebrafish                     | 1z3i | 3.0            | $e^{-128}$           | 33%/54%                      | [37]  |
| Sso1653 | <i>Sulfolobus sulfotaricus</i> Archaea           | 1z63 | 3.0            | $e^{-125}$           | 26%/44%                      | [39]  |
| RapA    | <i>Escherichia coli</i> Bacteria                 | 3dmq | 3.2            | $e^{-36}$            | 27%/46%                      | [52]  |
| Hef     | <i>Pyrococcus furiosus</i> Archaea               | 1wp9 | 2.9            | $e^{-16}$            | 28%/49%                      | [55]  |
| XPB     | <i>Archaeoglobus fulgidus</i> Archaea            | 2fwr | 2.6            | $e^{-16}$            | 15%/34%                      | [131] |
| Vasa    | <i>Drosophila melanogaster</i> Fruit fly         | 2db3 | 2.2            | $e^{-13}$            | 19%/38%                      | [132] |

was at subatomic resolution, the Rad54 coordinates could be used to model the orientation at atomic resolution. This and parallel biochemical experiments show that the chromo domains flanking the ATPase region contact the protrusions and are likely to block activity by occluding DNA from its binding site on the ATPase (Fig. 4A, B). This is consistent with earlier observations showing genetic linkages between adjacent domains and protrusion residues in Sth1 [42]. Hauk *et al.* propose that this represents ‘modular allostery’ [41,43], whereby DNA binding and ATPase activity are inhibited by structurally independent domains encoded either in sequences flanking the Snf2 ATPase or in independent subunits of the chromatin remodelling complex. The domains act as a switch or a ‘gate’ for translocase activity.

As all Snf2 family polypeptides contain at least one domain-size region adjacent to the ATPase [28], this suggests that a fundamental property of the Snf2 family could be as DNA-dependent ATPases whose activity can be modulated by adjacent inhibitory ‘gate’ domains (Fig. 4C, D). The highly conserved alpha helical organisation, but not sequences of protrusions observed in the sequence analysis [28], could provide distinctive surfaces for interactions with the inhibitory domains to set up the gating.

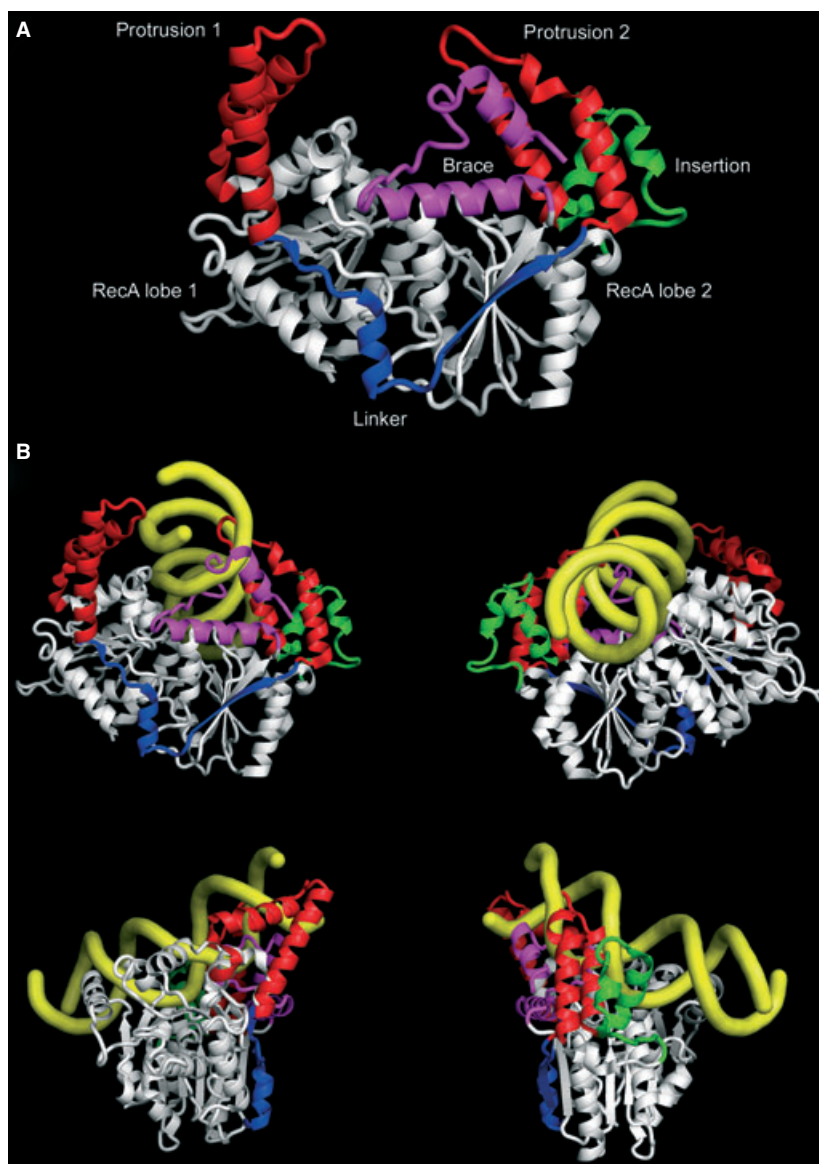
The high local ‘concentration’ of an adjacent inhibitory domain from the same polypeptide or complex means that only weak interfaces are required to stabilise inhibitory binding of the gate domain to the ATPase. This allows dynamic switching of the gate across a low-energy barrier when an alternative interaction for the inhibitory domain is brought into close proximity (Fig. 4C). Such a switch in gating could be driven by a higher affinity epitope for the inhibitory gate domain, such as methylated histones for the Chromo domain in Chd1, or the ARID domain of BAF250 for the HSA domain in Snf2 [44].

Alternatively, the switch could occur when a feature brought into proximity competes with the inhibitory binding of the gate domain and displaces it (Fig. 4D), as preferred by Hauk *et al.* [41] to explain their biochemical observations for *S. cerevisiae* Chd1.

### Snf2 family as context-sensitive DNA-dependent ATPases

Tight regulation of DNA-dependent ATPases is intuitive in biochemical terms. The expansion in genome size during the early evolution of eukaryotes required organisation of chromatin to package DNA at high density into a membrane-enclosed nucleus [45]. Adaptation of existing archaeal histones as nucleosomes [46] for this task would in turn require a remodelling machinery and this could be provided, in part, by the Snf2 family of ATP-driven, DNA-stimulated DNA translocases that already existed in archaea and bacteria [27,28]. Their distinctive modular allosteric regulation would reduce wasteful turnover of ATP in the high concentration of DNA substrate by providing a context-dependent switch via the inhibitory gate domain [41]. Subsequent specialisation in this context dependence would explain the diversity of Snf2 family members in eukaryotes that targets chromatin remodelling for highly specific functions.

