Functional Delineation of Three Groups of the ATP-dependent Family of Chromatin Remodeling Enzymes*

Received for publication, April 3, 2000, and in revised form, April 17, 2000
Published, JBC Papers in Press, April 21, 2000, DOI 10.1074/jbc.M002810200

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ATP-dependent chromatin remodeling enzymes antagonize the inhibitory effects of chromatin. We compare six different remodeling complexes: ySWI/SNF, yRSC, hSWI/SNF, xMi-2, dCHRAC, and dNURF. We find that each complex uses similar amounts of ATP to remodel nucleosomal arrays at nearly identical rates. We also perform assays with arrays reconstituted with hyperacetylated or trypsinized histones and isolated histone (H3/H4), tetramers. The results define three groups of the ATP-dependent family of remodeling enzymes. In addition we investigate the ability of an acidic activator to recruit remodeling complexes to nucleosomal arrays. We propose that ATP-dependent chromatin remodeling enzymes share a common reaction mechanism and that a key distinction between complexes is in their mode of regulation or recruitment.

The assembly of eukaryotic DNA into folded nucleosomal arrays is likely to have a major impact on the efficiency or regulation of nuclear processes that require access to the DNA sequence, including RNA transcription, DNA replication, recombination, and repair. In fact, it is now generally recognized that disruption or remodeling of chromatin structure is a rate-determining step for most of these nuclear DNA transactions (1–3). Two classes of highly conserved chromatin remodeling enzymes have been implicated as regulators of the repressive nature of chromatin structure, the first class includes enzymes that covalently modify the nucleosomal histones (e.g. acetylation, phosphorylation, methylation, ADP-ribosylation; reviewed in Ref. 4), and the second class is composed of multisubunit complexes that use the energy of ATP hydrolysis to disrupt histone-DNA interactions (reviewed in Refs. 5 and 6).

Each member of the ATP-dependent family of chromatin remodeling enzymes contains an ATPase subunit that is related to the SWI2/SNF2 subfamily of the DEAD/H superfamily of nucleic acid-stimulated ATPases (7). Seventeen members of the SWI2/SNF2 family have been identified in the yeast genome (6), and to date, four of these ATPases have been purified as subunits of distinct chromatin remodeling complexes ySWI/SNF (8, 9), yRSC (10), ISWI and ISW2 (11). Additional ATP-dependent remodeling complexes that harbor SWI2/SNF2 family members have been identified in Drosophila (dACTP (12), dNURF (13), dCHRAC (14), Brahma (15, 16), human (hSWI/SNF (17), hNURD (18–20), hRSF (21)), and frog (xMi-2 (22)). Although these complexes have a variable number of subunits (i.e. 3–15), and many different types of assays have been used to monitor the activity of individual complexes, each enzyme can apparently use the energy of ATP hydrolysis to alter chromatin structure and enhance the binding of proteins to nucleosomal DNA-binding sites (3, 5). Furthermore, in the case of the ySWI/SNF, Drosophila Brahma, and hSWI/SNF complexes, remodeling is required for transcriptional regulation of target genes in vivo (Refs. 23 and 24, for review, see Ref. 5).

ATP-dependent chromatin remodeling complexes have been further divided into three groups based on whether the sequence of the ATPase subunit is more related to yeast SWI2 (ySWI/SNF, yRSC, Brahma, and hSWI/SNF), Drosophila ISWI (ISW1, ISW2, dNURF, dCHRAC, dACTP, and hRSF), or human Mi-2 (hNURD, xMi-2) (reviewed in Ref. 3). Although each of these ATPases share a SWI2/SNF2-like ATPase domain, they harbor additional, unique sequence motifs adjacent to the ATPase domain that are characteristic of each group, the SWI2 group contains a bromodomain (25), the ISWI group contains a SANT domain (26), and the Mi-2 group contains a chromodomain (27). Differences among some groups are also apparent in the nucleic acid cofactor required for stimulation of ATPase activity. For enzymes that contain a SWI2-like ATPase (ySWI/SNF, yRSC, and hSWI/SNF), ATPase activity is stimulated equally well by “free” DNA or nucleosomes (8, 10, 28). In contrast, the ATPase activity of enzymes that contain an ISWI-like or Mi-2-like ATPase is optimally stimulated by nucleosomes (18–20, 22, 29). In the case of ISWI-like ATPases, this requirement for nucleosomes may reflect obligatory interactions with the trypsin-sensitive, histone N-terminal domains (30).

