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PROSPECTS

ATP-dependent Nucleosome Remodeling Complexes: Enzymes Tailored to Deal With Chromatin

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Abstract Chromatin remodeling complexes play a central role in the control of nuclear processes that utilize genomic DNA as a template including transcription, replication, recombination, and repair. Modulation of chromatin structure is mediated by a wide variety of enzymes which can affect nucleosome stability by either disrupting histone-DNA contacts or by covalently modifying histones and/or DNA. Although the biochemical properties of most chromatin-modifying enzymes have been well characterized and links between histone and DNA-modifying enzymes and ATP-dependent chromatin remodeling complexes have been established, the importance of their concerted action has just begun to emerge. As more and more genes are examined, new rules are being established about their transcriptional regulation, and it is becoming clear that diverse mechanisms are used to modify chromatin and either promote or hinder accessibility to DNA and histones. Moreover, the involvement of ATP-dependent chromatin remodelers in transcriptional regulation of cyclin genes and the association of misregulated expression of chromatin remodeling subunits with many cancers highlight the importance of chromatin remodeling complexes in the control of cell growth and proliferation. J. Cell. Biochem. 91: 1087–1098, 2004.

Key words: chromatin remodeling complexes; mSin3/HDAC co-repressors; methyl-CpG-binding domain; BRG1 and hBRM-associated factors; metastasis associated proteins

Recent advances in the field of chromatin have not only unraveled details about the organization of the four core histones within a nucleosomal core particle, but have also led to the discovery and characterization of numerous enzymatic activities that regulate chromatin structure. Packaging of genomic DNA into chromatin represents a major obstacle for DNA binding proteins and more recently this has also become true for enzymes that modify DNA and histones. Among the various chromatin-modifying enzymes, there are three structurally distinct categories: histone-modifying enzymes which covalently acetylate, phosphorylate, ubiquitinate, or methylate histones; DNA-modifying enzymes which methylate CpG-rich sequences and ATP-dependent chromatin remodeling complexes which can disrupt nucleosome structure and increase accessibility to DNA as well as histones. The functional characterization of these chromatin-modifying enzymes has dramatically changed our understanding of how nuclear processes such as transcription are regulated in vivo. In addition, the discovery of multisubunit complexes containing DNA-dependent ATPases in combination with histone deacetylases (HDAC), methyl-CpG-binding proteins (MBD), and activator as well as repressor histone methyltransferases (HMT) in higher eukaryotes suggests that ATP-dependent chromatin remodelers are not only involved in transcriptional activation but also transcriptional repression. Because the characteristics of each category of enzymes have been discussed in great detail [Ahringer, 2000; Jenuwein and Allis, 2001; Zhang and Reinberg, 2001; Becker and Hörz, 2002; Jones and Baylin, 2002; Narlikar et al., 2002], this review will focus on describing recent work that indicates a

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greater heterogeneity among chromatin remodeling complexes and their involvement in regulating chromatin structure.

CHROMATIN REMODELING COMPLEXES IN YEAST

Chromatin remodeling complexes were first identified by genetic screens in yeast as mutations that altered transcription of genes that were induced in response to extracellular signals [Winston and Carlson, 1992]. Subsequently, these mutant strains were named Swi/Snf because they affected expression of the *HO* and *SUC2* genes, which are important for mating type switching and sucrose fermentation, respectively. Other screens aimed at identifying suppressor mutations of the Swi/Snf phenotype resulted in the isolation of genes that encode histones and chromatin associated proteins such as SIN1 [Winston and Carlson, 1992]. These studies in combination with LexA-Swi/Snf fusion experiments provided the first clue that members of the Swi/Snf family of proteins are part of a complex that can activate transcription by relieving the repressive effects of chromatin [Laurent et al., 1991; Peterson and Herskowitz, 1992]. In accordance with this hypothesis, Swi/Snf proteins were shown to stimulate transcription of the *SUC2* gene by altering the structure of nucleosomes situated near the promoter region, and were later purified as part of a 12 subunit complex dubbed SWI/SNF [Hirschhorn et al., 1992; Cairns et al., 1994; Peterson et al., 1994]. Biochemical characterization of SWI/SNF, revealed that the complex can disrupt nucleosome structure and increase accessibility to nucleosomal DNA in an ATP-dependent manner [Côté et al., 1994]. Based on sequence homology to Swi/Snf subunits, Kornberg and colleagues were able to purify a second and more abundant yeast complex termed remodels the structure of chromatin (RSC), which contains the DNA-dependent ATPase Sth1 [Cairns et al., 1996]. Unlike SWI/SNF, RSC contains subunits that are essential for mitotic growth and exists in different forms which contain either Rsc1 or Rsc2 in the presence or absence of Rsc3 and Rsc30 [Cairns et al., 1999; Angus-Hill et al., 2001].

Homologs of SWI/SNF complexes were found in *Drosophila* and humans and share several conserved subunits including Swi2/Snf2, which provides the ATPase function (Fig. 1). The Swi2/Snf2 superfamily can be further divided into at least four families depending on whether they contain a bromodomain (Swi2/Snf2), two copies of a chromodomain (Mi-2), a SANT domain (ISWI), or a 450–550 amino acid insertion within the DNA-dependent ATPase domain (DOMINO). There are other ATPases that share homology with Swi2/Snf2 through their DNA-dependent ATPase domain, but that lack these signature motifs [Eisen et al., 1995]. Complexes containing members of the four families have been purified and studied extensively in vitro, and one common theme among all of them is that they are able to hydrolyze ATP and mobilize nucleosomes [Narlikar et al., 2002].

