Residues required for ATPase coupling in the Chd1 remodeler
Identification of Residues in Chromo-Helicase-DNA-Binding Protein 1 (Chd1) Required for Coupling ATP Hydrolysis to Nucleosome Sliding

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Background: Nucleosome sliding by chromatin remodelers requires ATP hydrolysis.

Results: Residues between the ATPase motor and DNA-binding domain of Chd1 are required for sliding but not nucleosome-stimulated ATP hydrolysis.

Conclusion: Residues outside the conserved ATPase core are required for efficiently utilizing the energy of ATP hydrolysis for sliding.

Significance: Understanding the role of ATPase-coupling residues will be critical for revealing nucleosome sliding mechanisms.

SUMMARY
Chromatin remodelers are ATP-dependent machines responsible for directionally shifting nucleosomes along DNA. We are interested in defining which elements of the Chd1 remodeler are necessary and sufficient for sliding nucleosomes. This work focuses on the polypeptide segment that joins the ATPase motor to the C-terminal DNA-binding domain. We identify amino acid positions outside the ATPase motor that, when altered, dramatically reduce nucleosome sliding ability and yet have only ~3-fold reduction in ATPase stimulation by nucleosomes. These residues therefore appear to play a role in functionally coupling ATP hydrolysis to nucleosome sliding, and suggest that the ATPase motor requires cooperation with external elements to slide DNA past the histone core.

In eukaryotes, genomic DNA is extensively packaged into chromatin, the basic unit of which is the nucleosome. By the nature of the DNA wrapping around the histone core, the nucleosome restricts access to genomic DNA and therefore must be reorganized for many basic cellular processes including DNA replication, recombination, repair, and gene transcription. Chromatin remodelers play a key role in altering chromatin accessibility, and are essential factors for nucleosome eviction, histone variant exchange, and nucleosome sliding (reviewed in (1-5)).

Chromatin remodelers encompass an increasingly diverse set of proteins and assemblies and have been classified into several families, the best characterized of which include SWI/SNF, ISWI, INO80, and CHD. Each family possesses a characteristic collection of auxiliary domains and/or subunits, yet all remodelers share a common SWI2/SNF2-type ATPase motor (6, 7). The ATPase motor has been shown to engage with nucleosomal DNA approximately two helical turns from the nucleosome dyad (superhelical location 2, SHL2), and ATP hydrolysis is essential for driving the nucleosome sliding reaction (8-10). Interestingly, the SWI2/SNF2-type ATPase motor is also found in proteins that do not slide nucleosomes but disrupt other protein-DNA complexes, such as bacterial RapA and eukaryotic Rad54 (11, 12). The conservation of the SWI2/SNF2-type ATPase motor outside the chromatin remodeler subfamilies suggests that remodelers possess additional elements that direct action of the ATPase motor to nucleosomes. While non-ATPase domains are believed to target chromatin remodelers to nucleosomes (13), it is
not known whether or how regions outside the ATPase motor might be essential for nucleosome sliding.

To define the minimal elements required for nucleosome sliding, we have focused on yeast Chd1 chromatin remodeler as a model system. Chd1 was named for three conserved motifs: a pair of N-terminal Chromodomains, a central Helicase-like ATPase motor, and a C-terminal DNA-binding domain (14). Like ISWI-type remodelers, Chd1 can assemble nucleosomes, generate evenly spaced nucleosomal arrays, and move mononucleosomes to the center of short DNA fragments (15, 16). For yeast Chd1, we previously showed that although deletion of the chromodomains or DNA-binding domain reduced nucleosome sliding ability, neither the chromodomains nor DNA-binding domain were essential for nucleosome sliding (17). In the course of our studies, we discovered that the protein segment between the ATPase motor and DNA-binding domain greatly influenced the efficiency of nucleosome sliding. Here we describe the biochemical characterization of a set of yeast Chd1 variant proteins that identify residues between the DNA-binding domain and ATPase motor critical for coupling nucleosome-stimulated ATP hydrolysis to nucleosome sliding.

**EXPERIMENTAL PROCEDURES**

**Mutagenesis and Protein purification**- Truncated constructs of *S. cerevisiae* Chd1 were topo-cloned into pDEST17 vectors following the manufacturer’s instructions (Invitrogen). N-terminal cloning primers included a Prescision Protease cleavage sequence (LEVLFQ/GP) immediately prior to residue 118 (*S. cerevisiae* numbering) to allow removal of the His tag and pDEST17-associated sequences. Variant Chd1 constructs containing internal deletions and amino acid substitutions were generated using the Quik-Change method (Strategene). The residues altered are denoted in Fig. 2.

Using the autoinduction method (18), Chd1 proteins were expressed in *Escherichia coli* BL21-star (DE3) cells (Invitrogen) containing two additional plasmids: a Trigger Factor chaperone overexpression plasmid (Li Ma and Guy Montelione, Rutgers University) and the RIL plasmid for rare tRNAs (Strategene). Two L of autoinduction media were inoculated with 20 ml of overnight culture, grown for 2 hours at 37°C, cooled on ice, and further cultured at 18°C for 18 hours. Cells were harvested by centrifugation and resuspended in buffer A (50 mM Tris pH 7.5, 500 mM NaCl, 10% glycerol, 10 mM imidazole) containing 1 mM PMSF, 1 mM benzamidine-HCl, and 1 mM DTT.