The need to remodel the chromatin substrate for genomic access provides a large number of possible roles for Snf2 family proteins and is understandably the focus of most functional investigations. However, there are a number of other potential uses for DNA translocases regulated by context in the eukaryotic nucleus. Examples include the role of the Rad54 subfamily in the establishment and progress of homologous recombination repair [47], the role of the Mot1 subfamily in TBP cycling at promoters [48,49] and the role of the ERCC6 subfamily in the passage of RNA polymerase through



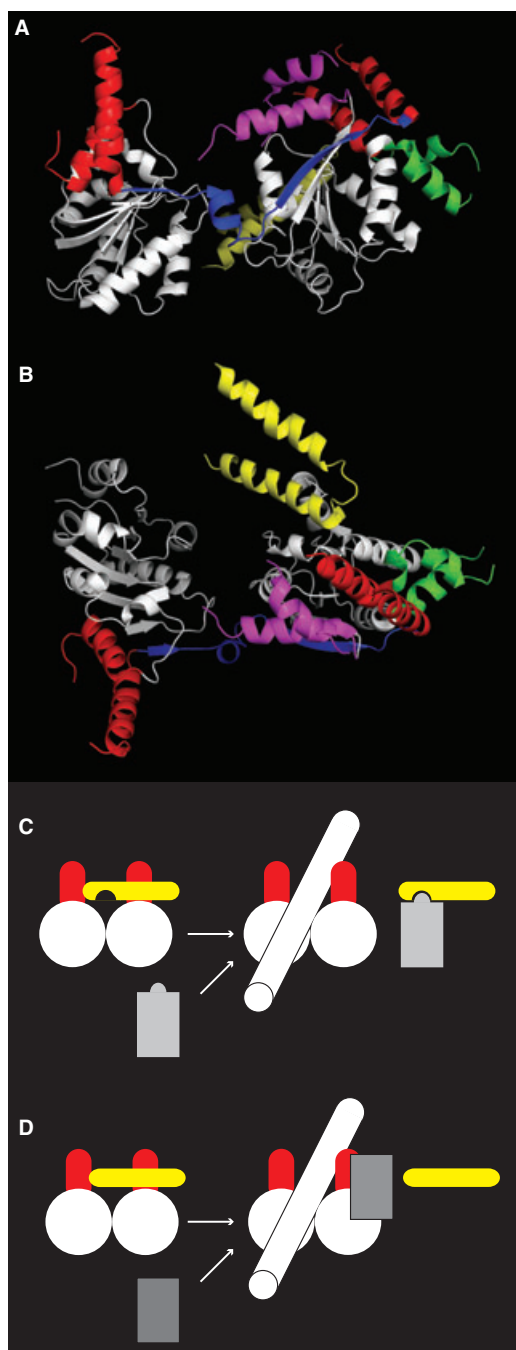
**Fig. 3.** Structural components of the Zebrafish Rad54 Snf2 family ATPase region. (A) Snf2 ATPase region showing RecA domain lobes (white), protrusions (red), linker (blue), brace (magenta) and insertion (green). From PDB code 1Z3I. (B) Modelling of the DNA path on Zebrafish Rad54 by alignment of RecA lobe 1 of the *S. solfataricus* SSO1653 structure. Structure PDB codes 1Z3I and 1Z63 [37,39].

DNA lesions [50]. Interestingly, Mot1 and ERCC6 sub-family members are the most similar to non-eukaryotic Snf2 family proteins (Fig. 2B) [28].

The biological function of archaeal *S. solfataricus* SSO1653 is unknown and the type strain has an inactivating transposon inserted in this gene. More distantly related bacterial RapA proteins are found at the edge of the Snf2 family and their *E. coli* archetype has been shown to have a role in RNA polymerase recycling at promoters [51]. The structure of RapA has been solved (Table 3) [52] and its core retains an Snf2 family-like organisation with a protrusion and brace on RecA lobe 2, although the protrusion on lobe 1 is less similar to other structures [53] (Fig. 5). The RapA structure

contains additional domains that could act as allosteric gates (Fig. 5C), and biochemical observations are consistent with gating [54].

The yet more distant archaeal *Pyrococcus furiosus* Hef protein is related to Mph1 in *S. cerevisiae* and to human FANCM. Hef protein is a true DNA helicase and also has a protrusion and potential brace on RecA lobe 2 [55] (see the supplementary data in [28]). Two distantly related non-Snf2 family helicases – archaeal *Archaeoglobus fulgidis* XPB and *Drosophila melanogaster* Vasa – mainly retain similarity to helicase motifs in the core RecA domains of the Snf2 family and lack any additional motifs in common with the Snf2 family (Table 3).



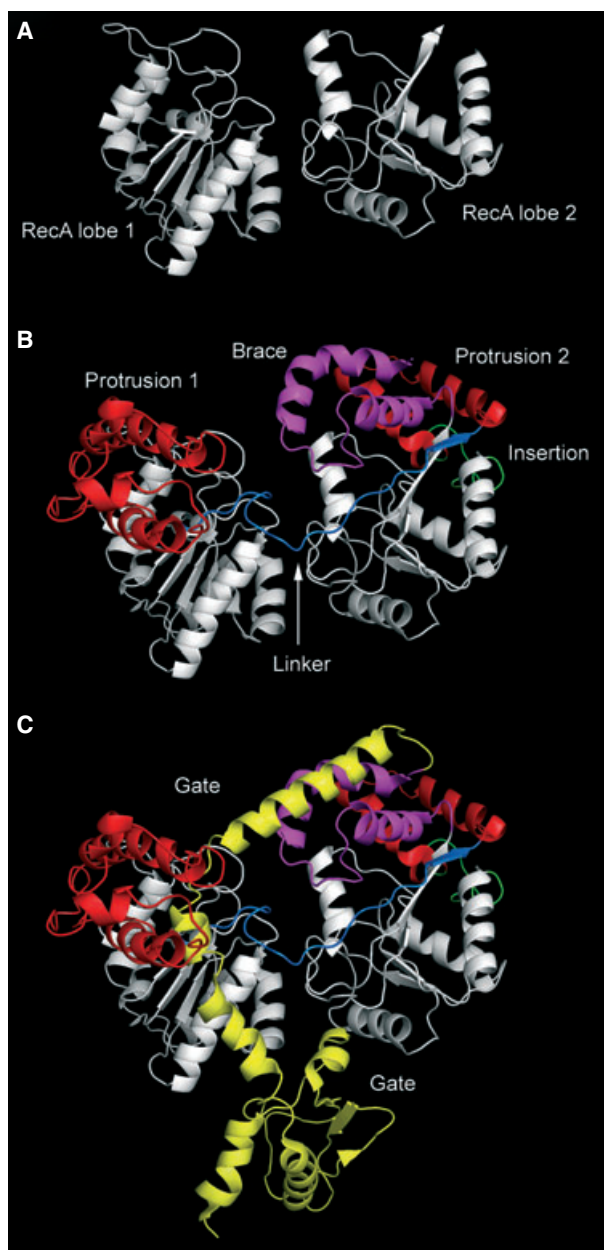
**Fig. 4.** Modular allostery by the inhibitory gate domain to prevent DNA binding to Snf2 ATPase. (A) *Saccharomyces cerevisiae* Chd1 ATPase region with orientation and colouring equivalent to that of Rad54 in Fig 3A. Chromowedge domain (yellow) is at the rear. (B) View from (A) rotated 120° to illustrate Chromowedge domain association with protrusion 2 and RecA lobe 2. (C) Schematic of modulation by switching of the 'gate' domain (yellow) on Snf2 ATPase through introduction of an alternative binding site (light grey). (D) Modulation by competition with the 'gate' domain (yellow) through introduction of a competitor for Snf2 ATPase binding (dark grey). Structure PDB code 3MWY [41].