Here we report the first direct comparison of the biochemical properties of six different chromatin remodeling enzymes (ySWI/SNF, yRSC, dCHRAC, dNURF, hSWI/SNF, and xMi-2) which encompass all three previously suggested groups. Surprisingly, each complex shows similar ATPase activity on nucleosomal array substrates, and they are each able to facilitate

* This work was supported by National Institutes of Health Grants GM49650 (to C. L. P.) and GM56244 (to A. N. I.) and by a fellowship from the Human Frontiers Science Program Organization (to C. L.). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked ‘advertisement’ in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

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18864 This paper is available on line at http://www.jbc.org
nucleosome mobilization within an array at nearly equivalent rates. We have also investigated the nucleosome substrate requirements for each enzyme by using arrays reconstituted with hyperacetylated or trypsinized histone octamers, as well as histone (H3/H4)$_2$ tetramers. ATPase and remodeling assays with these different substrates identify new common features, as well as new distinctions among enzymes. In addition, we test the ability of the GAL4-VP16 chimeric transcriptional activator to recruit these remodeling complexes to a nucleosomal array substrate. We report that ySWI/SNF is uniquely potent for recruitment by GAL4-VP16 in this assay. Our data are consistent with the differential regulation of ATP-dependent enzymes that each share a similar mechanism of nucleosome remodeling.

**EXPERIMENTAL PROCEDURES**

**Reagent Preparation**—The array DNA template contains 11 tandem, head-to-tail repeats of a 208-base pair sea urchin 5 S rRNA gene (31, 32). Template was isolated by digestion of plasmid pL7C (208-11S) or pCL8b (208-11S-Gal4) with NotI, HindIII, and HhaI (New England Biolabs) followed by fast protein liquid chromatography purification on Sephacryl-500 (Amersham Pharmacia Biotech) essentially as described previously (31, 32). Array DNA template was end-labeled by Klonen fill-in reaction with [α-32P]ATP as described (31, 32).

Chicken erythrocyte histone octamers were purified from chicken whole blood (Pel-Freez Biologicals) as described previously (33). Hyperacetylated histone octamers were purified from butyrate-treated HeLa cells as described (34). Trypsinized histone octamers and (H3/H4)$_2$ tetramers were purified as described (33, 35). (H3/H4)$_2$ tetramers were dialyzed against Buffer T (1 M NaCl, 10 mM Tris-HCl pH 8.0, 0.25 mM EDTA, 0.1 mM dithiothreitol) prior to array reconstitution.

ySWI/SNF complex was purified from yeast strains CY396 or Sin3 (H3/H4)$_2$ tetramers were purified as described (33, 35). (H3/H4)$_2$ tetramers were dialyzed against Buffer T (1 mM NaCl, 10 mM Tris-HCl pH 8.0, 1 mM dithiothreitol) prior to array reconstitution.