Cells were lysed by adding lysozyme having final concentration 1 mg/ml and disrupted by sonication on ice, and the lysate was clarified by centrifugation at 45000 × g for 20 min. The clarified lysate was applied onto a HisTrap column (GE Healthcare) pre-equilibrated with buffer A. The column was washed with 200 ml of buffer A, and Chd1 protein eluted with buffer A supplemented with 250 mM imidazole. Fractions containing Chd1 proteins were pooled and diluted 5-fold with buffer B (50 mM Tris pH 7.5 and 10% glycerol) and applied onto ion-exchange columns pre-equilibrated with buffer C (50 mM Tris pH 7.5, 100 mM NaCl, 10% glycerol). Chd1 118-939 was applied onto a Q-FF HiTrap column, whereas all other Chd1 proteins were applied to SP-FF HiTrap columns (GE Healthcare). After washing with 200 ml of buffer C, Chd1 proteins were eluted with a linear gradient to 50 % buffer D (50 mM Tris pH 7.5, 1 M NaCl, 10% glycerol). Fractions containing CHD1 proteins were pooled and incubated overnight with recombinant Precision protease at 4°C for removal of the N-terminal tag. Cleaved protein was then re-passed over a HisTrap affinity column pre-equilibrated with buffer A, collected in the flow-through, concentrated, and further purified on a Superdex S200 16/60 column (GE Healthcare). Size exclusion chromatography was carried out in buffer E (50 mM Tris pH 7.5, 300 mM NaCl, 10% glycerol and 1 mM DTT), at a flow rate of 1 ml/min. The peak fractions were analysed on SDS-PAGE, concentrated, and maintained at -80 °C until needed. Protein concentrations were determined by quantification of dilution series on an SDS-polyacrylamide gel and comparison against known standards.

**Nucleosome assembly**- Expression and purification of histone proteins were carried out as previous described (19). The assembly of histone octamers and nucleosomes were performed as described (ref. 19 and web protocol from the Tsukiyama Laboratory, [http://labs.fhcrc.org/tsukiyama/protocols.html](http://labs.fhcrc.org/tsukiyama/protocols.html)). The DNA used for nucleosomes was derived from
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the pGEM-601 plasmid. Fluorescently labeled DNA was generated by polymerase chain reaction amplification using a pair of primers with the following sequences: 5’-Cy5- CA GGA TGT ATA TAT CTG ACA CGT GCC TGG and 5’- GGA AAG CAT GAT TCT TCA CAC CGA GTT C (IDT). These primers amplified a 208 bp fragment with the 601 positioning sequence (20) at one end. Unlabeled DNA was used for ATPase assays, and was generated by digesting a 34-mer array of 601 sequences.

Nucleosome sliding - Nucleosome sliding was performed as described previously (17, 21, 22), with some variations. The buffer used for nucleosome sliding contained the following: 20 mM HEPES buffer pH 7.6, 50 mM KCl, 5 mM MgCl2, 5% sucrose, 0.1mg/ml BSA, and 1 mM DTT. Protein and nucleosome reagents were diluted with the above sliding buffer and reactions were initiated by addition of 2.5 mM ATP in the same buffer. All protein and nucleosome reagents were kept on ice, and then incubated at room temperature for 10 minutes prior to addition of ATP. Sliding reactions were quenched at desired time points by immediately placing on ice after addition of stop DNA (up to 25 µg of plasmid pJ201 containing a 34-mer array of 601 DNA sequences) and 25 mM EDTA in 20 mM HEPES buffer pH 7.5 containing 50 mM KCl, 0.1 mg/ml BSA, 5% sucrose, and 1 mM DTT. Reactions were then loaded onto 7% native polyacrylamide gels (60:1 acrylamide:bis) and run at 150 V for 4 hr at 4˚C. Gels were visualized using a Typhoon 9410 variable mode imager (GE Healthcare) and quantified with ImageJ.

Nucleosome binding - Increasing concentrations of Chd1 proteins were mixed with 100 nM Cy5 labeled (0-N-63) nucleosomes in buffer containing 20 mM HEPES (pH 7.6), 50 mM KCl, 5 mM MgCl2, 1 mM EDTA, 1 mM DTT, 0.1mg/ml BSA and 5% sucrose in a final volume of 10 µl. Reactions were then loaded onto 7% native polyacrylamide gels (60:1 acrylamide:bis) and run at 150 V for 4 hr at 4˚C. Gels were visualized using a Typhoon 9410 variable mode imager (GE Healthcare) and quantified with ImageJ.

Circular Dichroism measurements- Circular dichroism (CD) secondary structure measurements were collected on an Aviv Associates (Biomedical Lakewood, NJ) model 400 Circular Dichroism Spectropolarimeter device. Prior to CD measurements, protein samples were dialyzed overnight against 10 mM Tris pH 7.5 and 100 mM NaCl. Far-UV CD spectra were acquired in wavelength range 195-260 nm at 20˚C for 0.5

y = m1 + m2*(1 - exp(-m3*x)) + m4*(1 - exp(-m5*x)).

For each of these variants, the two rates differed by ~10-fold, and the faster rate was similar to that of Chd1ANC, but had an amplitude 3- to 5-fold lower than the slower rate. The appearance of a dominant, slower rate suggests that these amino acid changes altered a rate-limiting step in the sliding reaction, however with the experiments described here, we cannot say what stage of the cycle is altered. Sliding rates for the other Chd1 variants, which were both slower and failed to slide nucleosomes to the same extent under these conditions, were obtained by linearly fitting the fraction of nucleosomes shifted over the first 5 minutes (20 minutes for Chd1ANC∆932-940 and Chd1ANCV1). To report the observed initial sliding rate for all remodelers, the equations for the exponential fits were differentiated at time=0. For the single exponential fits, this yields the product of the rate times the total amount of nucleosomes shifted. For the double exponential fits, the initial rate is the sum of each rate times a fraction of the total amount of nucleosome shifted. Sliding experiments were performed three or more times, and the fits to individual reactions gave R values of ≥0.98. All nucleosome sliding gels shown are representative of three or more experiments.

Nucleosome binding- Several variants (Chd1ANC∆961-974, Chd1ANC∆977-994, Chd1ANC∆995-1005, Chd1ANCV3, and Chd1ANCV6) were also too fast to directly obtain initial sliding rates, and were fit to a double exponential equation,
mg/ml protein (Chd1_{ANC} or Chd1_{ANC}^{932-940}) in 400 µl buffer containing 10 mM Tris-HCl pH 7.5 and 100 mM NaCl, using a 1 mm path-length cuvette.