### Abundance and localisation of chromatin remodelling complexes

In addition to their diversity, remodelling complexes are surprisingly highly abundant nuclear components (Table 1) [56,57], although the proteome-wide data sets show significant overall variance [58] so caution should be exercised with quantitative interpretations. In fact, the ATPase subunit of Swi/Snf is by far the least abundant of the recognised nucleosome-active remodelling complexes in *S. cerevisiae* and its less extensive roles may have facilitated its historical identification. The combined abundance of the Snf2 subfamily members Sth1 and Snf2 equates to approximately one enzyme for every 35–50 nucleosomes, or less than one per gene, and may correlate with an occasional requirement for these enzymes to undertake specific activities such as nucleosome ejection. The chromatin-organising enzymes Isw1, Isw2 and Chd1 are together two to five times more abundant, perhaps reflecting a more general role for these enzymes in nucleosome spacing by sliding. Analogous protein-abundance estimates from human and mouse cells [59] suggests that SMARCA5/Snf2h, Chd1 and Chd4 are highly abundant in mammalian cells (data not shown). The Fun30, Ino80 and Swr1 enzymes, likely to have functions relating to histone exchange, also have similar abundance and the reason for requiring large numbers of histone exchangers per gene is not yet apparent. It may be that in addition to performing functions relating to the directed incorporation of histone variants, some of these enzymes could have a destabilising effect on chromatin by removing histone dimers, for example during the transit of polymerases.

Accessory subunits in multiprotein complexes, and flanking domains in the Snf2 family ATPase polypeptide, frequently encode subunits known to interact with chromatin, often with specificity for post-translational modifications or histone variants. This has led to the suggestion that a basic property of chromatin remodellers is that they recognise covalent histone modifications [10]. As discussed above, one function for histone recognition interactions may be to provide an allosteric regulatory mechanism to activate the remodeller in the presence of its substrate [41]. A second function may be the need for the remodelling complex to maintain affinity with the nucleosome substrate.

However, the most widely recognised function for chromatin-recognition domains in remodellers is to target remodelling complexes to sites of action. A potential problem is that most histone-binding domains have only a modest affinity for epitopes. This would be anticipated to result in significant non-specific



**Fig. 5.** Similarity of Rad54 and RapA structures. (A) Conserved RecA lobes in an equivalent orientation to Fig 3. (B) Characteristic Snf2 family structures including protrusions (red), linker (blue), insertion region (green) and brace (magenta). (C) Additional structure (yellow) potentially acting as a modulatory gate in *Escherichia coli* RapA. Structure PDB code 3DMQ [52].

interactions with chromatin not bearing the appropriate modification. However, the localisation of histone modification is often diffuse rather than punctuate, meaning that the local concentration of epitopes in specific regions of the nucleus may be sufficient to generate a localised enrichment in enzyme (see the accompanying review by Erdel and Rippe [60]). As some

remodelling enzymes contain epitopes that are capable of recognising similar modifications [61], and many modifications share similar distributions [62], there is the potential for multiplicity and redundancy of remodelling complexes associated with large-scale processes [60], such as the repair of DNA double-strand breaks [63], in establishing higher-order chromatin structures [64], or in transcription [65].

### Translocation by Snf2 family ATPase acting on nucleosomes

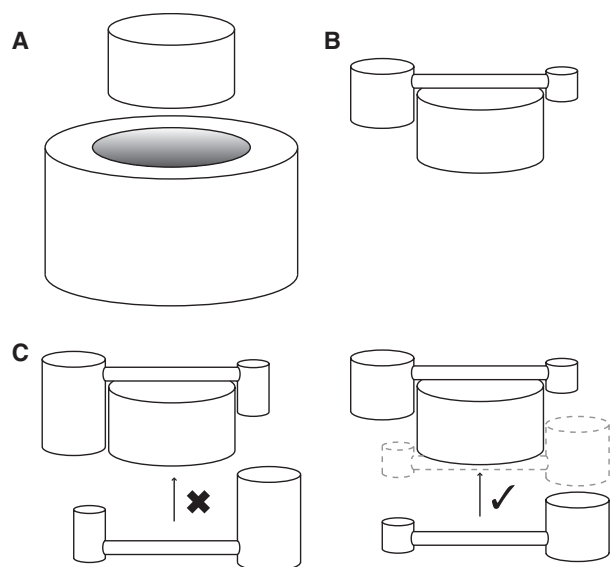
#### Significance of the remodelling mechanism

The pathway of chromatin remodelling has great functional significance because of its implications for the exposure of DNA sequences, the organisation of the genome and the exchange of histone proteins. First, chromatin packaging generally obscures DNA, so local recruitment of remodellers is required to facilitate access for genome-active processes. Second, genome-wide localisation shows that nucleosomes are very uniformly spaced despite the diversity of underlying DNA sequences. Deletion analysis in *S. cerevisiae* reveals that the spacing activity is contributed redundantly by Isw1, Isw2 and Chd1 [66], but can be locally manipulated by the effect of specialised sequences on remodelling [67]. Similar observations have been made in *Schizosaccharomyces pombe* for the role of the related Mit1 family member [68]. Third, remodelling has the potential to destabilise histone–DNA contacts that provide the link between histone post-translational modifications and bound DNA sequences [64]. This means that remodelling mechanisms must be structurally conservative to avoid erasing such epigenetic information.

#### Chromatin remodelling as a nucleosome response

The molecular mechanism of remodelling has been the subject of hypothesis for many years, possibly because the mechanical parallels are intuitively accessible and because the biochemical details of remodelling enzymes and chromatin substrates have been limiting.

Nucleosomes are the repeating molecular subunit of chromatin and therefore are likely to be the direct substrate for chromatin remodelling. A number of different outcomes have been proposed as an end result of remodelling on nucleosomes [10], principally the repositioning of the histone octamer relative to DNA (sliding), replacement of part or all of the octamer (exchange) or removal of part or all of the histone octamer (ejection). It is also formally possible that the



**Fig. 6.** Possible binding orientations for remodellers on nucleosomes. (A) Enveloping of nucleosome by remodeller. (B) Cantilevering of remodeller across nucleosome. (C) Examples of monomeric and dimeric remodeller binding to nucleosome by blocking (left) or enabling (right) of a symmetrical second site.

canonical nucleosome structure could be reconfigured to a stable alternative (switching), but this remains somewhat controversial [69–71].

Fundamentally, ATP-dependent chromatin remodelling is an enzymatic process with the remodeller accelerating the rate of change between a substrate and a product state. In contrast to textbook enzymology where individual covalent bonds are manipulated at localised sites, chromatin remodelling involves a non-covalent process on a 200 kDa substrate. This can lead to confusions of scale because the end product of remodelling, such as a slid nucleosome, may be the result of a large number of stepwise turnovers of the ATPase enzyme itself. A destabilised nucleosome is not the ‘transition state’ of an individual enzyme cycle; rather, it is the consequence of multiple enzyme cycle products building up on the nucleosome. This link between ATPase cycles of the remodelling enzyme and nucleosome outcomes is usually what is implied by ‘mechanism of remodelling’. It depends on how the ATPase cycle products are applied to the nucleosome, and the response of the nucleosome.

### Remodelling complex structure and substrate binding

A crucial element in the mechanism of remodelling is the dynamic potential of the multiple weak interactions

within the nucleosome structure itself, and how the chromatin remodeller directs these along a specific pathway. Compositional and structural information gathered for chromatin remodellers (Tables 1 and 3) suggests two general classes by which remodelling enzymes might engage with the nucleosome substrate.

First, large remodelling machinery could envelop the entire nucleosome to control its dynamic properties (Fig. 6A). As the nucleosome is a 200 kDa complex and volume scales with  $r^3$  for a simple sphere, this implies that an enveloping structure, with twice the radius of a nucleosome, will have approximately eight times its mass. Many multiprotein remodelling complexes are in the range of 1–2 MDa and EM image reconstructions are consistent with the ability to surround substrate nucleosomes [19].