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**RESULTS**

**ATPase and Remodeling Activities of Chromatin Remodeling Enzymes**—In order to quantify the nucleosome remodeling activity of ATP-dependent remodeling enzymes, we have developed a biochemical assay where nucleosome remodeling activity is coupled to restriction enzyme activity such that remodeling is revealed as an enhancement of restriction enzyme cleavage rates (31, 32). This assay uses a nucleosomal array substrate in which the central nucleosome of an 11-mer nucleosomal array contains a unique SalI/HincII site located at the predicted dyad axis of symmetry (31, 32). Restriction enzyme kinetics are biphasic in this system; the first phase is rapid and reflects the fraction of SalI/HincII restriction sites that are not occluded by a nucleosome (due primarily in our assays to nucleosomes that occupy minor translational positions; see Refs. 32, 42, and 43). The second phase is slow and reflects a dynamic equilibrium between the occluded and “open” nucleosomal DNA states (44, 45). In previous studies, addition of yeast SWI/SNF and ATP stimulated the second phase of SalI/HincII digestion 20–30-fold (32, 41). Recently we have found that SWI/SNF remodeling leads to a rapid redistribution of nucleosome positions within these arrays and that the apparent rate of remodeling determined in this assay provides an estimate of the rate of nucleosome mobilization (46).

Purified preparations of ySWI/SNF, yRSC, hSWI/SNF, dCHRAC, dNURF, and xMi-2 were analyzed in parallel for nucleosome-stimulated ATPase activity (see “Experimental Procedures”). Each complex was titrated in an ATPase reaction which contained 100 μM ATP and 12 nM of a reconstituted, 11-mer nucleosomal array. Surprisingly, the approximate concentration of each remodeling complex that was required to achieve equal velocities of ATP hydrolysis was similar for ySWI/SNF (2 nM), yRSC (2 nM), hSWI/SNF (5 nM), dCHRAC (2 nM), and dNURF (4 nM), each complex catalyzed the hydrolysis of 450–600 nmol of ATP/min (Fig. 1A, see also Ref. 41). xMi-2 was slightly less active in this assay as ~15 nM was required to achieve this level of ATPase activity (Fig. 1A). Given that our estimates of active enzyme concentrations are only approximate (see “Experimental Procedures”), the data shown in Fig. 1A indicate that each of these enzymes have nucleosome-stimulated ATPase activities that are similar within an order of magnitude. The similar levels of ATPase activity among complexes was unexpected given that each complex has different associated subunits, and, at least in the case of the hSWI/SNF complex, can have a large impact on the ATPase activity of the catalytic subunit (i.e. BRG1; Ref. 28).

To assess the capacity of the six different complexes to remodel an 11-mer nucleosomal array, each remodeling enzyme (1–5 nM ySWI/SNF, yRSC, hSWI/SNF, dCHRAC, dNURF, or 15 nM xMi-2) was added to 1.5 nM nucleosomal array and the
Fig. 1. Comparison of ATPase and remodeling activities of ATP-dependent chromatin remodeling complexes. A, ATPase assays. The indicated remodeling complexes (1–5 nM ySWI/SNF, hSWI/SNF, dNURF, dCHRAC, or 15 nM xMi-2) were analyzed in ATPase reactions that contained (closed symbols) or lacked (open symbols) 12 nM nucleosomal array, and ATP hydrolysis was monitored with time. Velocities of ATP hydrolysis were calculated from at least three reaction time points. B, nucleosomal array remodeling assays. HincII digestion of nucleosomal arrays incubated in the presence (closed symbols) or absence (open symbols) of the indicated remodeling complexes. HincII digestion rates were calculated from the slopes of plots of the natural logarithm of the fraction of uncut array versus time. These results are representative of multiple, independent experiments. Similar results were also obtained with at least two independent enzyme preparations for each complex except yRSC.

initial rates of HincII digestion were measured in parallel reaction time courses in the presence of ATP. We found that all six complexes enhanced the rate of HincII digestion essentially equivalently (Fig. 1B, see also Ref. 41 for a detailed comparison of ySWI/SNF and yRSC). The dCHRAC complex reproducibly yielded an approximately 2-fold lower rate of HincII digestion than all other complexes which probably reflects the fact that a significant amount of the ATPase activity of dCHRAC appears to be contributed by topoisomerase II (see below). Since the initial rate of HincII digestion provides an indirect measurement of the rate of remodeling, these data indicate that all six enzymes use similar amounts of ATP to remodel nucleosomal arrays at similar rates. Furthermore, since it appears that this coupled restriction enzyme-remodeling assay monitors the rate of nucleosome mobilization (46), all six enzymes can apparently redistribute nucleosomes within an array at comparable rates.