Recent yeast database searches have led to the identification of the ISWI-related proteins, Isw1p, Isw2p, and Ino80p, which are not essential for viability and associate with different remodeling complexes [Tsukiyama et al., 1999; Shen et al., 2000]. Isw1p and Isw2p contain the SANT (SWI3, ADA2, N-CoR, and TFIIIB B″) domain that is found in proteins involved in regulation of transcription and chromatin structure, and form distinct complexes that differ in their ability to remodel nucleosomes (Fig. 2). Both ISW1 and ISW2 complexes induce formation of regularly spaced nucleosomes, but only ISW1 increases accessibility to restriction enzymes on a nucleosomal array, indicating that both complexes have distinct features that might allow them to perform different functions in vivo.

Unlike other ISWI-related ATPases, Ino80 lacks a SANT domain and is incorporated into a multisubunit complex whose ATPase activity can be stimulated by both naked and nucleosomal DNA. INO80 complex contains actin and actin-related proteins (Arp), which have also been found in other chromatin-modifying complexes including SWI/SNF, RSC, dSWI/SNF, p400, BRG1, and BRM-based human SWI/SNF complexes (Fig. 2). Although it is believed that actin and Arps mediate interactions with the nuclear matrix, the functional relevance of these subunits in chromatin remodeling is poorly understood [Olave et al., 2002a]. Other conserved polypeptides associated with Ino80 include Rvb1 and Rvb2, two subunits related to the bacterial Holliday junction DNA helicase RuvB and eukaryotic TIP49a/RUVBL1/TAP54α, and TIP49b/RUVBL2/TAP54β DNA helicases. Rvb1 and Rvb2 are essential for viability and require Ino80 ATPase activity