Second, a simplified remodelling machinery could cantilever across the nucleosome (Fig. 6B) as a minimal alternative to envelopment. Arrangements equivalent to a cantilever have been modelled [72] using the HAND, Swi3 Ada2 N-CoR TFIIB (SANT) and SANT-like ISWI domain (SLIDE) domains of ISWI [73], although the very recent structure of *S. cerevisiae* Isw1 shows this binding near the linker and suggests that a more flexible part of the protein could reach across the nucleosome instead [25]. This domain arrangement is conserved between ISWI and Chd1 polypeptides [74].

One feature of both enveloping and cantilever complexes is that they achieve ‘template commitment’ to allow multiple ATPase cycles while retaining interactions with transiently destabilised nucleosomes as effects are accumulated [75,76]. A second feature of the stabilising interactions provided by the remodeller is that they will constrain the motions of the malleable nucleosome that could otherwise flex in different ways under an applied force. This feature may therefore be crucial to enable the remodelling process to follow a specific and defined mechanistic pathway.

### Multiple remodellers or multiple nucleosomes

Although diagrams such as those in Fig. 6A,B show a single remodeller engaged with a single nucleosome, the dyad symmetry of the nucleosome implies that chromatin remodellers should bind as dimers. Indeed, there is evidence that the ATPase subunits of some enzymes are dimeric [77,78], or bind to nucleosomes as dimers [24,76]. Alternatively, it is possible that remodeller association creates asymmetry, for example by blocking binding of a second enzyme (Fig. 6C). Asymmetry is observed in some RSC structures and in the Isw1a complex [17,25].

It is also possible that one chromatin remodelling complex could work on a dinucleosome substrate. Nucleosomes are typically found in genomes at high densities, meaning that following repositioning an encounter with a neighbour is a distinct possibility. It has been proposed that collisions between adjacent nucleosomes could act as a stage in the disassembly of nucleosomes [79–81]. Conversely, enzymes that act to space nucleosomes may stabilise chromatin. In this case, in order to prevent collisions, a means of sensing the adjacent nucleosome is required. Possible binding arrangements could include cooperation between remodellers on adjacent nucleosomes, or binding of a single remodeller to span adjacent linkers, as observed for Isw1a. However, in the simplest case, contacts with linker DNA adjacent to a nucleosome enzyme complex are important for full activity, so when adjacent nucleosomes interfere with these linker DNA contacts, movement in the direction of the adjacent nucleosome would be reduced.

## Mechanism of nucleosome dynamics

### Snf2 translocation on nucleosomes

When a remodelling complex is bound to a nucleosomal substrate, the core Snf2 ATPase motor provides a double-stranded DNA translocase that can move directionally on the DNA duplex (see the accompanying review by Croquette and colleagues [82]). Template commitment suggests that the complex also maintains contact with the histone components throughout the remodelling process. Termination will occur when the remodelling complex can no longer act on the nucleosome, for example because it has been disrupted or has reached a position where necessary flanking DNA is not available as a result of proximity with another nucleosome or some other barrier.

It is possible that DNA sequences may affect the outcome of remodelling [83], by affecting either the opportunity for engagement by Snf2-related enzymes or the response of the nucleosome to forces applied by the remodeller.

The capability of the Snf2 translocase for processive and directional movement is demonstrated by single-molecule observations showing rapid development of induced torsion and by biochemical experiments showing blockage by hairpins or single-stranded gaps [77,84–87]. Other SF2 double-stranded DNA translocases are observed to have apparent ‘kinetic’ step sizes distinguished by rate-determining steps down to 3–4 bp, and distinct ‘mechanical’ step sizes of 3–11 bp per ATP hydrolysis cycle [88]. Some estimates of step

sizes for Snf2 family proteins have been relatively large [86] but the advent of more sophisticated detection techniques has led to progressively smaller steps, with pauses every few base pairs being detected [76]. Further studies will be required to determine whether movements of several bases can be broken down into single base steps and to establish whether this applies to all Snf2-related enzymes.

The step size is of great interest as it has the potential to influence the amount of rotation generated during the remodelling process. A series of recent observations support the association of the ATPase region with nucleosomal DNA at superhelical location 2 (SHL  $\pm 2$ ) [22]. This includes evidence that DNA gaps appear to block the action of Snf2 and Iswi subfamily remodellers when introduced at this location [86,87,89] and directed crosslinking consistent with an interaction of the ATPase at SHL  $\pm 2$  [72,90]. This location coincides with an important structural feature within the nucleosome: the apparent high stability across the region between SHL -1.5 and SHL +1.5. Stability is reflected in the high uniformity and reduced dynamics of the region in crystal structures on multiple DNA sequences [91], increased number of histone–DNA contacts in the region [92] and histone SIN mutations reducing contact with DNA, which also accelerates nucleosome sliding [93]. This has been taken to suggest that Snf2-related enzymes target a region of the nucleosome that is rate limiting for dynamics.

### A dynamic histone octamer?

The most commonly proposed mechanisms indirectly imply that DNA is being remodelled across a static histone octamer surface. However, recent interest in nucleosome dynamics has accumulated evidence that various parts of the histone octamer may readily flex and change their binding to DNA. For example, the most external turns of DNA are known to be readily released in the process of site exposure [94,95], and H2A–H2B dimers can be displaced [96] such that they even become exchangeable at a significant rate during remodelling [97]. Tetramers of H3 and H4 have been observed to adopt conformations that differ from those observed within octamer and nucleosome structures [98–100]. It is possible that a concerted pathway occurs during remodelling, involving rearrangements in the histone octamer that weaken histone–DNA contacts, thus altering the energetics of DNA passage across its surface. The repertoire of mechanisms proposed for remodelling may be able to be expanded from the widely discussed twist-defect

and bulge-diffusion models and variations on them (reviewed in [101] and in the accompanying review by Blossy and Schiessel [102]).

## Conclusion

The biochemistry of chromatin remodelling remains a highly energetic and fruitful field. The complexity of understanding the behaviours of dynamic mechanical enzymes on dynamic mechanical substrates poses significant demands on biochemical techniques more suited to homogenous and stable molecules. Likewise, mechanistic thinking has been coloured by conceptual models of rigid bodies that hide details of local structure and malleability.

The growing sophistication of both experimental and conceptual analyses is therefore crucial to a full understanding of the process. The biochemistry of the cell involves a number of fundamental processes for which a universal and highly conserved solution has evolved as a result of the complexity of factors involved. It appears that directed alteration of the chromatin structure by Snf2 family enzymes is the means to the end required for dynamics of high-density chromatin packaging in eukaryotes.