A hallmark of our nucleosomal array assay is that the SWI/SNF-dependent enhancement of restriction enzyme accessibility requires continuous ATP hydrolysis (32, 46). This requirement reflects a state of constant redistribution of nucleosome positions in the presence of ATP, and the subsequent inactivation of SWI/SNF “freezes” a random positioning of nucleosomes which is characterized by a general occlusion of restriction enzyme sites (46). We carried out similar remodeling/reversal assays with hSWI/SNF, dCHRAC, dNURF, or xMi-2 and in all cases the enhanced rates of HincII digestion were lost after ATP was enzymatically removed with apyrase (data not shown; for analysis of yRSC, see Ref. 41). Thus, these results indicate that all six complexes use the energy of ATP hydrolysis to create a dynamic, reversible state of nucleosome mobilization. Our results are consistent with previous demonstrations of mononucleosome mobilization catalyzed by ySWI/SNF (46, 49), dCHRAC (47) or dNURF (48).

Nucleosome Moiety Requirements of the Chromatin Remodeling Complexes—Previous studies have demonstrated that optimal ATPase activity of dNURF (29), dCHRAC (14), and xMi-2/NURD (18–20, 22) complexes requires nucleosomes, whereas the ATPase activities of hSWI/SNF (17), ySWI/SNF (8), and yRSC (10, 41) complexes are stimulated equally well by free DNA. Furthermore, in the case of the dNURF complex, the nucleosome stimulation of ATPase activity requires one or more trypsin-sensitive histone N-terminal domain(s) (30). To further define the nucleosome moiety requirements for all six complexes, we reconstituted nucleosomal arrays with hyperacetylated or trypsinized histone octamers, as well as with histone (H3/H4)2 tetramers. To ensure that each type of array reconstitution was of similar quality, all reconstitutions were analyzed for extent of DNA repeat saturation and correct positioning with multiple restriction enzyme mapping and native polyacrylamide gel electrophoresis (see “Experimental Procedures”). We then measured the ability of these arrays to stimulate the ATPase activity of each complex (Fig. 2A). As expected, ySWI/SNF and yRSC complex hydrolyzed ATP with similar kinetics on all substrates, including free DNA (Fig. 2A; see also, Ref. 41). Likewise, the ATPase activity of the hSWI/SNF complex was stimulated by all substrates, with the exception that activity was consistently 40–50% less in the presence of arrays reconstituted with histone (H3/H4)2 tetramers (Fig. 2A).

In agreement with previous studies, we also found that the ATPase activity of the dNURF complex was maximally stimulated only by nucleosomal arrays (for analysis of ATPase activity with DNA or hyperacetylated substrates, see Ref. 30); little ATPase activity was detected with arrays reconstituted with trypsinized histone octamers or histone (H3/H4)2 tetramers. Given that the ATPase activity of dNURF requires one or more histone N-terminal domain(s) (Fig. 2A; see also, Ref. 30), the lack of ATPase activity in the presence of the histone (H3/H4)2 tetramer arrays suggested that the N-terminal domains of the histone H2A/H2B dimers might play a key role. However, nucleosomal arrays reconstituted with hybrid histone octamers composed of intact histone (H3/H4)2 tetramers and tail-less histone H2A/H2B dimers yielded maximal stimulation of dNURF ATPase activity.2 Thus, the inability of (H3/H4)2 tetramer arrays to stimulate the ATPase activity of dNURF does

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2. P. Horn and C. L. Peterson, unpublished observation.
not reflect a key role for the N-terminal domains of histones H2A/H2B dimers.