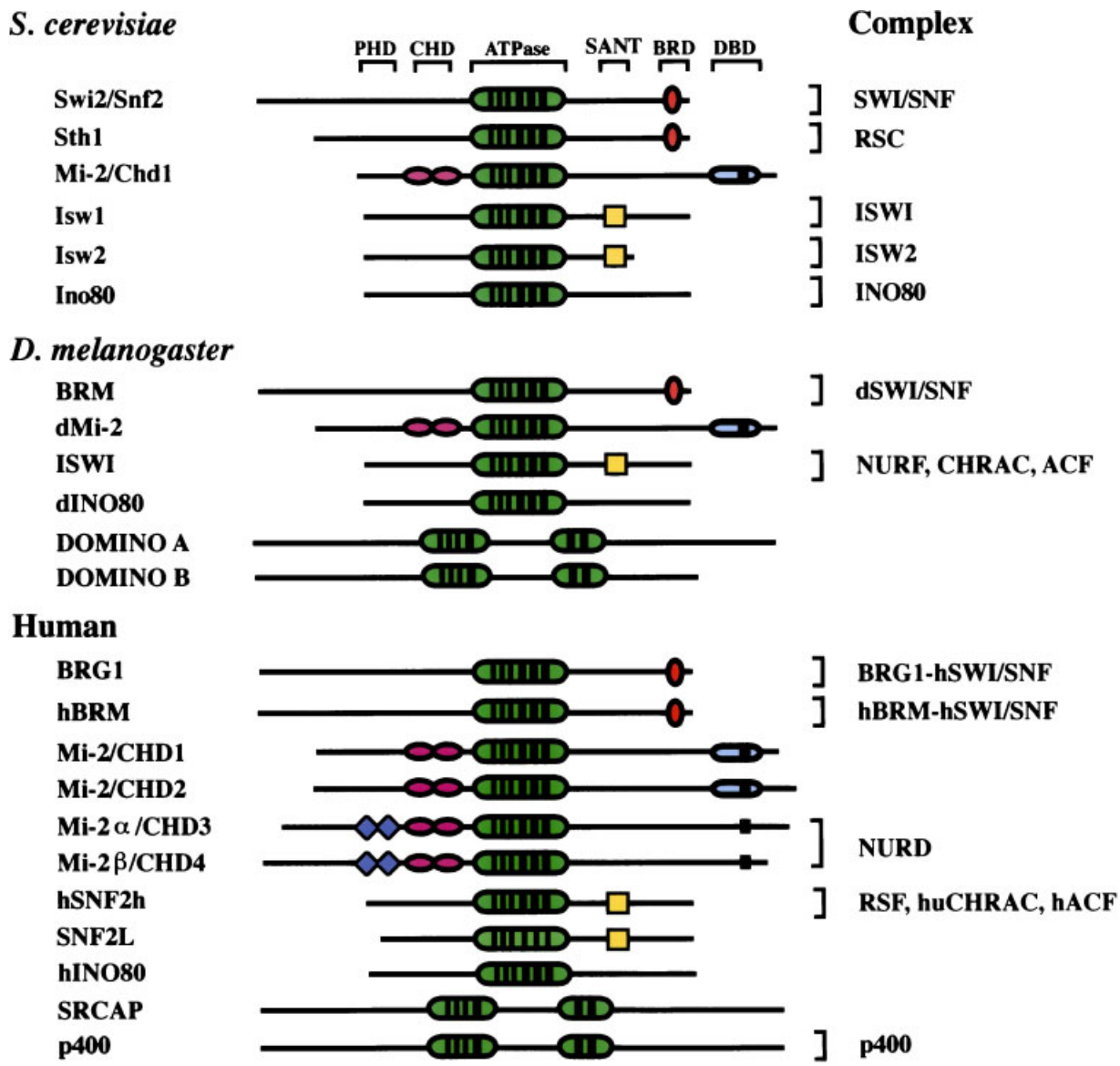
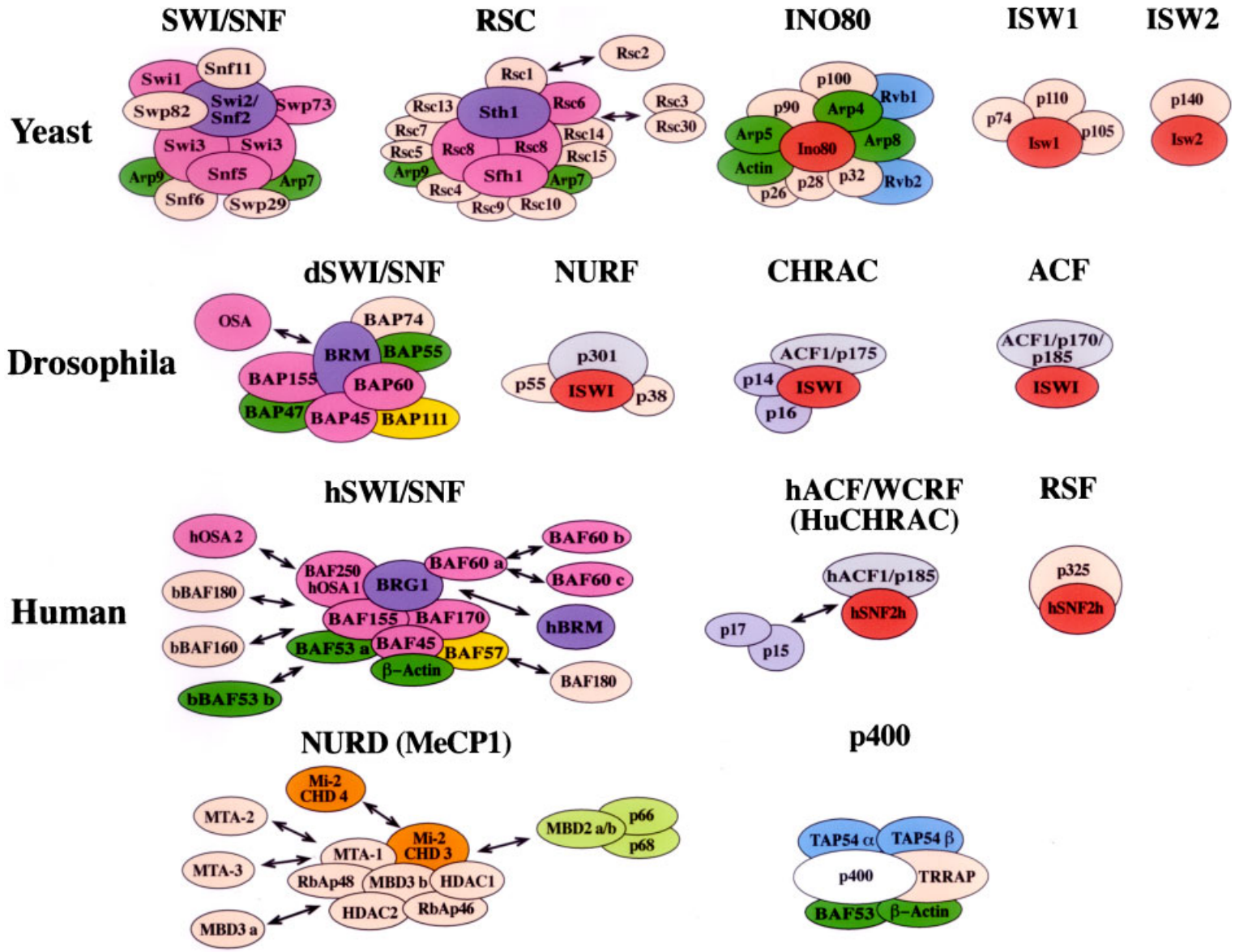