ATPase assays— ATPase rates were monitored using an NADH-coupled system as previously described (23). All reagents were kept on ice and preincubated at room temperature for 10 min before initiation of the reactions with the addition of 2.5 mM ATP and 5.0 mM MgCl₂. The concentration of Chd1 proteins was 50 nM, with the indicated amount of (0-N-63) nucleosome substrates. The DNA substrate was a 208 bp duplex equivalent to that used for nucleosome reconstitution. The reported DNA- and nucleosome-stimulated ATPase rates were obtained after subtraction of basal rates in the absence of substrates. Substrate-stimulated and basal ATPase rates are reported in Table I.

RESULTS

A segment between the ATPase motor and DNA-binding domain of Chd1 contributes to nucleosome sliding independently of the DNA-binding domain. We previously showed that the truncated yeast Chd1 protein used for crystal structure determination (residues 142-939) could slide nucleosomes, indicating that the DNA-binding domain was not essential for nucleosome sliding (17). Nucleosome sliding activity of this truncated Chd1 remodeler, however, was extremely poor and required comparatively high (micromolar) concentrations, likely due to inefficient localization accompanying loss of the DNA-binding domain. In the course of optimizing construct borders for crystallization, we found that inclusion of residues 940-978, a region distinct from the ATPase motor, noticeably improved nucleosome sliding activity.

In yeast Chd1, structural information is now available for the chromodomain-ATPase motor (17) and the DNA-binding domain (24). Approximately 87 amino acid residues separate the ATPase motor (which includes a ~50 residue C-terminal bridge segment that folds against the ATPase core and ends around residue 922) from the DNA-binding domain (residues 1009-1274). The C-terminal residues of the crystallization construct (residues 923-939) were disordered in the crystal structure and encompass part of a strictly conserved tryptophan at position 932. Following residue 955 of the 87 residue linker, sequence conservation drops off until the beginning of the DNA-binding domain. To investigate the extent that residues between the ATPase motor/C-terminal bridge and DNA-binding domain may contribute to nucleosome sliding, four truncated Chd1 proteins were studied (Fig. 1A). All protein constructs lacked the first 117 residues, which are not conserved among Chd1 orthologs and not required for nucleosome sliding or centering (17).

To investigate nucleosome sliding activities, end-positioned mononucleosomes were generated using the 601 positioning sequence (20) flanked by 63 additional basepairs on one side (referred to as 0-N-63). The ATP-dependent mobilization of these end-positioned nucleosomes was monitored by native PAGE, where slowed migration indicates a shift of nucleosomes towards the center of a DNA fragment (21). In accordance with previously reported nucleosome-centering activity of Chd1 (16), a yeast Chd1 protein that possessed all three conserved domains (spanning residues 118-1274), was able to efficiently shift the majority of end-positioned nucleosomes to more centrally located positions on the DNA fragment (Fig. 1B, lanes 3-7). Chd1 produced two major up-shifted nucleosome bands. This behavior is similar to movement of 601-containing nucleosomes by ISWI-type remodelers such as NURF, which was shown with high resolution mapping to correspond to two centrally located positions (8). To simplify the nomenclature of proteins containing internal deletions described later, the Chd1 protein construct spanning residues 118 to 1274 will be hereafter referred to as Chd1_{ANC}, which signifies that it possesses deletions at both the N- and C-term)
lacked the C-terminal DNA-binding domain (Chd1_{118-978} and Chd1_{118-1014}) displayed intermediate activities. These longer chromodomain-ATPase proteins were significantly more active in sliding than Chd1_{118-939}, requiring ~100-fold less protein to mobilize a similar fraction of nucleosomes (compare lanes 13, 21, and 31). This marked improvement in sliding activity suggested that, independently of the C-terminal DNA-binding domain, some element in the segment linking the ATPase motor to the DNA-binding domain contributed to efficient nucleosome sliding.

The four truncated yeast Chd1 proteins were also assayed for ATPase activities in the presence and absence of DNA and nucleosome substrates (Fig. 1C). ATP hydrolysis by Chd1 is regulated by the N-terminal chromodomains and required for sliding. When the chromodomain-ATPase interface is intact, maximal ATPase stimulation requires nucleosome substrates (17). This preference for nucleosome substrates over naked DNA was observed with Chd1_{ANC}, which showed a hydrolysis rate of 308 ± 4 ATP·min⁻¹ for 250 nM (0-N-63) nucleosomes compared with 46 ± 1 ATP·min⁻¹ for an equal amount of DNA. In contrast, at the same enzyme concentration (50 nM remodeler) the other three C-terminally truncated proteins did not show significant levels of ATPase stimulation by DNA or nucleosome substrates relative to the no substrate control (Fig. 1C). This absence of detectable ATPase activation of Chd1_{118-939}, Chd1_{118-978} and Chd1_{118-1014} highlights the severity of deleting the DNA-binding domain. Given the complications of comparing truncated Chd1 proteins with extremely low sliding and ATPase activities due to a commonly missing DNA-binding domain, we sought to investigate the importance of the segment connecting the ATPase motor and DNA-binding domain with internal deletions and point substitutions.

Efficient nucleosome sliding is highly dependent on the N-terminal portion of the segment between the ATPase motor and DNA-binding domain. To determine which portions of the segment between the ATPase motor and DNA-binding domain contribute most significantly to nucleosome sliding, five deletion constructs were generated (Fig. 2A). Given the significant reduction in nucleosome sliding and ATPase activities in the absence of the DNA-binding domain, the Chd1_{ANC} construct, which maintains chromodomains, ATPase motor, and DNA-binding domain, was used as the parent into which deletions were introduced.

To compare the impact of internal deletions on nucleosome sliding activities, Chd1 proteins were tested over a 1000-fold concentration range for their ability to shift end-positioned nucleosomes (Fig. 3). The parent protein, Chd1_{ANC}, shifted approximately half of the nucleosomes to a single, more slowly migrating band within 5 minutes at the lowest remodeler concentration (1 nM). At longer time points and higher Chd1_{ANC} concentrations, the majority (>80%) of end-positioned nucleosomes were shifted to one or two primary bands representing more centrally positioned nucleosomes.