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## References

- 1 Stern M, Jensen R & Herskowitz I (1984) Five SWI genes are required for expression of the HO gene in yeast. *J Mol Biol* **178**, 853–868.
- 2 Nasmyth K, Stillman D & Kipling D (1987) Both positive and negative regulators of HO transcription are required for mother-cell-specific mating-type switching in yeast. *Cell* **48**, 579–587.
- 3 Neigeborn L & Carlson M (1984) Genes affecting the regulation of SUC2 gene expression by glucose repression in *Saccharomyces cerevisiae*. *Genetics* **108**, 845–858.
- 4 Abrams E, Neigeborn L & Carlson M (1986) Molecular analysis of SNF2 and SNF5, genes required for expression of glucose-repressible genes in *Saccharomyces cerevisiae*. *Mol Cell Biol* **6**, 3643–3651.
- 5 Sternberg PW, Stern MJ, Clark I & Herskowitz I (1987) Activation of the yeast HO gene by release from multiple negative controls. *Cell* **48**, 567–577.
- 6 Neigeborn L, Rubin K & Carlson M (1986) Suppressors of SNF2 mutations restore invertase derepression and cause temperature-sensitive lethality in yeast. *Genetics* **112**, 741–753.
- 7 Kruger W, Peterson CL, Sil A, Coburn C, Arents G, Moudrianakis EN & Herskowitz I (1995) Amino acid substitutions in the structured domains of histones H3 and H4 partially relieve the requirement of the yeast SWI/SNF complex for transcription. *Genes Dev* **9**, 2770–2779.
- 8 Hirschhorn JN, Brown SA, Clark CD & Winston F (1992) Evidence that SNF2/SWI2 and SNF5 activate transcription in yeast by altering chromatin structure. *Genes Dev* **6**, 2288–2298.
- 9 Winston F & Carlson M (1992) Yeast SNF/SWI transcriptional activators and the SPT/SIN chromatin connection. *Trends Genet* **8**, 387–391.
- 10 Clapier CR & Cairns BR (2009) The biology of chromatin remodeling complexes. *Annu Rev Biochem* **78**, 273–304.
- 11 Foury F & Goffeau A (1979) Genetic control of enhanced mutability of mitochondrial DNA and gamma-ray sensitivity in *Saccharomyces cerevisiae*. *Proc Natl Acad Sci U S A* **76**, 6529–6533.
- 12 Cairns BR, Schlichter A, Erdjument-Bromage H, Tempst P, Kornberg RD & Winston F (1999) Two functionally distinct forms of the RSC nucleosome-remodeling complex, containing essential AT hook, BAH, and bromodomains. *Mol Cell* **4**, 715–723.
- 13 Vary JC Jr, Gangaraju VK, Qin J, Landel CC, Kooperberg C, Bartholomew B & Tsukiyama T (2003) Yeast Isw1p forms two separable complexes *in vivo*. *Mol Cell Biol* **23**, 80–91.
- 14 Lowary PT & Widom J (1998) New DNA sequence rules for high affinity binding to histone octamer and sequence-directed nucleosome positioning. *J Mol Biol* **276**, 19–42.
- 15 Luger K, Rechsteiner TJ, Flaus AJ, Waye MM & Richmond TJ (1997) Characterization of nucleosome core particles containing histone proteins made in bacteria. *J Mol Biol* **272**, 301–311.
- 16 Asturias FJ, Chung WH, Kornberg RD & Lorch Y (2002) Structural analysis of the RSC chromatin-remodeling complex. *Proc Natl Acad Sci U S A* **99**, 13477–13480.
- 17 Leschziner AE, Saha A, Wittmeyer J, Zhang Y, Bustamante C, Cairns BR & Nogales E (2007) Conformational flexibility in the chromatin remodeler RSC observed by electron microscopy and the orthogonal tilt reconstruction method. *Proc Natl Acad Sci USA* **104**, 4913–4918.
- 18 Skiniotis G, Moazed D & Walz T (2007) Acetylated histone tail peptides induce structural rearrangements in the RSC chromatin remodeling complex. *J Biol Chem* **282**, 20804–20808.

- 19 Chaban Y, Ezeokonkwo C, Chung WH, Zhang F, Kornberg RD, Maier-Davis B, Lorch Y & Asturias FJ (2008) Structure of a RSC-nucleosome complex and insights into chromatin remodeling. *Nat Struct Mol Biol* **15**, 1272–1277.
- 20 Leschziner AE, Lemon B, Tjian R & Nogales E (2005) Structural studies of the human PBAF chromatin-remodeling complex. *Structure* **13**, 267–275.
- 21 Dechassa ML, Zhang B, Horowitz-Scherer R, Persinger J, Woodcock CL, Peterson CL & Bartholomew B (2008) Architecture of the SWI/SNF-nucleosome complex. *Mol Cell Biol* **28**, 6010–6021.
- 22 Hota SK & Bartholomew B (2011) Diversity of operation in ATP-dependent chromatin remodelers. *Biochim Biophys Acta*, doi: 10.1016/j.bbasm.2011.05.007.
- 23 Kasten MM, Clapier CR & Cairns BR (2011) SnapShot: chromatin remodeling: SWI/SNF. *Cell* **144**, 310e1.
- 24 Racki LR, Yang JG, Naber N, Partensky PD, Acevedo A, Purcell TJ, Cooke R, Cheng Y & Narlikar GJ (2009) The chromatin remodeller ACF acts as a dimeric motor to space nucleosomes. *Nature* **462**, 1016–1021.
- 25 Yamada K, Frouws TD, Angst B, Fitzgerald DJ, DeLuca C, Schimmele K, Sargent DF & Richmond TJ (2011) Structure and mechanism of the chromatin remodelling factor ISW1a. *Nature* **472**, 448–453.
- 26 Fan HY, Trotter KW, Archer TK & Kingston RE (2005) Swapping function of two chromatin remodeling complexes. *Mol Cell* **17**, 805–815.
- 27 Eisen JA, Sweder KS & Hanawalt PC (1995) Evolution of the SNF2 family of proteins: subfamilies with distinct sequences and functions. *Nucleic Acids Res* **23**, 2715–2723.
- 28 Flaus A, Martin DM, Barton GJ & Owen-Hughes T (2006) Identification of multiple distinct Snf2 subfamilies with conserved structural motifs. *Nucleic Acids Res* **34**, 2887–2905.
- 29 Hargreaves DC & Crabtree GR (2011) ATP-dependent chromatin remodeling: genetics, genomics and mechanisms. *Cell Res* **21**, 396–420.
- 30 Ahel D, Horejsi Z, Wiechens N, Polo SE, Garcia-Wilson E, Ahel I, Flynn H, Skehel M, West SC, Jackson SP *et al.* (2009) Poly(ADP-ribose)-dependent regulation of DNA repair by the chromatin remodeling enzyme ALC1. *Science* **325**, 1240–1243.
- 31 Brzeski J & Jerzmanowski A (2003) Deficient in DNA methylation 1 (DDM1) defines a novel family of chromatin-remodeling factors. *J Biol Chem* **278**, 823–828.
- 32 Law MJ, Lower KM, Voon HP, Hughes JR, Garrick D, Viprakasit V, Mitson M, De Gobbi M, Marra M, Morris A *et al.* (2010) ATR-X syndrome protein targets tandem repeats and influences allele-specific expression in a size-dependent manner. *Cell* **143**, 367–378.
- 33 Gorbalenya AE & Koonin EV (1993) Helicases: amino acid sequence comparisons and structure-function relationships. *Curr Opin Struct Biol* **3**, 419–429.
- 34 Singleton MR, Dillingham MS & Wigley DB (2007) Structure and mechanism of helicases and nucleic acid translocases. *Annu Rev Biochem* **76**, 23–50.
- 35 Flaus A & Owen-Hughes T (2001) Mechanisms for ATP-dependent chromatin remodelling. *Curr Opin Genet Dev* **11**, 148–154.
- 36 Eberharder A & Becker PB (2004) ATP-dependent nucleosome remodelling: factors and functions. *J Cell Sci* **117**, 3707–3711.
- 37 Thoma NH, Czyzewski BK, Alexeev AA, Mazin AV, Kowalczykowski SC & Pavletich NP (2005) Structure of the SWI2/SNF2 chromatin-remodeling domain of eukaryotic Rad54. *Nat Struct Mol Biol* **12**, 350–356.
- 38 Richmond E & Peterson CL (1996) Functional analysis of the DNA-stimulated ATPase domain of yeast SWI2/SNF2. *Nucleic Acids Res* **24**, 3685–3692.
- 39 Durr H, Korner C, Muller M, Hickmann V & Hopfner KP (2005) X-ray structures of the *Sulfolobus solfataricus* SWI2/SNF2 ATPase core and its complex with DNA. *Cell* **121**, 363–373.
- 40 Durr H, Flaus A, Owen-Hughes T & Hopfner KP (2006) Snf2 family ATPases and DExx box helicases: differences and unifying concepts from high-resolution crystal structures. *Nucleic Acids Res* **34**, 4160–4167.
- 41 Hauk G, McKnight JN, Nodelman IM & Bowman GD (2010) The chromodomains of the Chd1 chromatin remodeler regulate DNA access to the ATPase motor. *Mol Cell* **39**, 711–723.
- 42 Szerlong H, Hinata K, Viswanathan R, Erdjument-Bromage H, Tempst P & Cairns BR (2008) The HSA domain binds nuclear actin-related proteins to regulate chromatin-remodeling ATPases. *Nat Struct Mol Biol* **15**, 469–476.
- 43 Pufall MA & Graves BJ (2002) Autoinhibitory domains: modular effectors of cellular regulation. *Annu Rev Cell Dev Biol* **18**, 421–462.
- 44 Trotter KW, Fan HY, Ivey ML, Kingston RE & Archer TK (2008) The HSA domain of BRG1 mediates critical interactions required for glucocorticoid receptor-dependent transcriptional activation *in vivo*. *Mol Cell Biol* **28**, 1413–1426.
- 45 Minsky A, Ghirlando R & Reich Z (1997) Nucleosomes: a solution to a crowded intracellular environment? *J Theor Biol* **188**, 379–385.
- 46 Sandman K & Reeve JN (2006) Archaeal histones and the origin of the histone fold. *Curr Opin Microbiol* **9**, 520–525.
- 47 San Filippo J, Sung P & Klein H (2008) Mechanism of eukaryotic homologous recombination. *Annu Rev Biochem* **77**, 229–257.