Fig. 1. Members of the Swi2/Snf2 superfamily. All members of the family contain a DNA-dependent ATPase domain (green) and either a bromodomain (BRD in red), two copies of chromodomain (CHD in pink), or a SANT domain (yellow). Members of the Mi-2 family which contain a C-terminal DNA binding domain (DBD, in light blue), a partial region of homology within the DBD (black box), or two copies of a plant homeodomain (PHD in dark blue) are also shown. ATPases that have been purified and shown to be part of a complex are indicated by a bracket. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

since a catalytically inactive form of Ino80 abolishes the DNA helicase function of the complex [Shen et al., 2000]. Detailed analysis of the INO80 complex both in vivo and in vitro showed that it functions differently than other ISWI-containing complexes in that it is involved in processing damaged DNA. Rvb1 and two related proteins have been found in other complexes including the mammalian p400 complex and the TIP60 histone acetylase complex [Ikura et al., 2000; Fuchs et al., 2001]. Both p400 and TIP60 complexes share several subunits including the c-Myc and E2F binding protein, TRRAP, the DOMINO-related ATPase p400, the TIP60 associated proteins (TAP) 54α and β, β-actin and the actin related protein, BAF53. Pre-incubation of TIP60 complex with the non-hydrolyzable ATP analog ATPαS inhibits its helicase activity, suggesting that p400 ATPase activity is required for efficient strand removal [Ikura et al., 2000]. Therefore, it appears that RuvB-like DNA helicases associate with different


SWI/SNF
RSC
INO80
ISW1
ISW2
Yeast
Swi1
Snf11
Swp82
Swi2/
Snf2
Swp73
Swi3
Swi3
Arp9
Snf5
Arp7
Snf6
Swp29
Rsc1
Rsc2
Rsc13
Sth1
Rsc6
Rsc3
Rsc30
Rsc7
Rsc14
Rsc5
Rsc8
Rsc8
Rsc15
Arp9
Sfh1
Arp7
Rsc4
Rsc9
Rsc10
p100
Rvb1
p90
Arp4
Arp5
Ino80
Arp8
Actin
p28
p32
Rvb2
p26
p110
p74
Isw1
p105
p140
Isw2
dSWI/SNF
NURF
CHRAC
ACF
Drosophila
OSA
BAP74
BRM
BAP55
BAP155
BAP60
BAP47
BAP45
BAP111
p301
p55
ISWI
p38
ACF1/p175
p14
ISWI
p16
ACF1/p170/
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ISWI
hSWI/SNF
hACF/WCRF
(HuCHRAC)
RSF
Human
hOSA 2
BAF250
hOSA 1
BRG1
BAF60 a
BAF60 b
BAF60 c
bBAF180
BAF155
BAF170
hBRM
bBAF160
BAF53 a
BAF45
BAF57
β–Actin
BAF180
bBAF53 b
hACF1/p185
hSNF2h
p17
p15
p325
hSNF2h
NURD (MeCP1)
p400
Mi-2
CHD 4
MTA-2
MBD2 a/b
p66
p68
Mi-2
CHD 3
MTA-1
MTA-3
RbAp48
MBD3 b
HDAC1
HDAC2
RbAp46
MBD3 a
TAP54 α
TAP54 β
p400
TRRAP
BAF53
β–Actin

Fig. 2.

members of the Swi2/Snf2 superfamily. In yeast, Rvb1 and Rvb2 associate with the ISWI-related ATPase Ino80, while in mammals TAP54α/TAP54β form a complex with the DOMINO-like ATPase p400. Although, it is unclear at this time whether the p400 complex is involved in processing damaged DNA, a role in DNA repair has been ascribed to the TIP60 and INO80 complexes [Ikura et al., 2000; Shen et al., 2000]. These studies suggest that the combination of DNA helicases, ATPases and/or HATs provides a cell with specialized nucleosome remodeling complexes that can be used to resolve the constraints imposed by chromatin during DNA damage repair.

CHROMATIN REMODELERS ARE HIGHLY CONSERVED IN *DROSOPHILA*

Members of the four families of ATPases have been identified in *Drosophila* and complexes associated with brahma (BRM) and imitation switch (ISWI) have been purified and tested for their ability to remodel chromatin. Like Swi2/Snf2, BRM is associated with a large multisubunit complex that contains homologs of yeast and human SWI/SNF subunits [Papoulas et al., 1998]. BRM complex also contains actin and Arps, as well as the BRM-associated factor 111 (BAP111), which is related to the BRG1 and hBRM-associated factor, BAF57, a subunit capable of binding four way junction DNA. Deletion of the BAF57 N-terminal high mobility group (HMG) domain or a single point mutation that abolishes DNA binding causes derepression of the T cell *CD4* gene, suggesting that BAF57 is involved in transcriptional repression [Chi et al., 2002]. Chromatin immunoprecipitation studies indicate that BAF57 and BRG1 are recruited to the CD4 silencer, which suppresses CD4 expression. More recently, work from our laboratory has shown that BAF57 mediates interaction of BRG1 and hBRM complexes with mSin3A and B co-repressors and that these interactions are important for proper regulation of the Myc/Max/Mad target gene *cad* [Pal et al., 2003]. In addition, mapping studies showed that interaction with mSin3 proteins occurs through the C-terminal half of BAF57, which harbors the kinesin-like coiled coil region [S. Pal and S. Sif, unpublished]. Taken together, these studies suggest that the BAF57 N-terminal HMG domain is involved in DNA binding, whereas the C-terminal half is involved in recruiting the mSin3/HDAC co-repressor complex. Based on the evolutionary conservation that exists between *Drosophila* and mammalian BRM complexes, it is conceivable that BAP111 might perform a similar function in the BRM complex.