The five internal deletions impacted nucleosome sliding activity to different extents. The two proteins with deletions closest to the ATPase motor, Chd1_{ANC}_{932-940} and Chd1_{ANC}_{941-955}, were the least effective in sliding nucleosomes. Even after a 2-hour incubation, the highest (1000 nM) concentration of Chd1_{ANC}_{932-940} was unable to shift all nucleosomes away from the end of the DNA fragment at all concentrations tested (Fig. 3, lane 19). Chd1_{ANC}_{941-955}, in contrast, was able to shift nucleosomes away from the end-position to a similar extent as Chd1_{ANC}, but more slowly and requiring higher remodeler concentrations. For instance, in a 5 minute reaction, 10 nM of Chd1_{ANC} shifted >80% of end-positioned nucleosomes, whereas 1000 nM of Chd1_{ANC}_{941-955} left a significant fraction of nucleosomes unshifted (compare lanes 4 and 28). However, after two hours, the majority of end-positioned nucleosomes were shifted by 100 and 1000 nM Chd1_{ANC} (lanes 27 and 29).

The other three proteins, Chd1_{ANC}_{961-974}, Chd1_{ANC}_{977-994}, and Chd1_{ANC}_{995-1005}, were much less impacted by the internal deletions, and showed similar patterns of shifted nucleosomes to those generated by Chd1_{ANC}. These sliding experiments revealed that not all portions of the 87 residue segment connecting the ATPase motor with the DNA-binding domain were equally important for nucleosome sliding.

Two variants, W932A and D933K/D934K, show severe defects in the rates of nucleosome sliding. To quantify the extent that disruptions in the segment linking the ATPase motor with the DNA-
binding domain altered sliding activity of Chd1, time-course experiments were used to calculate initial nucleosome sliding rates. Sliding reactions were followed by native PAGE over a two hour period, and the fraction of end-positioned nucleosomes shifted was determined at each time point (Fig. 4). As before, sliding of these end-positioned (0-N-63) nucleosomes yielded two primary bands, which were consistent with the repositioning of the histone octamer towards more central locations on the DNA fragment (21). The lower of the two shifted bands always appeared earlier in the reaction, and in general the majority of the starting material shifted to this intermediate band before appearance of the upper shifted band (Supplemental Figure 1). For the purposes of calculating initial sliding rates, the fraction of shifted nucleosomes at each point was considered to be the measured intensities of all shifted bands divided by the sum of all nucleosome bands in each lane. Progress curves were fit to single or double exponentials as shown in Fig. 4. Initial sliding rates were calculated from exponential or linear fits to the data (Table I; see Experimental Procedures).

Using 1 nM remodeler and 100 nM (0-N-63) nucleosomes, Chd1ANC displayed an initial sliding rate of $11.7 \pm 1.1 \text{nM} \cdot \text{min}^{-1}$. The three deletions closest to the DNA-binding domain, Chd1ANC$^{961-974}$, Chd1ANC$^{977-994}$, and Chd1ANC$^{995-1005}$, showed initial sliding rates that were approximately 50-80% that of Chd1ANC (Table I). The similar rates of these three internally deleted variants suggests that specific residues within the segment spanning 961-1005 are likely not making critical interactions with the rest of the remodeler or nucleosome substrate. Instead, these similar rates may reflect the general shortening of the distance between the ATPase motor and DNA-binding domain common to these deletion variants, which removed 11 to 18 residues.

Given the extremely low sliding activity observed for the two remodeler variants with the most deleterious internal deletions, Chd1ANC$^{932-940}$ and Chd1ANC$^{941-955}$, (Fig. 3, lanes 12-29), 10- or 100-fold higher remodeler concentrations were used to follow nucleosome sliding. At 10 nM concentration, Chd1ANC$^{941-955}$ shifted nucleosomes with an apparent initial rate of $1.4 \pm 0.3 \text{nM} \cdot \text{min}^{-1}$ (Fig. 4A). Adjusting for enzyme concentration, this variant therefore shifted nucleosomes approximately 100-fold more slowly than observed for Chd1ANC. For Chd1ANC$^{932-940}$, the initial rate was even slower, only $0.34 \pm 0.03 \text{nM} \cdot \text{min}^{-1}$ for 100 nM remodeler, or approximately 3500-fold worse than observed for Chd1ANC (Table I).

To more finely dissect the contributions of residues encompassed in the most severe deletions, we generated eight Chd1ANC variants in the region spanning residues 932 to 955. These variants, referred to as Chd1ANC$^{V1}$ – Chd1ANC$^{V8}$, possessed the same number of residues as Chd1ANC but had one to four amino acid substitutions (Fig. 2B). This region is highly charged, and a distinctive feature includes alternating clusters of acidic and basic residues. Comparing amino acid sequences of Chd1 orthologs in this region shows a conservation of electrostatic character, and a few positions (notably Trp932, Ile935, Ile936, and Pro937) are strongly conserved in identity. Charge-reversal substitutions were introduced to probe the importance of alternating positive and negatively charged clusters, and alanine substitutions were introduced to test the importance of conserved hydrophobic side chains.

Similar to the five variants with internal deletions, the eight variant remodelers with point substitutions also showed a broad range of remodeling activities, with the most disruptive changes located in the N-terminal portion of the 932-955 segment (Fig. 4B). By far the least active variant was Chd1ANC$^{V1}$. This variant, a single Trp→Ala substitution at position 932, shifted nucleosomes as poorly as the worst internal deletion variant, Chd1ANC$^{932-940}$, with 100 nM remodeler producing an initial rate of $0.38 \pm 0.13 \text{nM} \cdot \text{min}^{-1}$, roughly 3000-fold worse than Chd1ANC. Thus, removal of the indole ring of Trp932 appeared to account for most or all of the lost sliding activity observed for Chd1ANC$^{932-940}$. In contrast, simultaneous substitution of the highly conserved Ile935, Ile936, and Pro937 to alanine (variant Chd1ANC$^{V3}$) did not greatly diminish sliding activity (Fig. 4B; Table I).

Nucleosome sliding was also significantly diminished for Chd1ANC$^{V2}$, which was approximately 500-fold slower than Chd1ANC. Although the dramatic change in charge that accompanies two charge-reversal substitutions for Chd1ANC$^{V2}$ (Asp933→Lys and Asp934→Lys) might be expected to interfere with activity, the other five charge-reversal clusters (Chd1ANC$^{V4}$ to Chd1ANC$^{V8}$) had much more moderate effects on
sliding rates. In fact, none of the charge reversals in the 941-955 region were as poor as the deletion encompassing this region, suggesting that a combined loss of charged residues in this region was likely important in reducing sliding activity of Chd1\textsubscript{ANC}\textsuperscript{941-955}.