- 48 Sikorski TW & Buratowski S (2009) The basal initiation machinery: beyond the general transcription factors. *Curr Opin Cell Biol* **21**, 344–351.
- 49 Viswanathan R & Auble DT (2011) One small step for Mot1; one giant leap for other Swi2/Snf2 enzymes? *Biochim Biophys Acta*, doi: 10.1016/j.bbagr.2011.05.012.
- 50 Laine JP & Egly JM (2006) When transcription and repair meet: a complex system. *Trends Genet* **22**, 430–436.
- 51 Sukhodolets MV, Cabrera JE, Zhi H & Jin DJ (2001) RapA, a bacterial homolog of SWI2/SNF2, stimulates RNA polymerase recycling in transcription. *Genes Dev* **15**, 3330–3341.
- 52 Shaw G, Gan J, Zhou YN, Zhi H, Subburaman P, Zhang R, Joachimiak A, Jin DJ & Ji X (2008) Structure of RapA, a Swi2/Snf2 protein that recycles RNA polymerase during transcription. *Structure* **16**, 1417–1427.
- 53 Jin DJ, Zhou YN, Shaw G & Ji X (2011) Structure and function of RapA: a bacterial Swi2/Snf2 protein required for RNA polymerase recycling in transcription. *Biochim Biophys Acta*, doi: 10.1016/j.bbagr.2011.03.003.
- 54 Yawn B, Zhang L, Mura C & Sukhodolets MV (2009) RapA, the SWI/SNF subunit of *Escherichia coli* RNA polymerase, promotes the release of nascent RNA from transcription complexes. *Biochemistry* **48**, 7794–7806.
- 55 Nishino T, Komori K, Tsuchiya D, Ishino Y & Morikawa K (2005) Crystal structure and functional implications of *Pyrococcus furiosus* hef helicase domain involved in branched DNA processing. *Structure* **13**, 143–153.
- 56 Ghaemmghami S, Huh WK, Bower K, Howson RW, Belle A, Dephoure N, O'Shea EK & Weissman JS (2003) Global analysis of protein expression in yeast. *Nature* **425**, 737–741.
- 57 Weiss M, Schrimpf S, Hengartner MO, Lercher MJ & von Mering C (2010) Shotgun proteomics data from multiple organisms reveals remarkable quantitative conservation of the eukaryotic core proteome. *Proteomics* **10**, 1297–1306.
- 58 von der Haar T (2008) A quantitative estimation of the global translational activity in logarithmically growing yeast cells. *BMC Syst Biol* **2**, 87.
- 59 Schwanhaussner B, Busse D, Li N, Dittmar G, Schuchhardt J, Wolf J, Chen W & Selbach M (2011) Global quantification of mammalian gene expression control. *Nature* **473**, 337–342.
- 60 Erdel F & Rippe K (2011) ISWI chromatin remodellers in mammalian cells – where, when and why? *FEBS J*.
- 61 Ferreira H, Flaus A & Owen-Hughes T (2007) Histone modifications influence the action of Snf2 family remodelling enzymes by different mechanisms. *J Mol Biol* **374**, 563–579.
- 62 Liu CL, Kaplan T, Kim M, Buratowski S, Schreiber SL, Friedman N & Rando OJ (2005) Single-nucleosome mapping of histone modifications in *S. cerevisiae*. *PLoS Biol* **3**, e328.
- 63 van Attikum H & Gasser SM (2005) ATP-dependent chromatin remodeling and DNA double-strand break repair. *Cell Cycle* **4**, 1011–1014.
- 64 Korber P & Becker PB (2010) Nucleosome dynamics and epigenetic stability. *Essays Biochem* **48**, 63–74.
- 65 Koerber RT, Rhee HS, Jiang C & Pugh BF (2009) Interaction of transcriptional regulators with specific nucleosomes across the *Saccharomyces* genome. *Mol Cell* **35**, 889–902.
- 66 Xella B, Goding C, Agricola E, Di Mauro E & Caserta M (2006) The ISWI and CHD1 chromatin remodelling activities influence ADH2 expression and chromatin organization. *Mol Microbiol* **59**, 1531–1541.
- 67 Whitehouse I & Tsukiyama T (2006) Antagonistic forces that position nucleosomes *in vivo*. *Nat Struct Mol Biol* **13**, 633–640.
- 68 Lantermann AB, Straub T, Stralfors A, Yuan GC, Ekwall K & Korber P (2010) *Schizosaccharomyces pombe* genome-wide nucleosome mapping reveals positioning mechanisms distinct from those of *Saccharomyces cerevisiae*. *Nat Struct Mol Biol* **17**, 251–257.
- 69 Shukla MS, Syed SH, Montel F, Faivre-Moskalenko C, Bednar J, Travers A, Angelov D & Dimitrov S (2010) Remosomes: RSC generated non-mobilized particles with approximately 180 bp DNA loosely associated with the histone octamer. *Proc Natl Acad Sci U S A* **107**, 1936–1941.
- 70 Schnitzler G, Sif S & Kingston RE (1998) Human SWI/SNF interconverts a nucleosome between its base state and a stable remodeled state. *Cell* **94**, 17–27.
- 71 Floer M, Wang X, Prabhu V, Berrozpe G, Narayan S, Spagna D, Alvarez D, Kendall J, Krasnitz A, Stepanisky A *et al.* (2010) A RSC/nucleosome complex determines chromatin architecture and facilitates activator binding. *Cell* **141**, 407–418.
- 72 Kagalwala MN, Glaus BJ, Dang W, Zofall M & Bartholomew B (2004) Topography of the ISW2-nucleosome complex: insights into nucleosome spacing and chromatin remodeling. *EMBO J* **23**, 2092–2104.
- 73 Grune T, Brzeski J, Eberharter A, Clapier CR, Corona DF, Becker PB & Muller CW (2003) Crystal structure and functional analysis of a nucleosome recognition module of the remodeling factor ISWI. *Mol Cell* **12**, 449–460.
- 74 Ryan DP, Sundaramoorthy R, Martin D, Singh V & Owen-Hughes T (2011) The DNA-binding domain of the Chd1 chromatin-remodelling enzyme contains SANT and SLIDE domains. *EMBO J*, **30**, 2596–2609.
- 75 Gangaraju VK, Prasad P, Srour A, Kagalwala MN & Bartholomew B (2009) Conformational changes associ-