An interaction between the trithorax group gene *osa*, which encodes a protein related to yeast Swi1 and human BAF250, and components of the *Drosophila* BRM complex has been documented both biochemically and genetically [Collins et al., 1999]. However, only a subset of BRM complexes contain OSA. Although, it appears that the heterogeneity of BRM-based complexes is limited, ISWI-based complexes appear to be more diverse and include the nucleosome remodeling factor (NURF), the chromatin accessibility complex (CHRAC) and the ATP-utilizing chromatin assembly, and remodeling factor (ACF) [Becker and Hörz, 2002]. All ISWI-based complexes have an ATPase activity that is strictly stimulated by nucleosomes, except for yeast Ino80 whose ATPase activity can be stimulated by both free and nucleosomal DNA [Shen et al., 2000]. Previous work showed that NURF requires the presence of all four histone N-terminal tails for efficient nucleosome remodeling and lacks the ability to form regularly spaced nucleosomes [Georgel et al., 1997; Tsukiyama et al., 1999]. However, CHRAC and ACF can induce regular spacing of nucleosomes and depend only on the integrity of H4 N-terminal tail [Eberharter et al., 2001]. In stark contrast, BRG1 and hBRM

Fig. 2. ATP-dependent chromatin remodeling complexes. All chromatin remodeling complexes purified to date and their associated subunits are shown. Swi2/Snf2-related ATPases are indicated in purple, ISWI-related ATPases are depicted in red, Mi-2-related ATPases are colored orange, and DOMINO-like ATPases are shown in white. Subunits conserved between Swi/Snf complexes are shown in pink, while subunits specific to each complex are indicated in peach. Actin and actin-related proteins (Arp) are shown in green. Yellow indicates the subunits that are conserved in *Drosophila* (BAP111) and humans (BAF57). Rvb1/Rvb2 and TAP54α/TAP54β DNA helicases are shown in blue. Gray shows the highly conserved *Drosophila* and human ACF1 subunit, while light purple shows the histone fold subunits found in CHRAC. Light green depicts the three subunits (MBD2, p66 and p68) found in MeCP1. bBAF indicates brain specific SWI/SNF subunits found in association with BRG1 and BRM. Arrows show the interactions of individual subunits with their respective complexes. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

complexes can disrupt tailless nucleosomes [Guyon et al., 1999]. Other differences reside in the fact that ISWI-based complexes can only catalyze small step (few base pairs) sliding of histone octamers in cis, whereas Swi2/Snf2-related complexes induce transfer of histone octamers in cis and in trans [Becker and Hörz, 2002; Narlikar et al., 2002]. Thus, it appears that different chromatin remodeling complexes recognize different features of the nucleosome, and that this in turn might impact how chromatin is modulated during transcription, replication, recombination, and repair.

HUMAN CHROMATIN REMODELING COMPLEXES ARE FUNCTIONALLY DIVERSE

Human cells contain two Swi2/Snf2-related ATPases, BRG1, and hBRM, which associate with several conserved subunits [Kwon et al., 1994; Wang et al., 1996; Sif et al., 1998]. To date, two genes that encode proteins related to BAF53 and BAF250/hOSA1 have been identified in addition to the previously characterized alternatively spliced forms of BAF60 [Wang et al., 1996; Inoue et al., 2002; Olave et al., 2002b]. BAF53b is highly related to the more ubiquitous BAF53a and is expressed exclusively in postmitotic cells in the murine central nervous system, whereas human OSA2 is related to BAF250/hOSA1 and is expressed in all tissues. In addition, purification of BRG1 and BRM-associated subunits from mouse brain showed that two novel polypeptides, p160 and p180, co-exist in complexes containing bBAF53b [Olave et al., 2002b]. These findings suggest that in addition to the combinatorial heterogeneity, more diversity is achieved by tissue-specific expression of particular BAFs. Hence, more genes can be regulated through differential recruitment of various hSWI/SNF complexes. Although, BRG1 and hBRM complexes contain similar subunits, they do show some differences. For example, the more abundant BRG1 complex is kept intact during mitosis, while the hBRM complex is targeted for degradation [Sif et al., 1998]. There are also different requirements for these complexes during development. Gene knockout experiments have shown that BRG1 cannot be deleted unless it is expressed from an ectopic source, suggesting that the BRG1 complex is essential for viability [Sumi-Ichinose et al., 1997; Bultman et al., 2000]. BRM on the other hand is dispensable for cell growth since deletion of both alleles does not affect viability [Reyes et al., 1998]. Mice that lack BRM show increased levels of BRG1 in certain tissues and altered control of cellular proliferation. These results suggest that BRG1 can functionally substitute for BRM, and that BRM could be involved in negative regulation of cell proliferation. These studies also suggest that the BRG1 and BRM complexes are regulated and targeted differently, and as such, might affect gene expression patterns during different stages of cell growth and differentiation.

Human ISWI-based complexes are highly related to their *Drosophila* counterparts and display similar biochemical activities. Human ACF contains two subunits, hSNF2h and hACF1, which are highly related to *Drosophila* ISWI and ACF1, respectively [Bochar et al., 2000; LeRoy et al., 2000; Poot et al., 2000]. Similarly, human CHRAC contains the two subunits of hACF in combination with p15 and p17, two histone fold proteins related to *Drosophila* CHRAC14 and CHRAC16 subunits [Poot et al., 2000]. Therefore, human and *Drosophila* CHRAC and ACF complexes appear to be functionally and structurally conserved. Human cells also contain remodeling and spacing factor (RSF), a two subunit hSNF2h-containing complex that can remodel nucleosomes and activate transcription [LeRoy et al., 1998]. The second subunit of RSF is a 325 kDa polypeptide whose function remains unknown, but it is possible that it might stimulate the catalytic activity of hSNF2h. This has already been reported for *Drosophila* ACF1, a component of ACF and CHRAC [Eberharter et al., 2001].