Deletion of residues 932-940 of Chd1 does not disrupt overall secondary structure. Considering the extremely poor sliding activity of Chd1\textsubscript{ANC}\textsuperscript{932-940}, it seemed possible that removal of these residues may interfere with proper domain folding and therefore activity of the ATPase motor. To investigate this, the secondary structural profiles of Chd1\textsubscript{ANC}, Chd1\textsubscript{ANC}\textsuperscript{V1}, and Chd1\textsubscript{ANC}\textsuperscript{932-940} were monitored by circular dichroism (CD). As shown in Fig. 5, the secondary structural profiles of all three proteins are virtually identical. With the small size of the internal deletion \(\Delta932-940\), a difference in the CD profile would not be expected to be detectable from removal of these nine residues alone unless these residues were important for stabilizing a folded domain. The similarity in these curves suggests that these residues are not required for maintaining any large folded domains in the Chd1 protein.

Deletion of residues 932-940 does not compromise nucleosome binding. Although the region most deleterious to nucleosome sliding (residues 932-940) lies closer to the ATPase motor than the DNA-binding domain, we wanted to exclude the possibility that changes in this region reduced affinity for nucleosomes. The DNA-binding domain is needed for the Chd1 remodeler to stably associate with nucleosomes, and this interaction requires extranucleosomal DNA (24). To see whether the deletion \(\Delta932-940\) influenced the ability of Chd1 to associate with nucleosomes containing extranucleosomal DNA, increasing concentrations of both Chd1\textsubscript{ANC} and Chd1\textsubscript{ANC}\textsuperscript{932-940} were incubated with (0-N-63) nucleosomes and monitored by native PAGE (Fig. 6). Both Chd1 proteins super-shifted nucleosomes over the same concentration range, demonstrating a similar overall nucleosome binding ability.

Chd1 variants deficient in nucleosome sliding display significant nucleosome-stimulated ATPase activity. The results described above demonstrate that particular regions between the ATPase motor and DNA-binding domain are important in nucleosome sliding. However, the nucleosome sliding experiments do not reveal the stage at which these deletions and substitutions interfere with sliding. Like all other remodelers, nucleosome sliding by Chd1 is an ATP-dependent process, and therefore influencing the rate at which ATP hydrolysis occurs would be a straightforward route by which perturbations in a remodeler could reduce the rate of nucleosome sliding. Using an NADH-coupled assay, ATP hydrolysis rates were measured over a range of nucleosome and DNA concentrations. For Chd1\textsubscript{ANC}, we observed near saturation above 100 nM (0-N-63) nucleosome or DNA substrates. In agreement with previous findings (17), Chd1\textsubscript{ANC} was preferentially activated by nucleosomes, with approximately 7-fold higher maximal ATPase activity in the presence of 500 nM nucleosomes (293 ± 20 ATP·min\(^{-1}\)) compared with an equal amount of naked DNA (43 ± 2 ATP·min\(^{-1}\)).

Interestingly, all Chd1 variants showed significant levels of DNA- and nucleosome-stimulated ATPase activities (Fig. 7, Table 1). For the five Chd1 proteins with internal deletions, Chd1\textsubscript{ANC\textsuperscript{977-994}} possessed nucleosome-stimulated activity indistinguishable from Chd1\textsubscript{ANC} (284 ± 17 ATP·min\(^{-1}\)), Chd1\textsubscript{ANC\textsuperscript{961-974}} and Chd1\textsubscript{ANC\textsuperscript{995-1005}} had intermediate values (202 ± 24 and 186 ± 10 ATP·min\(^{-1}\), respectively), and Chd1\textsubscript{ANC\textsuperscript{932-940}} and Chd1\textsubscript{ANC\textsuperscript{941-955}} had the lowest rates (107 ± 5 and 101 ± 6 ATP·min\(^{-1}\), respectively), approximately 35% that of Chd1\textsubscript{ANC} (Fig. 7A, Table 1). The eight variants with amino acid substitutions showed a much narrower distribution, approximately 40-60% that of Chd1\textsubscript{ANC} (Fig. 7B, Table 1).

With the exception of Chd1\textsubscript{ANC\textsuperscript{V5}}, all Chd1 variants showed a DNA-stimulated ATPase activity equal to or greater than Chd1\textsubscript{ANC}. Importantly, for all proteins the nucleosome-stimulated ATP hydrolysis rates were significantly higher than DNA-stimulated rates. This means that all remodelers were all able to engage nucleosomes sufficiently to distinguish them from naked DNA. Therefore, for the Chd1 variants with nucleosome sliding rates ~1% or less than that of Chd1\textsubscript{ANC} (Chd1\textsubscript{ANC\textsuperscript{932-940}}, Chd1\textsubscript{ANC\textsuperscript{941-955}}, Chd1\textsubscript{ANC\textsuperscript{V1}}, Chd1\textsubscript{ANC\textsuperscript{V2}}), the defects in sliding did not appear to result from a gross inability to bind or recognize nucleosome substrates.