- ated with template commitment in ATP-dependent chromatin remodeling by ISW2. *Mol Cell* **35**, 58–69.
- 76 Blosser TR, Yang JG, Stone MD, Narlikar GJ & Zhuang X (2009) Dynamics of nucleosome remodelling by individual ACF complexes. *Nature* **462**, 1022–1027.
- 77 Strohner R, Wachsmuth M, Dachauer K, Mazurkiewicz J, Hochstatter J, Rippe K & Langst G (2005) A 'loop recapture' mechanism for ACF-dependent nucleosome remodeling. *Nat Struct Mol Biol* **12**, 683–690.
- 78 Awad S, Ryan D, Prochasson P, Owen-Hughes T & Hassan AH (2010) The Snf2 Homolog Fun30 Acts as a Homodimeric ATP-dependent Chromatin-remodeling Enzyme. *J Biol Chem* **285**, 9477–9484.
- 79 Boeger H, Griesenbeck J & Kornberg RD (2008) Nucleosome retention and the stochastic nature of promoter chromatin remodeling for transcription. *Cell* **133**, 716–726.
- 80 Engholm M, de Jager M, Flaus A, Brenk R, van Noort J & Owen-Hughes T (2009) Nucleosomes can invade DNA territories occupied by their neighbors. *Nat Struct Mol Biol* **16**, 151–158.
- 81 Dechassa ML, Sabri A, Pondugula S, Kassabov SR, Chatterjee N, Kladde MP & Bartholomew B (2010) SWI/SNF has intrinsic nucleosome disassembly activity that is dependent on adjacent nucleosomes. *Mol Cell* **38**, 590–602.
- 82 Lavelle C, Praly E, Bensimon D, Le Cam E & Croquette V (2011) Nucleosome remodelling machines and other molecular motors observed at single molecule level. *FEBS J*.
- 83 van Vugt JJ, de Jager M, Murawska M, Brehm A, van Noort J & Logie C (2009) Multiple aspects of ATP-dependent nucleosome translocation by RSC and Mi-2 are directed by the underlying DNA sequence. *PLoS ONE* **4**, e6345.
- 84 Whitehouse I, Flaus A, Cairns BR, White MF, Workman JL & Owen-Hughes T (1999) Nucleosome mobilization catalysed by the yeast SWI/SNF complex. *Nature* **400**, 784–787.
- 85 Langst G & Becker PB (2001) ISWI induces nucleosome sliding on nicked DNA. *Mol Cell* **8**, 1085–1092.
- 86 Zofall M, Persinger J, Kassabov SR & Bartholomew B (2006) Chromatin remodeling by ISW2 and SWI/SNF requires DNA translocation inside the nucleosome. *Nat Struct Mol Biol* **13**, 339–346.
- 87 Saha A, Wittmeyer J & Cairns BR (2005) Chromatin remodeling through directional DNA translocation from an internal nucleosomal site. *Nat Struct Mol Biol* **12**, 747–755.
- 88 Lohman TM, Tomko EJ & Wu CG (2008) Non-hexameric DNA helicases and translocases: mechanisms and regulation. *Nat Rev Mol Cell Biol* **9**, 391–401.
- 89 Schwanbeck R, Xiao H & Wu C (2004) Spatial contacts and nucleosome step movements induced by the NURF chromatin remodeling complex. *J Biol Chem* **279**, 39933–39941.
- 90 Dang W & Bartholomew B (2007) Domain architecture of the catalytic subunit in the ISW2-nucleosome complex. *Mol Cell Biol* **27**, 8306–8317.
- 91 Tan S & Davey CA (2011) Nucleosome structural studies. *Curr Opin Struct Biol* **21**, 128–136.
- 92 Davey CA, Sargent DF, Luger K, Maeder AW & Richmond TJ (2002) Solvent mediated interactions in the structure of the nucleosome core particle at 1.9 Å resolution. *J Mol Biol* **319**, 1097–1113.
- 93 Flaus A, Rencurel C, Ferreira H, Wiechens N & Owen-Hughes T (2004) Sin mutations alter inherent nucleosome mobility. *EMBO J* **23**, 343–353.
- 94 Polach KJ & Widom J (1995) Mechanism of protein access to specific DNA sequences in chromatin: a dynamic equilibrium model for gene regulation. *J Mol Biol* **254**, 130–149.
- 95 Li G, Levitus M, Bustamante C & Widom J (2005) Rapid spontaneous accessibility of nucleosomal DNA. *Nat Struct Mol Biol* **12**, 46–53.
- 96 Bohm V, Hieb AR, Andrews AJ, Gansen A, Rocker A, Toth K, Luger K & Langowski J (2010) Nucleosome accessibility governed by the dimer/tetramer interface. *Nucleic Acids Res* **39**, 3093–3102.
- 97 Bruno M, Flaus A, Stockdale C, Rencurel C, Ferreira H & Owen-Hughes T (2003) Histone H2A/H2B dimer exchange by ATP-dependent chromatin remodeling activities. *Mol Cell* **12**, 1599–1606.
- 98 Bowman A, Ward R, El-Mkami H, Owen-Hughes T & Norman DG (2010) Probing the (H3-H4)<sub>2</sub> histone tetramer structure using pulsed EPR spectroscopy combined with site-directed spin labelling. *Nucleic Acids Res* **38**, 695–707.
- 99 Sekulic N, Bassett EA, Rogers DJ & Black BE (2010) The structure of (CENP-A-H4)<sub>2</sub> reveals physical features that mark centromeres. *Nature* **467**, 347–351.
- 100 Bancaud A, Wagner G, Conde ESN, Lavelle C, Wong H, Mozziconacci J, Barbi M, Sivolob A, Le Cam E, Mouawad L *et al.* (2007) Nucleosome chiral transition under positive torsional stress in single chromatin fibers. *Mol Cell* **27**, 135–147.
- 101 Flaus A & Owen-Hughes T (2003) Mechanisms for nucleosome mobilization. *Biopolymers* **68**, 563–578.
- 102 Blosser R & Schiessel H (2011) The dynamics of the nucleosome: thermal effects, external forces, and ATP. *FEBS J*.
- 103 Cote J, Quinn J, Workman JL & Peterson CL (1994) Stimulation of GAL4 derivative binding to nucleosomal DNA by the yeast SWI/SNF complex. *Science* **265**, 53–60.
- 104 Smith CL, Horowitz-Scherer R, Flanagan JF, Woodcock CL & Peterson CL (2003) Structural analysis of

