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# Analysis of Nucleosome Repositioning by Yeast ISWI and Chd1 Chromatin Remodeling Complexes*[S]

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**ISWI proteins form the catalytic core of a subset of ATP-dependent chromatin remodeling activities in eukaryotes from yeast to man. Many of these complexes have been found to reposition nucleosomes but with different directionalities. We find that the yeast Isw1a, Isw2, and Chd1 enzymes preferentially move nucleosomes toward more central locations on short DNA fragments whereas Isw1b does not. Importantly, the inherent positioning properties of the DNA play an important role in determining where nucleosomes are relocated to by all of these enzymes. However, a key difference is that the Isw1a, Isw2, and Chd1 enzymes are unable to move nucleosomes to positions closer than 15 bp from a DNA end, whereas Isw1b can. We also find that there is a correlation between the inability of enzymes to move nucleosomes close to DNA ends and the preferential binding to nucleosomes bearing linker DNA. These observations suggest that the accessibility of linker DNA together with the positioning properties of the underlying DNA play important roles in determining the outcome of remodeling by these enzymes.**

Nucleosomes are the fundamental subunits of eukaryotic chromatin. The assembly of DNA into chromatin fulfils important functions in both packaging DNA into nuclei and regulating access to genetic information. Crystallographic structures of nucleosomes provide a detailed picture of how DNA is bound to the surface of the histone octamer (1). However, in solution nucleosomes exhibit dynamic properties that include the ability to spontaneously relocate to different positions on DNA fragments (2–4). The positioning of nucleosomes has the potential to positively or negatively regulate access to DNA and consequently all genetic processes.

In addition to undergoing spontaneous thermal movements, nucleosomes can be repositioned by ATP-dependent chromatin remodeling enzymes. These enzymes consist of a catalytic subunit with a region of homology to the yeast Snf2 protein and a variable number of accessory subunits. Snf2 family proteins fall into distinct subfamilies. For example the ISWI subfamily is named after its founding member, the *Drosophila* ISWI protein (5). The ISWI protein was subsequently found to be a component of several distinct protein complexes that have the ability to alter chromatin structure in an ATP-dependent reaction (6). Related ISWI complexes have since been identified in a broad spectrum of eukaryotes from yeast to humans (7). These complexes have been found to function in a range of processes ranging from the regulation of transcription and DNA replication to the maintenance of chromatin structure (7).

Characterization of ISWI-driven chromatin remodeling reactions has revealed that one outcome is the repositioning of nucleosomes along DNA (8–10). Although the redistribution of nucleosomes may represent the major means by which these complexes alter chromatin structure, this is not necessarily the case for other subfamilies of Snf2 proteins which have been shown to cause other transitions in chromatin structure (11–15). In addition, there are differences in the way that different ISWI containing complexes redistribute nucleosomes. For example, while the *Drosophila* ISWI-containing complex NURF and its isolated catalytic subunit redistributes nucleosomes to positions closely related to those observed in thermal nucleosome redistribution reactions (4, 16), the ACF, CHRAC, and Isw2 complexes have been reported to preferentially move nucleosomes to positions closer to the center of short DNA fragments (9, 17, 18).

Yeast Chd1p represents another, less well characterized subfamily of remodelers, which shows genetic interactions with ISWI factors (19, 20). This subfamily is also represented in multicellular eukaryotes and in *Drosophila* and yeast appears to be mainly monomeric (21, 22).

The differences in the directionality with which nucleosomes are redistributed are likely to significantly influence the functions for which these complexes are used. To understand what these differences, we have systematically analyzed the positions to which nucleosomes are redistributed in different contexts using yeast Isw1a, Isw1b, Isw2, and Chd1 remodeling enzymes. We find that the enzymes that relocate nucleosomes to more central locations preferentially engage with nucleosomes bearing linker DNA. This may explain why these enzymes are unable to move nucleosomes to positions close to DNA ends where linker DNA would be lost. In addition to this inability to move nucleosomes close to DNA ends we find that the inherent nucleosome positioning properties of the DNA play an important role in determining where nucleosomes are moved to.

## EXPERIMENTAL PROCEDURES

*Nucleosome Binding*—Binding of Isw1a and Isw1b was monitored in 10% glycerol, 50 mM Tris-Cl, pH 8.0, 50 mM NaCl, 3 mM $MgCl_2$, and 100 $\mu$g/ml bovine serum albumin. For Chd1 reactions contained 2.5% Ficoll, 50 mM Tris-Cl, pH 8.0, 50 mM NaCl, and 3 mM $MgCl_2$. All reactions contained 0.5 nM 5′-$^{32}$P-labeled nucleosomes (concentrations were obtained by scintillation counting of nucleosomes and comparing the value to that of the parent DNA, whose concentration was obtained by absorbance at 260 nm). Reactions were set up on ice and electrophoresed through 0.2× Tris borate-EDTA, 5% acrylamide gels for 4 h at 150 V at 4 °C with running buffer recirculation.

*Enzymes*—Isw1a and Isw1b were purified from yeast strains (YTT1168 and YTT1167) in which the Ioc3p or Ioc2p factors, respec-

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[S] The on-line version of this article (available at http://www.jbc.org) contains supplemental Figs. 1–3.
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tively, were expressed as fusions with the TAP[2] double affinity tag (23). Chd1 and Isw2 were purified from Chd1-TAP or Isw2-TAP strains, which were purchased from Euroscarf. Remodelers were purified by IgG and calmodulin affinity chromatography from 20 liters of yeast (24). Supplemental Fig. 1 illustrates the purity of these complexes.