Although these deletions and amino acid substitutions lie outside the canonical ATPase domain, it is possible that these variations...
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influenced ATP binding. To investigate this, nucleosome-stimulated ATPase activities were measured for Chd1\textsubscript{ANC} and Chd1\textsubscript{ANC}\textsuperscript{Δ932-940} with varying ATP concentrations (Fig. 7C). Under these conditions, the rates of ATP hydrolysis saturated over a similar range of ATP concentrations, yielding apparent $K_M$ values of $54 \pm 6$ and $58 \pm 6$ $\mu$M for Chd1\textsubscript{ANC} and Chd1\textsubscript{ANC}\textsuperscript{Δ932-940}, respectively. Therefore, the deletion that most severely reduced the maximum rate of nucleosome-stimulated hydrolysis did not appear to significantly alter the association of the ATPase motor with ATP.
DISCUSSION

Chromatin remodelers possess ATP-dependent DNA translocase motor domains required for shifting nucleosomes along DNA. To understand the mechanism of nucleosome sliding, it is essential to define all remodeler components that cooperate to shift the DNA duplex past the histone core. Here we identify several conserved residues in the Chd1 remodeler that are essential for efficiently coupling ATP hydrolysis to nucleosome sliding. Deletion of short segments C-terminal to the ATPase motor (Δ932-940 and Δ941-955) or substitution of amino acids in this region (variants V1-Trp932Ala and V2-Asp933Lys/Asp934Lys) severely attenuated the ability to slide nucleosomes (Figs. 3 and 4; Table I). However, despite these losses in nucleosome sliding ability, these changes only reduced the levels of nucleosome-stimulated ATPase activity by ~3-fold (Fig. 7).

Due to the dependence of nucleosome sliding on ATP hydrolysis, utilization of ATP for a native enzyme is expected to directly correlate with productive engagement of nucleosomal and DNA substrates. By characterizing Chd1 proteins with internal deletions and amino acid substitutions, we have identified residues of the Chd1 remodeler required for coupling the energy derived from ATP hydrolysis to nucleosome sliding. Dividing the rate of nucleosome sliding by the rate of ATP hydrolysis yields the relative “coupling efficiency,” which is effectively the amount of sliding achieved per ATP hydrolyzed. The most severe disruptions (Δ932-940 and Trp932Ala) reduced coupling efficiency to 0.08% that of the parental remodeler, Chd1\textsuperscript{ANC} (Table I), which means that ~1250-fold more ATP was hydrolyzed for an equivalent amount of nucleosome sliding activity. Similarly, poor coupling efficiency was observed for the substitution variant Asp933Lys/Asp934Lys in this region, which required 250-fold more ATP than Chd1\textsuperscript{ANC} for sliding.

Amino acid substitutions and deletions that uncouple ATP hydrolysis from mechanical disruptions of nucleic acid substrates have been reported for Chd1 and SWI/SNF remodelers, as well as related helicase superfAMILY ATPases. These ATPase coupling residues are located both within the ATPase motor, as well as on segments outside the conserved ATPase core. Within the SWI/SNF ATPase motor, deletion of a conserved eight residue segment, “STRAGGLG”, found in the second lobe of the motor, severely reduced nucleosome sliding activity yet did not interfere with DNA-stimulated ATP hydrolysis (25). The STRAGGLG region corresponds to helicase motif V, one of the seven conserved motifs found in helicase superfamily 1 (SF1) and superfamily 2 (SF2) proteins (26). Motif V forms a loop on the second domain of the ATPase motor, and can make interactions both with a bound nucleic acid substrate and the first domain of the ATPase motor (27). In a different SF2 protein, the Prp22 helicase, amino substitutions in motif V had a similar effect as for SWI/SNF, resulting in equal or higher RNA-stimulated ATPase activity yet little or no duplex unwinding ability (28).

For other SF2 helicases, single amino acid substitutions in helicase motifs II and III, also located in the central cleft of the ATPase motor, uncoupled ATP hydrolysis from helicase activity. Substitution of the fourth residue in motif II, which for several families is DEx(D/H), significantly diminished or abolished helicase activity while preserving ATPase activity for eIF4A, NHP-II, and NS3 (29-31). Single or double alanine substitutions introduced into motif III, which is (S/T)AT for many helicases, uncoupled ATPase and helicase activities for eIF4A, NHP-II, and Prp22 (29, 30, 32).

Outside the conserved ATPase motifs, mutations that uncoupled ATP hydrolysis from duplex unwinding have been reported for the SF1 helicase PcrA (33). The ATPase motor of PcrA binds to single-stranded DNA and can translocate along it through an inch-worm opening and closing of the ATPase cleft (34). Two domains (1B and 2B) unique to the PcrA/UvrD family contact double-stranded DNA outside the ATPase motor, and have been proposed to both bind and partially destabilize the DNA duplex (33, 34). Several amino acid substitutions in these domains diminished the efficiency of duplex unwinding 20- to 30-fold without significantly reducing ssDNA-stimulated ATP hydrolysis. While there is still some debate over whether these domains aid or negatively regulate helicase activity (34-36), this uncoupling demonstrates that the efficiency by which ATP hydrolysis is used to do work can be strongly influenced by domains outside the conserved ATPase core.
Residues required for ATPase coupling in the Chd1 remodeler

Similar to the findings reported here, two regions outside the ATPase motors of Chd1 and SWI/SNF remodelers have recently been shown to be required for coupling ATP hydrolysis to nucleosome sliding. Several combinations of point substitutions in the DNA-binding domain of Chd1 were found to severely reduce nucleosome sliding ability, while diminishing nucleosome-stimulated ATP hydrolysis less than two-fold (24). These substitutions weakened binding to both naked DNA and to nucleosomes. It was suggested that this ATPase uncoupling may signify a role of the DNA-binding domain in maintaining remodeler processivity (allowing multiple turnover events that may be required for sliding), or alternatively, by providing a point of contact on DNA against which the ATPase motor may generate tension by torquing or pulling the DNA (24). Although we observed nucleosome sliding using Chd1 proteins that lacked the DNA-binding domain, nucleosome-stimulated ATP hydrolysis rates were not significantly above background (Figure 1). The low activities of these proteins (which had to be compensated by increased remodeler concentrations) presumably stemmed at least in part from poorer binding to nucleosome substrates. The low nucleosome-stimulated ATPase activities made it difficult to determine with confidence whether ATP hydrolysis was occurring without sliding, and therefore we were unable to evaluate whether deletion of the entire DNA-binding domain uncoupled ATP hydrolysis from nucleosome sliding.

For SWI/SNF, a conserved 58 residue segment C-terminal to the ATPase motor referred to as the SnAC domain (for Snf2 ATPase Coupling) was found to be important for both ATPase and nucleosome sliding ability (37). The SnAC domain was found to not be required for nucleosome binding or recruitment, nor essential for assembly of SWI/SNF subunits. Though deletion of the SnAC domain significantly reduced nucleosome-stimulated ATPase activity (~10-fold relative to wild-type), a role in ATPase coupling was evident from the absence of detectable nucleosome sliding activity.