The dCHRAC complex, like dNURF, contains ISWI, which is a nucleosome-stimulated ATPase. In addition, dCHRAC also contains topoisomerase II which is a DNA-stimulated ATPase (14). Thus, the ATPase activity associated with dCHRAC is a composite of ISWI and topoisomerase II which complicates the analysis of the substrate preferences of this complex (Fig. 2A). The ATPase activity of dCHRAC was stimulated by all substrates, although ATPase activity is reproducibly higher in the presence of nucleosomal or hyperacetylated arrays. Since the ATPase activity of dNURF is only stimulated by a nucleosomal or hyperacetylated substrate (Fig. 2A, see also Ref. 30), our data suggest that only 30–40% of the overall ATPase activity of CHRAC is due to the ISWI subunit, and the remaining DNA-stimulated ATPase activity is due to topoisomerase II.

The ATPase activity of the xMi-2 complex was distinct from both the SWI/SNF (ySWI/SNF, yRSC, hSWI/SNF) and ISWI groups (dNURF, dCHRAC) of ATPases (Fig. 2A). Like the ISWI group, the ATPase activity of xMi-2 was maximally stimulated by nucleosomal arrays, although free DNA did stimulate a significant amount of ATPase activity (27% of the nucleosomal level). In contrast to the ISWI group, arrays reconstituted with trypsinized histones were still able to stimulate the ATPase activity of xMi-2 to nearly 70% the level of intact nucleosomal arrays. Likewise, arrays reconstituted with hyperacetylated histones or histone (H3-H4)2 tetramers were more similar to the nucleosomal arrays. Thus the observed preference for nucleosomal arrays does not reflect an obligatory interaction with the histone N-terminal domains. Thus, based on a preference for a nucleosomal substrate and a lack of histone tail dependence, xMi-2 appears to define a third group of the ATP-dependent chromatin remodeling family.

We also performed coupled restriction enzyme-remodeling assays for most of the different array substrates and each remodeling complex. As shown in Fig. 2B, remodeling of the different substrate arrays paralleled the ATPase activity of the complexes except in three cases. First, although arrays reconstituted with histone (H3-H4)2 tetramers were able to stimulate the ATPase activity of ySWI/SNF and hSWI/SNF, the

Fig. 2. Nucleosome moiety requirements for ATP-dependent chromatin remodeling enzymes. A, ATPase assays. The indicated remodeling enzymes were added to ATPase assays that contained either 208-11S DNA template (DNA) or 208-11S arrays reconstituted with histone octamers, hyperacetylated histone octamers, trypsinized histone octamers, or isolated (H3-H4)2 tetramers. Each reaction represented an ATPase time course, and ATP hydrolysis velocities were calculated for each substrate. Data is presented as a percentage of the ATPase velocity exhibited with the nucleosomal array substrate. Data shown for yRSC is the result of a single experiment which essentially repeated our prior study (41). *, denotes that ATPase assays with DNA and hyperacetylated array substrates were not performed with dNURF (see Ref. 30 for detailed analysis). B, nucleosomal array remodeling assays. HincII digestion rates were determined for each enzyme on each array substrate. For each substrate, rates were calculated from the slopes of plots of the natural logarithm of the fraction of uncut array versus time. Data is presented as a percentage of the remodeler-dependent HincII digestion rate of the nucleosomal array. With the exception of yRSC, the results shown include experiments with at least two independent enzyme preparations for each complex. *, denotes that remodeling of tetramer arrays was not performed with yRSC. In addition, remodeling data shown for yRSC with hyperacetylated and trypsinized nucleosomal arrays is from Logie et al. (41) and is shown for comparison purposes only.
apparent rate of remodeling of these tetramer arrays was reduced 10–50-fold compared with remodeling of nucleosomal arrays (Fig. 2B; see also Ref. 39 for an extensive discussion). Second, although dCHRAC showed high levels of ATPase activity with all substrates, it was not able to remodel arrays reconstituted with either trypsinized histones or with the histone (H3-H4)2 tetramers (Fig. 2B). These results suggest that the ATPase activity that is presumably contributed by the topoisomerase II subunit of dCHRAC is not sufficient to enhance restriction enzyme accessibility in these array assays. Furthermore, the results from this remodeling analysis indicate that dCHRAC and dNURF, which each contain the ISWI ATPase, have indistinguishable histone moieties. And finally, although the ATPase activity of xMi-2 was stimulated well by arrays reconstituted with (H3-H4)2 tetramers (Fig. 2A), these arrays were only poorly remodeled by xMi-2 (Fig. 2B).