- the yeast SWI/SNF chromatin remodeling complex. *Nat Struct Biol*, **10**, 141–145.
- 105 Cairns BR, Lorch Y, Li Y, Zhang M, Lacomis L, Erdjument-Bromage H, Tempst P, Du J, Laurent B & Kornberg RD (1996) RSC, an essential, abundant chromatin-remodelling complex. *Cell* **87**, 1249–1260.
- 106 Gelbart ME, Rechsteiner T, Richmond TJ & Tsukiyama T (2001) Interactions of Isw2 chromatin remodeling complex with nucleosomal arrays: analyses using recombinant yeast histones and immobilized templates. *Mol Cell Biol* **21**, 2098–2106.
- 107 Tran HG, Steger DJ, Iyer VR & Johnson AD (2000) The chromo domain protein chd1p from budding yeast is an ATP-dependent chromatin-modifying factor. *EMBO J* **19**, 2323–2331.
- 108 Stockdale C, Flaus A, Ferreira H & Owen-Hughes T (2006) Analysis of nucleosome repositioning by yeast ISWI and Chd1 chromatin remodeling complexes. *J Biol Chem* **281**, 16279–16288.
- 109 Mizuguchi G, Shen X, Landry J, Wu WH, Sen S & Wu C (2004) ATP-driven exchange of histone H2AZ variant catalyzed by SWR1 chromatin remodeling complex. *Science* **303**, 343–348.
- 110 Krogan NJ, Keogh MC, Datta N, Sawa C, Ryan OW, Ding H, Haw RA, Pootoolal J, Tong A, Canadien V *et al.* (2003) A Snf2 family ATPase complex required for recruitment of the histone H2A variant Htz1. *Mol Cell* **12**, 1565–1576.
- 111 Kobor MS, Venkatasubrahmanyam S, Meneghini MD, Gin JW, Jennings JL, Link AJ, Madhani HD & Rine J (2004) A protein complex containing the conserved Swi2/Snf2-related ATPase Swr1p deposits histone variant H2A.Z into euchromatin. *PLoS Biol* **2**, E131.
- 112 Luk E, Ranjan A, FitzGerald PC, Mizuguchi G, Huang Y, Wei D & Wu C (2010) Stepwise histone replacement by SWR1 requires dual activation with histone H2A.Z and canonical nucleosome. *Cell* **143**, 725–736.
- 113 Neves-Costa A, Will WR, Vetter AT, Miller JR & Varga-Weisz P (2009) The SNF2-family member Fun30 promotes gene silencing in Heterochromatic loci. *PLoS ONE* **4**, e8111.
- 114 Shen X, Mizuguchi G, Hamiche A & Wu C (2000) A chromatin remodelling complex involved in transcription and DNA processing. *Nature* **406**, 541–544.
- 115 Papamichos-Chronakis M, Watanabe S, Rando OJ & Peterson CL (2011) Global regulation of H2A.Z localization by the INO80 chromatin-remodeling enzyme is essential for genome integrity. *Cell* **144**, 200–213.
- 116 Clever B, Interthal H, SchmuckliMaurer J, King J, Sigrist M & Heyer WD (1997) Recombinational repair in yeast: functional interactions between Rad51 and Rad54 proteins. *EMBO J* **16**, 2535–2544.
- 117 Shah PP, Zheng XZ, Epshtein A, Carey JN, Bishop DK & Klein HL (2010) Swi2/Snf2-related translocases prevent accumulation of toxic Rad51 complexes during mitotic growth. *Mol Cell* **39**, 862–872.
- 118 Klein HL (1997) RDH54, a RAD54 homologue in *Saccharomyces cerevisiae*, is required for mitotic diploid-specific recombination and repair and for meiosis. *Genetics* **147**, 1533–1543.
- 119 Chi P, Kwon Y, Moses DN, Seong C, Sehorn MG, Singh AK, Tsubouchi H, Greene EC, Klein HL & Sung P (2009) Functional interactions of meiotic recombination factors Rdh54 and Dmc1. *DNA Repair* **8**, 279–284.
- 120 Johnson RE, Henderson ST, Petes TD, Prakash S, Bankmann M & Prakash L (1992) *Saccharomyces-cerevisiae* Rad5-encoded DNA-repair protein contains DNA helicase and zinc-binding sequence motifs and affects the stability of simple repetitive sequences in the genome. *Mol Cell Biol* **12**, 3807–3818.
- 121 Blastyak A, Pinter L, Unk I, Prakash L, Prakash S & Haracska L (2007) Yeast Rad5 protein required for postreplication repair has a DNA helicase activity specific for replication fork regression. *Mol Cell* **28**, 167–175.
- 122 Guzder SN, Sung P, Prakash L & Prakash S (1997) Yeast Rad7-Rad16 complex, specific for the nucleotide excision repair of the nontranscribed DNA strand, is an ATP-dependent DNA damage sensor. *J Biol Chem* **272**, 21665–21668.
- 123 Reed SH, Akiyama M, Stillman B & Friedberg EC (1999) Yeast autonomously replicating sequence binding factor is involved in nucleotide excision repair. *Genes Dev* **13**, 3052–3058.
- 124 Alvaro D, Lisby M & Rothstein R (2007) Genome-wide analysis of Rad52 foci reveals diverse mechanisms impacting recombination. *PLoS Genet* **3**, 2439–2449.
- 125 Zhang ZM & Buchman AR (1997) Identification of a member of a DNA-dependent ATPase family that causes interference with silencing. *Mol Cell Biol* **17**, 5461–5472.
- 126 Vangoel AJ, Verhage R, Swagemakers SMA, Vandeputte P, Brouwer J, Troelstra C, Bootsma D & Hoeijmakers JHJ (1994) Rad26, the functional *saccharomyces-cerevisiae* homolog of the cockayne-syndrome-B gene Ercc6. *EMBO J* **13**, 5361–5369.
- 127 Reid J & Svejstrup JQ (2004) DNA damage-induced Def1-RNA polymerase II interaction and Def1 requirement for polymerase ubiquitylation *in vitro*. *J Biol Chem* **279**, 29875–29878.
- 128 Poon D, Campbell AM, Bai Y & Weil PA (1994) Yeast Taf170 is encoded by MOT1 and exists in a TATA box-binding protein (TBP)-TBP-associated factor complex distinct from transcription factor IID. *J Biol Chem* **269**, 23135–23140.
- 129 Auble DT, Hansen KE, Mueller CG, Lane WS, Thorner J & Hahn S (1994) Mot1, a global repressor of RNA polymerase II transcription, inhibits TBP

- binding to DNA by an ATP-dependent mechanism. *Genes Dev* **8**, 1920–1934.
- 130 Gumbs OH, Campbell AM & Weil PA (2003) High-affinity DNA binding by a Mot1p-TBP complex: implications for TAF-independent transcription. *EMBO J* **22**, 3131–3141.
- 131 Fan L, Arvai AS, Cooper PK, Iwai S, Hanaoka F & Tainer JA (2006) Conserved XPB core structure and motifs for DNA unwinding: implications for pathway selection of transcription or excision repair. *Mol Cell* **22**, 27–37.
- 132 Sengoku T, Nureki O, Nakamura A, Kobayashi S & Yokoyama S (2006) Structural basis for RNA unwinding by the DEAD-box protein *Drosophila* Vasa. *Cell* **125**, 287–300.