For Chd1, we have shown that residues outside of the folded ATPase domain play a critical role in productively utilizing the energy released during ATP hydrolysis for sliding nucleosomes. The crystal structure of the chromodomain-ATPase fragment of Chd1 revealed that the canonical fold of the ATPase motor was followed by a C-terminal, ~50 residue bridge segment that traversed one surface of the ATPase motor, ending at approximately residue 922 (17). The cluster of residues that are reported here to be most important for ATPase coupling (Trp932, Asp933, and Asp934) are therefore located just C-terminal to this bridge segment, and though included in the crystallization construct, were not visible in the crystal structure. At present, it is not known whether these residues directly contact the ATPase motor during sliding, potentially stabilizing an active organization similar to what may occur through helicase motifs II, III, and V, or if these residues instead contact the nucleosomal substrate. Using a fluorescently labeled peptide encompassing residues 932-955, we were unable to detect interactions with mononucleosomes using native PAGE (data not shown). If this region were to contact the nucleosome, similar to the scenarios proposed for the Chd1 DNA-binding domain (24), these residues might be important for destabilizing histone-DNA or histone-histone interactions required for nucleosome sliding.

The severe defects in nucleosome sliding with just single (Trp932Ala) and double (Asp933Lys, Asp934Lys) amino acid substitutions suggest that this cluster forms a specific and critical interaction with either the remodeler or nucleosome. In contrast, for the residues that follow (941-955), it seems that coupling depends on the overall electrostatic nature of the segment, as charge-reversal substitutions had little effect compared with deletion of the entire segment (Fig. 4; Table I). A notable feature of this segment is the alternating clusters of positively and negatively charged residues (Fig. 2B), which may be well suited for complementing the highly charged surfaces presented by histones and DNA.

Over the past few years, Chd1 and ISWI-type remodelers have come to be recognized as remarkably similar enzymes, first for their common abilities to assemble, space, and center nucleosomes (15, 16), and more recently for sequence and structural homology. ISWI-type remodelers have significant sequence homology to the C-terminal bridge segment following the Chd1
ATPase motor, suggesting that they too possess a similar structure (17). Likewise, the DNA-binding domain of Chd1 was found to consist of a SANT and SLIDE domain pair, analogous to the HAND-SANT-SLIDE DNA-binding domain first reported for ISWI (24, 38). Given the importance of the ATPase coupling residues described here for nucleosome sliding, one might expect ISWI remodelers to possess a functionally similar element. Like Chd1, ISWI orthologs possess a segment of highly conserved residues between the C-terminal bridge and the beginning of the DNA-binding domain (Supplemental Figure S2A). While this segment in ISWI does not have clear sequence homology to the 932-955 segment of Chd1 described here, it does possess several conserved aromatic residues flanked by charged amino acids that may play roles similar to Trp932, Asp933, and Asp934 in ATPase coupling.

In SWI/SNF, the ATPase coupling SnAC domain is also C-terminal to the ATPase motor (S. cerevisiae Snf2 residues 1312-1370 (ref. 37)), and may be functionally analogous to the residues required for ATPase coupling for Chd1. The SnAC domain also lacks clear homology to residues 932-955 of Chd1, though interestingly, the most debilitating substitution was Tyr1359Ala, which like Trp932 of Chd1, is followed by two polar residues, one of them acidic (Tyr1359-Asn1360-Asp1361; Supplemental Figure S2B). The sequence differences among segments involved in ATPase coupling could be indicative of functionally distinct interactions with the nucleosome or remodeler. Alternatively, sequence differences may reflect some degeneracy in binding that still allows the ATPase motor to productively shift DNA past the histone core. Continuing to identify residues required by remodelers for utilizing ATP hydrolysis to move nucleosomes, and beginning to uncover the mechanisms by which such coupling occurs will be necessary for pushing our understanding of chromatin remodeling forward.
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Residues required for ATPase coupling in the Chd1 remodeler

Figure Legends:

Figure 1:
Analysis of S. cerevisiae Chd1 proteins progressively truncated at the C-terminus.
(A) Schematic representation of Chd1 constructs.
(B) Nucleosome sliding assays using end-positioned (0-N-63) Cy5-labeled nucleosomes. Nucleosome centering is observed as an up-shift of the nucleosome band from the initial position.
(C) ATPase activities of Chd1 proteins measured in the presence of buffer alone, naked DNA, or mononucleosome substrates. The same 208 bp DNA sequence containing the core 601 nucleosome positioning sequence at one end was used for the DNA alone and mononucleosome substrates. All experiments were performed in triplicate and shown as means with standard errors.

Figure 2:
Design for internal deletions and amino acid substitutions between the Chd1 ATPase motor and DNA-binding domain.
(A) Schematic representations for S. cerevisiae Chd1 deletion constructs. Note that the sizes of domains and deletions are not drawn to scale.
(B) Sequence alignment for several Chd1 orthologs, highlighting the residues altered in eight variant proteins of S. cerevisiae Chd1. The sequence alignment was generated using ClustalX (39) and TEXshade (40).

Figure 3:
Internal deletions between the ATPase motor and DNA-binding domain become progressively more deleterious towards the ATPase motor.
The indicated concentrations of each Chd1 protein were incubated with end-positioned (0-N-63) Cy5 labeled nucleosomes in the presence or absence of ATP, and sliding reactions were quenched by addition of prechilled 25μg competitor DNA and 25 mM EDTA after 5 or 120 min. The gels shown are representative of three or more experiments.

Figure 4:
Quantification of nucleosome sliding rates for Chd1 proteins with internal deletions and amino acid substitutions.
(A) Quantification of nucleosome sliding reactions using Chd1 proteins with or without internal deletions incubated with 100 nM end-positioned (0-N-63) Cy5-labeled nucleosomes. Nucleosome sliding reactions were initiated by adding 2.5 mM ATP and 5 mM MgCl2, and quenched with prechilled competitor DNA and 25 mM EDTA at the indicated time points and monitored using native acrylamide gels (see Supplemental Figure 1). The inset shows the compressed data points within the first 5 min. Error bars represent standard deviations from three experiments.
(B) Quantification of nucleosome sliding reactions using Chd1 variants containing amino acid substitutions as defined in Fig 2B, performed and analyzed as described for (A).