Targeting of the Chromatin Remodeling Complexes by Transcriptional Activators—Recently we have shown that the remodeling activity of ySWI/SNF can be targeted to reconstituted nucleosomal arrays by GAL4 derivatives that contain an acidic transcriptional activation domain (50). For these targeting assays we used a modified array DNA template which contains five high affinity GAL4-binding sites adjacent to the 5′ S repeat that harbors the HincII/Sall site (208-11S-GAL4; see Ref. 50). Reconstitution of nucleosomal arrays with this DNA template positions the GAL4-binding sites in the linker region between two positioned nucleosomes (50). Targeting of remodeling activity is then assayed in HincII reactions which contain a 32P-labeled 208-11S-GAL4 array and 15-fold molar excess of an unlabeled 208-11S array (which lacks GAL4 sites). In the absence of targeting, the remodeling enzyme is sequestered by the excess unlabeled, competitor array and there is little stimulation of HincII digestion kinetics. Targeting of remodeling activity is scored by any stimulation of HincII digestion kinetics due to a functional GAL4 derivative (50). Note in this assay that a GAL4 derivative does not affect HincII digestion kinetics in the absence of remodeling complex or when the labeled and unlabeled arrays lack GAL4-binding sites. Previously, using this assay we were able to detect targeting of ySWI/SNF remodeling activity by GAL4-VP16 and GAL4-AH acidic activators (50).

We wished to investigate whether other members of the ATP-dependent family of remodeling enzymes could also be recruited by acidic activators in our reconstituted nucleosomal array system. Each of the six remodeling complexes were added in parallel to HincII targeting assays which contained 0.2 nM 32P-labeled 208-11S-GAL4 nucleosomal array, 3 nM unlabeled 208-11S nucleosomal array, and 10 nM of a GAL4 derivative (Fig. 3). Under these reaction conditions, little remodeling of the labeled 208-11S-GAL4 array was observed in the absence of activator-dependent targeting. Likewise, addition of the isolated GAL4 DNA-binding domain did not enhance HincII digestion kinetics in the presence or absence of remodeling enzyme (data not shown). Furthermore, similar to our previous studies (50), the remodeling activity of ySWI/SNF was effectively targeted to the 208-11S-GAL4 array by both the GAL4-VP16 and GAL4-AH acidic activators, as visualized by an activator- and ySWI/SNF-dependent stimulation of HincII digestion kinetics (Fig. 3 and data not shown; see also Table I). In contrast, the remodeling activity of yRSC, hSWI/SNF, dNURF, dCHRAC, or xMi-2 was not significantly targeted by either GAL4-VP16 or GAL4-AH activators (Fig. 3A and data not shown; see also Table I). In fact, we reproducibly observed some activator-dependent inhibition of remodeling by dCHRAC and yRSC (Fig. 3). Similar results were obtained in several independent experiments and with a range of remodeler concentrations. In the case of hSWI/SNF we also failed to observe targeting in this assay using an immunoaffinity purified form of this enzyme (51).3 Thus, for the six purified remodeling complexes tested here, only the remodeling activity of ySWI/SNF is effectively targeted by prototype acidic activators.