Other human ATP-dependent chromatin remodeling complexes include nucleosome remodeling and deacetylase (NURD) complex which contains in addition to either Mi-2/CHD3 or Mi-2/CHD4, HDACs 1 and 2, retinoblastoma associated (RbA) proteins p46 and RbAp48, metastasis associated (MTA) protein 1 or 2, and MBD3 [Ahringer, 2000]. NURD can alter nucleosome structure and deacetylate histones, and like ISWI-based complexes its ATPase activity is stimulated more efficiently by nucleosomal DNA [Wade et al., 1998; Zhang et al., 1998]. NURD differs from ACF, CHRAC, and RSF by its inability to induce nucleosome spacing [Zhang et al., 1998]. Functional characterization of NURD from different species revealed that different subunits perform distinct func-

tions. In human NURD, MTA-2 was shown to enhance the histone deacetylase activity of the core complex, which consists of HDACs 1 and 2, RbAp46 and RbAp48; while MBD3 played a role of a bridging molecule that mediates the interaction between MTA-2 and the histone deacetylase core complex [Zhang et al., 1999]. In Xenopus NURD, both MTA-1-like and MBD3 can bind DNA, with MBD3 showing preference for methylated DNA [Wade et al., 1999]. Human NURD contains predominantly MBD3b, a shorter version of MBD3a that lacks a portion of the conserved MBD domain [Zhang et al., 1999]. Therefore, it appears that there are different NURD complexes that contain either MBD3a or MBD3b in combination with either MTA-1 or -2, and that depending on which MBD3/MTA polypeptide pair present the DNA binding ability of NURD might be altered. NURD provided the first example for coupling chromatin remodeling with HDAC activity and MBD, both of which are known to induce gene silencing. However, knowing that mammalian MBD3 is unable to bind DNA, it was unclear how human NURD could bind methylated DNA until it was shown that MBD2 could interact with NURD [Wade et al., 1999; Zhang et al., 1999]. Recent work confirmed these results and showed that the MeCP1 complex, which contains in addition to all NURD subunits, MBD2, p66 and p68, is able to bind and remodel methylated nucleosomal DNA more efficiently than unmethylated DNA [Ng et al., 1999; Feng and Zhang, 2001]. Therefore, MeCP1 appears to combine the chromatin remodeling and histone deacetylase activities of NURD with the methyl-CpG-binding properties of MBD2.

It is important to note that other MBD-containing proteins such as MBD1, MBD4, and MeCP2 can also bind methylated DNA, but it is not known whether these MBD can interact with chromatin remodeling complexes [Hendrich and Bird, 1998]. MeCP2 has been shown to induce transcriptional repression by recruiting mSin3/HDAC co-repressor complexes [Nan et al., 1998], and we and others have shown that mSin3A/HDAC can be found in association with BRG1 and hBRM chromatin remodeling complexes [Sif et al., 2001; Kuzmichev et al., 2002; Pal et al., 2003]. Thus, it is possible that MeCP2 might target mSin3A/HDAC-containing BRG1 and hBRM complexes. The coupling of ATP-dependent chromatin remodeling complexes with HDAC and MBD shows the wide variety of mechanisms used to induce gene silencing, and suggests that modulation of chromatin structure at specific loci might require the gathering of multiple nucleosome modifying activities. Evidence in support of this view comes from the purification of a large supercomplex that interacts with the human trithorax protein ALL-1, which was originally identified through its involvement in acute leukemia [Nakamura et al., 2002]. In this instance, various chromatin-modifying enzymes are assembled into a single supercomplex to regulate ALL-1 target gene expression. Biochemical characterization of the ALL-1 supercomplex, which contains hBRM, NURD, hSNF2h, mSin3A/HDAC, TFIID, and homologs of the yeast histone methyltransferase Set1 complex, revealed that it can remodel nucleosomes, deacetylate histones, acetylate H2A and H4, and methylate H3K4. These studies demonstrate that the ALL-1 supercomplex can function both as an activator as well as a repressor of transcription, and suggest that different ATP-dependent chromatin remodeling complexes might be brought together either individually or in combination with other chromatin-modifying enzymes to regulate expression of specific target genes (Fig. 3).