Figure 5:
Deletion of residues 932-940 does not disrupt global folding of Chd1.
CD spectra for Chd1ANC, Chd1ANCV1, and Chd1ANC del932-940. Chd1ANCV1 data points (gray circles) are obscured by Chd1ANC del932-940 (white circles). The curves represent the averages of three scans.

Figure 6:
Deletion of residues 932-940 does not reduce nucleosome binding affinity.

Nucleosome binding abilities of Chd1\textsubscript{ANC} and Chd1\textsubscript{ANC}\textsuperscript{\Delta932\textendash940} were monitored by native PAGE. Increasing concentrations of Chd1 proteins were incubated with 25 nM (0-N-63) Cy5-labeled nucleosomes for 2 hours at room temperature. The gels shown are representative of three experiments.

**Figure 7:**

Chd1 proteins with internal deletions and substitutions show significant DNA- and nucleosome-stimulated ATPase activities.

(A) ATPase activities of Chd1 proteins with and without internal deletions. ATPase rates were measured in the presence of buffer alone, 100 or 500 nM naked DNA, or 100 or 500 nM mononucleosome substrates. The DNA used for ATPase stimulation was the same 208 bp DNA fragment used for producing (0-N-63) mononucleosome substrates. All experiments were performed three or more times and are shown as means with standard errors.

(B) ATPase activities of variant Chd1 proteins, as described in (A). All experiments were performed three or more times and are shown as means with standard errors.

(C) ATP titration curves for the Chd1 proteins with and without the deleterious internal deletion (\(\Delta932\textendash940\)). ATP hydrolysis rates were measured in the presence of 100 nM (0-N-63) mononucleosome at the indicated ATP concentrations.
Table I. Rates of ATP hydrolysis and nucleosome sliding for yeast Chd1 proteins described in this study.

| Chd1 variant | initial nucleosome sliding rate (min⁻¹) | normalized initial sliding rate (min⁻¹) | basal ATPase rate (min⁻¹) | DNA-stimulated ATPase rate* (min⁻¹) | nucleosome-stimulated ATPase rate* (min⁻¹) | nucleosome-stimulated hydrolysis relative to ∆NC (%) | coupling efficiency (%) |
|--------------|----------------------------------------|----------------------------------------|--------------------------|-----------------------------------|-------------------------------------------|--------------------------------------------------|------------------------|
| ∆NC         | 11.7 ± 1.1                             | 100 ± 13                               | 5 ± 2                    | 43 ± 2                            | 293 ± 20                                   | 100 ± 10                                         | 100 ± 17               |
| ∆932-940    | 0.0034 ± 0.0003                        | 0.029 ± 0.004                          | 28 ± 2                   | 35 ± 3                            | 107 ± 5                                    | 36 ± 3                                            | 0.081 ± 0.012          |
| ∆941-955    | 0.14 ± 0.03                            | 1.2 ± 0.3                              | 19 ± 3                   | 35 ± 7                            | 101 ± 6                                    | 34 ± 3                                            | 3.5 ± 0.9              |
| ∆961-974    | 6 ± 2                                  | 50 ± 14                                | 13 ± 3                   | 83 ± 6                            | 202 ± 24                                   | 69 ± 10                                            | 72 ± 23               |
| ∆977-994    | 9 ± 2                                  | 81 ± 17                                | 5 ± 2                    | 106 ± 8                           | 284 ± 17                                   | 97 ± 9                                            | 84 ± 19               |
| ∆995-1005   | 7.8 ± 0.9                              | 67 ± 10                                | 5.8 ± 1.0                | 37 ± 6                            | 186 ± 10                                   | 63 ± 6                                            | 105 ± 18              |
| V1          | 0.0038 ± 0.0013                        | 0.033 ± 0.011                          | 26 ± 2                   | 41 ± 3                            | 120 ± 10                                   | 41 ± 4                                            | 0.08 ± 0.03           |
| V2          | 0.022 ± 0.009                          | 0.19 ± 0.08                            | 24 ± 4                   | 40 ± 5                            | 125 ± 3                                    | 43 ± 3                                            | 0.4 ± 0.2             |
| V3          | 8 ± 2                                  | 67 ± 17                                | 6.1 ± 1.0                | 38 ± 4                            | 137 ± 2                                    | 47 ± 3                                            | 143 ± 39              |
| V4          | 1.2 ± 0.3                              | 10 ± 3                                 | 11 ± 2                   | 35 ± 4                            | 135 ± 5                                    | 46 ± 4                                            | 23 ± 7                |
| V5          | 0.8 ± 0.09                             | 6.7 ± 1.0                              | 20 ± 2                   | 24 ± 6                            | 119 ± 6                                    | 41 ± 3                                            | 16.5 ± 2.8            |
| V6          | 8 ± 3                                  | 67 ± 23                                | 12.3 ± 1.2               | 91 ± 3                            | 186 ± 17                                   | 64 ± 7                                            | 106 ± 38              |
| V7          | 2.8 ± 0.6                              | 24 ± 6                                 | 21 ± 2                   | 41 ± 5                            | 169 ± 7                                    | 58 ± 5                                            | 41 ± 11               |
| V8          | 1.1 ± 0.4                              | 9 ± 3                                  | 8.2 ± 1.4                | 44 ± 2                            | 118 ± 7                                    | 40 ± 4                                            | 23 ± 9                |

* The reported DNA- and nucleosome-stimulated activities were calculated by subtracting basal ATPase activities (without substrate) from total ATPase activities in the presence of 500 nM DNA or nucleosome substrates.
Figure 1
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Figure 2
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Figure 3
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Figure 4
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Figure 5
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Figure 6
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Figure 7
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Identification of residues in chromo-Helicase-DNA-binding protein 1 (Chd1) required for coupling ATP hydrolysis to nucleosome sliding
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