**DISCUSSION**

Our results indicate that six different members of the ATP-dependent family of chromatin remodeling enzymes use similar levels of ATP hydrolysis to rapidly establish a dynamic state of enhanced nucleosome mobilization. This “fluid” chromatin state is characterized by an enhanced accessibility of restriction enzymes and DNA binding transcription factors. Furthermore, the nearly identical rates of nucleosomal array remodeling (Fig. 1B) and the common requirement for histone H2A/H2B dimers (Fig. 2) are consistent with a similar remodeling mechanism for all members of this ATP-dependent family. Although the mechanistic details of “remodeling” are not clear, all of these enzymes can apparently transduce the energy of ATP hydrolysis into an enhanced mobilization of nucleosomes

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3 P. Horn, R. E. Kingston, and C. L. Peterson, unpublished observations.
within linear arrays (as suggested in Ref. 52).

In contrast to our studies with nucleosomal arrays, some differences between remodeling complexes have been observed with mononucleosome substrates. For example, ySWI/SNF, NURF, and recombinant ISWI have been shown to move a histone octamer from a central position to an end position (46–48), whereas dCHRAC and ySWI/SNF can also move histone octamers in the opposite direction (47, 49). dCHRAC, however, also contains the ISWI ATPase, and thus these differences are not intrinsic to the catalytic subunit or to the basic mechanism of remodeling. Alternatively, the differences in the direction of histone octamer movement may reflect the propensity of some complexes (such as dCHRAC) to bind to DNA ends. In this scenario, protection of the DNA ends may block end-directed movements and favor movements from the ends to more central locations. In contrast, on nucleosomal arrays, where free DNA ends do not flank individual nucleosomes, we propose that the direction of histone octamer movement is random for all remodeling complexes. This situation is consistent with our observation that the rates of nucleosome remodeling are similar for all complexes.

We were surprised to discover that arrays reconstituted with histone (H3-H4), tetramers are not efficiently remodeling by any of the complexes tested. In the absence of remodeling enzyme, arrays of (H3-H4) tetramers are digested at rates only 3–5-fold faster than nucleosomal arrays (data not shown; see also Ref. 39), whereas ATP-dependent remodeling of nucleosomal arrays typically yields 20–30-fold increases in restriction enzyme rates. Thus, arrays of (H3-H4) tetramers still provide a potent barrier to factor access and, furthermore, the inability to score remodeling of tetramer arrays is not due to a high level of restriction enzyme cleavage in the absence of remodeling enzyme. Interestingly this requirement for the histone H2A-H2B dimers also does not reflect an obligatory need for the N-terminal domains of these two histones, since xMi-2, ySWI/SNF, and hSWI/SNF are insensitive to removal of all the N-terminal domains (Fig. 2A) (41, 53). Instead, we favor a model in which all of these enzymes require a canonical nucleosome structure either for substrate recognition or for the mechanism of remodeling. For instance, these enzymes may need to interact with two adjacent gyres of DNA in order to induce nucleosome mobilization (see also, Ref. 39).

Our data also suggest the delineation of three groups within the ATP-dependent family: 1) a SWI/SNF group (ySWI/SNF, yRSC, and hSWI/SNF) whose ATPase activity does not require an intact nucleosome and whose remodeling function is independent of the histone tails; 2) an ISWI group (dCHRAC, dNURF) whose ATPase activity requires an intact nucleosome and whose remodeling function is histone tail dependent; and 3) a Mi-2 group (xMi-2) whose optimal ATPase activity requires an intact nucleosome and whose remodeling function is histone tail independent. In fact, we found that the remodeling activity of xMi-2 was actually enhanced by removal of the histone N-terminal domains (Fig. 2B). Similar results have been obtained previously using a subset of the complexes tested here as well as recombinant BRG1 (ATPase subunit of hSWI/SNF) and ISWI (8, 14, 17, 28, 30, 41, 53). We note, however, that recombinant ISWI also shows significant stimulation of ATPase activity by free DNA (54). Furthermore, like dNURF and dCHRAC, the histone N-terminal domains promote efficient remodeling by ySWI/SNF and yRSC complexes under different reaction conditions where these enzymes must be catalytic (41). Thus, although the different nucleosome moiety requirements are important for defining distinctions among enzymes, these distinctions are likely to reflect subtle differences in nucleosome recognition or in regulation of the remodeling cycle (41, 56), rather than key differences in the basic remodeling mechanism.