CHROMATIN REMODELING COMPLEXES AND THE CONTROL OF CELL GROWTH AND PROLIFERATION

Recent studies have shown that inactivation of BRG1, hBRM, BAF57, and BAF45/Ini1 is associated with leukemias, lymphomas, lung, and breast cancers, as well as early childhood cancer [Versteege et al., 1998; Wong et al., 2000; DeCristofaro et al., 2001; Reisman et al., 2003]. It is not clear how inactivating mutations of human SWI/SNF subunits can contribute to tumorigenesis, but one mechanism might involve interaction with the Rb family of tumor suppressor proteins. Both BRG1 and hBRM can interact with Rb proteins and induce cell cycle arrest [Muchardt and Yaniv, 2001]. Since BRG1 and hBRM can activate transcription, it was not clear how interaction with Rb could repress E2F target genes and cause growth arrest. However, the findings which showed that Rb-mediated transcriptional repression was dependent on HDAC activity provided the first link between BRG1/hBRM complexes and HDAC [Brehm et al., 1998; Luo et al., 1998; Magnaghi-Jaulin

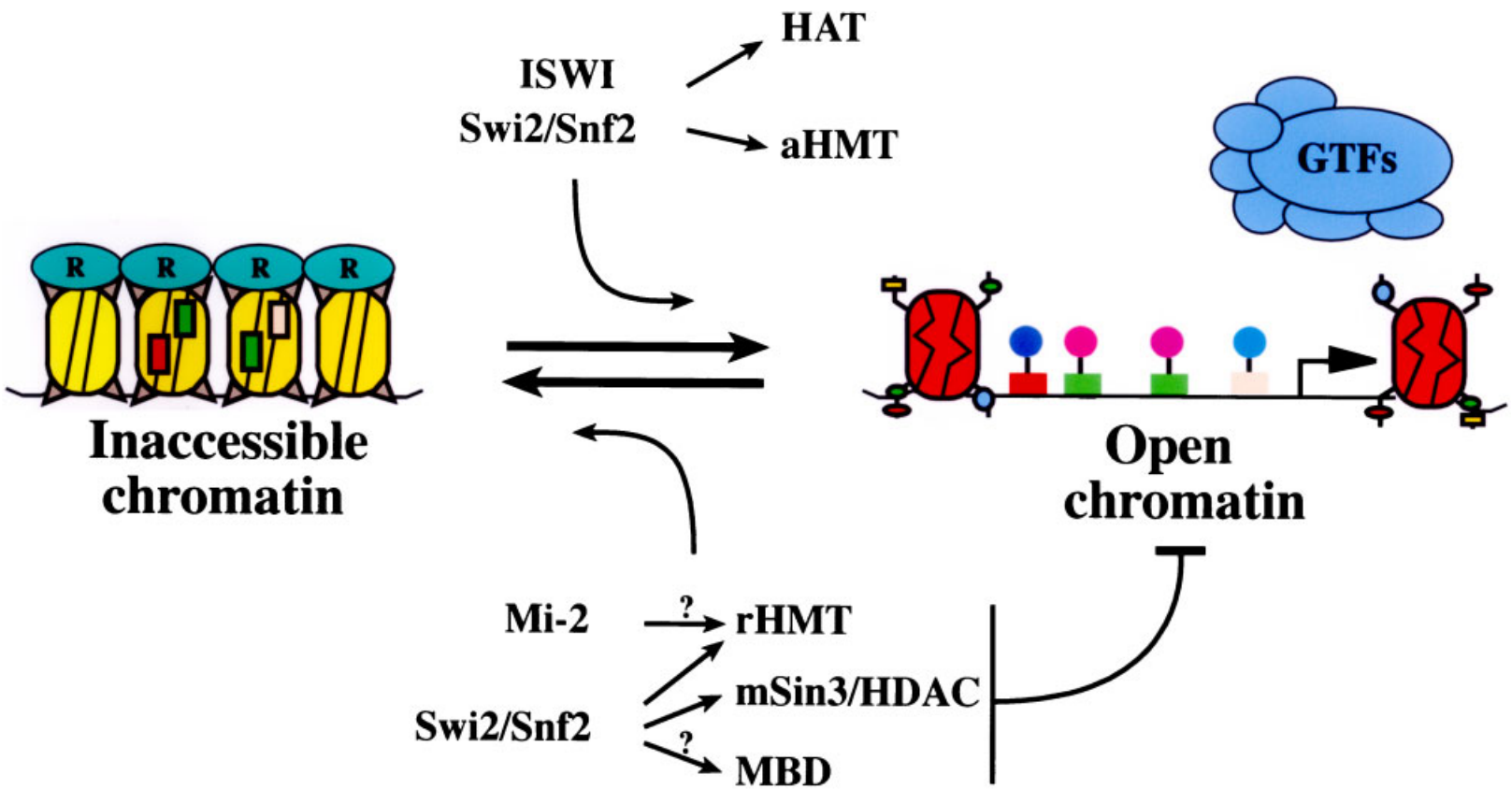

Fig. 3. Model for regulation of chromatin structure. Swi2/Snf2 and ISWI-based ATP-dependent chromatin remodeling complexes can alter nucleosome structure either individually or in combination with either histone acetyltransferases (HAT) or activator HMT (aHMT). As a result, chromatin becomes more accessible and specific and general transcription factors (GTFs) can bind to their sites. This reaction is not limited to transcription, but can also occur to enhance DNA binding of factors involved in replication, recombination and repair. To restore chromatin structure, ATP-dependent chromatin remodelers such as Mi-2 based complexes, which contain HDACs and MBD, as well as Swi2/Snf2-based complexes like BRG1 and hBRM, which can interact with mSin3/HDAC and repressor HMT (rHMT), can stabilize nucleosome structure by enhancing interaction between deacetylated histone N-terminal tails and DNA. Repressor (R) proteins such heterochromatin protein (HP1) can further stabilize nucleosomes (shown in yellow) by decreasing accessibility and binding to DNA. Activating histone modifications are shown in different colors on the histone N-terminal tails of disrupted nucleosomes (depicted in orange). Question marks indicate interactions that have not been established yet. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