Molecular phylogenetic analysis has been used to organize the SWI2/SNF2 family of DNA-stimulated ATPases into multiple subfamilies (7). These studies included sequence comparisons among different SWI2/SNF2 ATPase domains as well as among sequences N-terminal or C-terminal to the ATPase domain. Interestingly, ATPases from three of the subfamilies defined by phylogenetic analysis are the catalytic subunits associated with the three groups of ATP-dependent remodeling enzymes delineated by our biochemical analyses (e.g. SWI2, ISWI, Mi-2). This correspondence between such completely different experimental approaches was not expected, since the homology among the ATPase domains of SWI2, ISWI, and Mi-2/CHD proteins is very high (7). One possibility is that a small number of amino acid changes can lead to large differences in nucleic acid substrate requirements (i.e. nucleosomes versus free DNA). Consistent with this view, previous studies have found that ATPase domain swaps between two members of the same subfamily (i.e. brahma and SWI2/SNF2) yield a SWI2 protein that retains function in vivo in yeast, whereas swaps between members of different families (i.e. ISWI and SWI2/SNF2) are not functional (15). Alternatively, sequence elements that are unique to each subfamily that lie outside of the ATPase domain (i.e. bromodomains, SANT domains, chromodomains) might also contribute to interactions with the histone N-terminal domains or other nucleosomal components.

Although our comparative analysis delineates three groups of ATP-dependent remodeling enzymes, our data also suggests that individual enzymes within a single group are likely to be subject to differential modes of regulation. For instance, we found that ySWI/SNF was recruited by an acidic activator in our nucleosomal array system, whereas other members of the SWI/SNF group (e.g. yRSC, hSWI/SNF) were not. We anticipate that yRSC and hSWI/SNF can be recruited by other types of activators in this assay. Likewise, members of the ISWI or Mi-2 groups are likely to be recruited by nonacidic activators or by transcriptional repressors. These ideas are consistent with several previous studies. First, acidic activators are unable to recruit yRSC complex to an immobilized DNA template from a yeast nuclear transcription extract (50). Second, xMi-2 complexes are believed to function in transcriptional repression (36, 57) and thus it is not surprising that an acidic activator is unable to recruit xMi-2. And finally, hSWI/SNF has recently been demonstrated to be targeted in vivo by the glucocorticoid receptor (58), an isoform of C/EBP-β (59) and erythroid kruppel-like factor (60).

Clearly, acidic activators are likely to recruit ATP-dependent remodeling complexes in Drosophila and mammalian cells. One possibility is that there exists additional, uncharacterized members of the ATP-dependent remodeling family that can be recruited by acidic activators and which might play a key role in acidic activator function. It is also possible that regulatory subunits, which might facilitate interactions with acidic activators, have been lost during purification of one or more of the remodeling complexes that we have tested here. Alternatively, several of the more abundant complexes (e.g. dCHRAC and yRSC) may establish more global domains of fluid chromatin, and thus they may not rely on gene-specific targeting proteins.

Acknowledgments—We thank members of the Peterson laboratory for helpful discussions throughout the course of this work. We are especially grateful to Bradley Cairns (University of Utah) for the generous gift of yRSC complex.

REFERENCES

1. Cairns, B. R. (1998) Trends Biochem. Sci. 23, 20–25
2. Burns, L. G., and Peterson, C. L. (1997) Biochem. Biophys. Acta 1350, 159–168
3. Muchardt, C., and Yaniv, M. (1999) J. Mol. Biol. 293, 187–198