et al., 1998]. These studies suggested that BRG1 and hBRM complexes might inhibit cell cycle progression by actively remodeling and deacetylating chromatin of genes that control cell growth and proliferation. BRG1 and hBRM complexes that contain mSin3A/HDAC have been purified and shown to be able to remodel chromatin and deacetylate histones [Sif et al., 2001; Kuzmichev et al., 2002; Pal et al., 2003]. In addition, HDAC-containing BRG1 complex has also been implicated in transcriptional repression of genes involved in cell cycle regulation such as *cdc2*, cyclins E, A, and D1, and the Myc/Max/Mad target gene *cad* which is involved in nucleotide biosynthesis and is important for G1 to S transition [Zhang et al., 2000; Zhang et al., 2002; Pal et al., 2003]. These experiments clearly show that ATP-dependent chromatin remodelers can associate with co-repressor complexes and be recruited to inhibit transcription. Therefore, inactivation of components of the BRG1 and hBRM complexes could disrupt regulation of pathways controlled by Myc and Rb.

NURD complexes contain different isoforms of Mi-2, MBD2, and MBD3. In addition to the heterogeneity generated by these three subunits, members of the MTA protein family can also be found in association with distinct NURD complexes further increasing the repertoire of Mi-2-based chromatin remodelers [Zhang et al., 1998; Wade et al., 1999; Feng and Zhang, 2001; Fujita et al., 2003]. Since silencing by DNA methylation also involves histone deacetylation, and since NURD can be targeted to methylated DNA through its interaction with MBD2, a protein that has been identified as a colon cancer antigen, it is conceivable that increased expression of MTA and/or MBD proteins could enhance the repressive effects of NURD at aberrantly methylated DNA sites. Consequently, this could lead to increased silencing of genes involved in regulating cell growth. A recent report by Wade and co-workers identified another MTA protein, MTA-3, that can interact with NURD in the absence of MTA-1 and -2 [Fujita et al., 2003]. In this study, MTA-3 was shown to induce E-cadherin expression in an estrogen receptor dependent manner via a mechanism that involves transcriptional repression of Snail. E-cadherin is a transmembrane protein whose function is tightly linked to adherens junction formation [Perez-Moreno et al., 2003]. The cytoplasmic tail of E-cadherin binds β-catenin which in turn interacts with α-catenin, and it is through this network of intracellular protein-protein interactions that E-cadherin couples the intracellular actin cytoskeleton of neighboring cells and maintains cell-cell adhesion. Repression of E-cadherin could therefore result in reduced cell adherence, a phenotype that has been observed in E-cadherin deficient mice; although no tumorigeneis was observed in these mice [Young et al., 2003]. However, previous work has shown that decreased expression of α-catenin and E-cadherin is associated with human oesophageal cancer [Kadowaki et al., 1994]. Thus, as estrogen and/or estrogen receptor levels decrease, NURD would lose its ability to control expression of E-cadherin. This in turn could contribute to reduced cell-cell adhesion and enhanced metastasis.

SUMMARY AND FUTURE DIRECTIONS

Although a great deal has been learned about the subunit composition of most chromatin remodeling complexes and the mechanisms by which they alter nucleosome structure, there is limited information on their ability to remodel methylated nucleosomes. The recent discovery of HMTs and their implication in both transcriptional activation as well as repression, and the findings which show that HMTs and MBDs can interact with ATP-dependent chromatin remodelers raise some very important questions. For example, MBD-containing complexes such as NURD and MeCP1 can bind and remodel methylated chromatin; however, it is not known if Swi2/Snf2 and ISWI-based complexes are able to remodel methylated chromatin. It is also not clear whether MDBs can mediate the interaction of Swi2/Snf2 and ISWI-based complexes with methylated chromatin. So far, it appears that there is a great abundance of HMTs, which can be classified either as activators or repressors of transcription depending on the residue(s) they methylate, that can target the N-terminal tail of histones H3 and H4. Except for yeast Dot1p and mammalian SETDB1 HMTs, which can efficiently methylate nucleosomal histone H3, it is not known whether any of the other HMTs can modify histones that are incorporated into chromatin, and whether ATP-dependent chromatin remodeling complexes are involved in facilitating histone methylation.

Significant progress has been made in understanding how chromatin remodelers regulate chromatin structure, but less work has been done on identifying their target genes. In light of the recent reports which link alterations in chromatin remodeling complexes with many forms of cancer, it is going to be important to find target genes regulated by these various remodelers and decipher how their misregulated expression induces tumorigenesis. For instance by directly silencing Snail, NURD is able to control expression of components of the intercellular junctional complex, suggesting that NURD activity might be involved in the control of metastasis and invasive growth. Whether NURD affects other components involved in the maintenance of adhesion between cells is still not clear, and the role played by other members of the Swi2/Snf2 superfamily in tumorigenesis remains obscure. Answering these questions will undoubtedly provide more insight into the mechanisms by which ATP-dependent chromatin remodeling complexes regulate cell growth and proliferation.

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