*Nucleosomes*—Histone octamers were assembled from individual *Xenopus laevis* histones expressed in bacteria (25). Octamers for high resolution repositioning assays contained a S47C mutation in H4 and a C110A change in H3. These octamers were subsequently reacted with the thiol-reactive EDTA derivative (EDTA-2-aminoethyl)2-pyridyl disulfide (26). Octamers were reconstituted into nucleosomes using PCR prepared DNA derived from the MMTV nucleosome A (4) or 601.3 (27) positioning sequences. Reconstitutions were performed at 1 $\mu$M concentration and pH 7.5 by stepwise dialysis from 2 M NaCl or KCl to 0.85, 0.65, 0.5, and finally 0 M.

*Mononucleosome Repositioning Assays*—Nucleosome repositioning was carried out in 10-$\mu$l reactions containing 20 mM Tris-Cl, pH 8, 50 mM KCl, 1 mM $MgCl_2$, 1 mM MgATP, 1 pmol of nucleosome ($^{32}$P-end-labeled at a single 5′-DNA end) and various concentrations of enzyme as described in the legends to Figs. 1–5. After 20-min incubation at 30 °C reactions were stopped by transfer to ice and addition of competitor DNA (0.1 $\mu$g/$\mu$l final) and additional salt (200 mM KCl final). One-tenth of the reaction was electrophoresed through 0.2× Tris borate-EDTA, 5% acrylamide gels for 3.5 h at 300 V at 4 °C with running buffer recirculation; the remainder was subjected to site-directed mapping to determine the exact nucleosome position (28). This involved the addition of 1 $\mu$l of 40 $\mu$M ammonium ferrous sulfate and 5 $\mu$l each of 19.2 mM ascorbic acid and 0.2% hydrogen peroxide to each reaction followed by a 1-h incubation on ice. DNA was extracted with phenol:chloroform: isoamyl alcohol (25:24:1), recovered by ethanol precipitation, and resuspended in 5 $\mu$l of formamide loading dye (80% formamide, 10 mM NaOH, 1 mM EDTA, 0.1% xylene cyanol, 0.1% bromphenol blue). Samples were run on 8% acrylamide sequencing gels containing 6 M urea, fixed with 10% methanol, 10% acetic acid, dried, and exposed to image plates. Markers for sequencing gels were obtained by limited cleavage at G residues of the appropriate DNA template.

## RESULTS

*Differences in the Directionality of Nucleosome Redistribution by Isw1b, Isw1a, Isw2, and Chd1*—To test the ability of the purified Isw1 and Chd1 enzymes to catalyze nucleosome sliding, recombinant *X. laevis* histone octamers were assembled onto DNA fragments containing the MMTV nucleosome A positioning sequence flanked by different lengths of linker DNA. The DNA fragments used were designed such that the nucleosomes were initially flanked by 54-bp linkers on either side, off center (54 bp on one side and 18 bp on the other), or located at one end of a DNA fragment with 54 bp of linker DNA on one side and none on the other. The position of these nucleosomes was analyzed by native acrylamide gel electrophoresis following treatment with Isw1a, Isw1b, or Chd1 and ATP. The Isw1a complex caused nucleosomes initially located at the end and off center locations to run slower on the gel in an ATP dependent manner (Fig. 1*A*, *lanes 1–8*). However, no obvious ATP-dependent change was observed for the centrally located nucleosome (Fig. 1*A*, *lanes 9–12*). Conversely, treatment with the Isw1b complex and ATP caused the off-center and centrally located nucleosomes to increase in mobility, whereas no obvious change occurred with the end positioned nucleosome (Fig. 1*B*). The activity of Chd1 was similar to Isw1a except less alteration to the 54A18 nucleosome was observed (Fig. 1*C*).

It is known that when a histone octamer is positioned close to the end of a DNA fragment the mobility through a gel is greater than when the octamer is close to the center of the same piece of DNA (3). Therefore these observations suggest that Isw1a and Chd1 relocate nucleosomes closer to the center of the DNA ("end to center" type sliding) and that Isw1b causes nucleosomes to move to locations that are near to, or at, the ends of the DNA ("center to end" type sliding). To study this in more detail, site-directed nucleosome mapping was used to determine the positions nucleosomes were relocated to at high resolution. Briefly, this involves the tethering of cysteaminyl EDTA to a recombinant histone octamer via a thiol group introduced at H4 cysteine 47 (29). The chelation of $Fe^{2+}$ ions by this reagent provides a means of generating localized hydroxyl radicals that are capable of cleaving DNA but only within a range of less than 1.5 nm. The site at which the reagent is attached on histone H4 comes closest to DNA 2 bp from either side of the nucleosome dyad, meaning that the sites of cleavage can be used to determine nucleosome positions at base pair resolution.

In Fig. 2*A* the positions to which Isw1a and Isw1b reposition nucleosomes on an DNA fragment designed to position nucleosomes initially at an off center location with 54 bp of DNA on one side and 18 bp on the other. The untreated nucleosomes display a characteristic pattern of strong and weak DNA cleavage separated by 7 bp, indicating that nucleosomes assembled onto this DNA fragment were initially positioned predominantly at +70 relative to the MMTV transcription start site (Fig. 2*A*, *lanes 1*, *6*, and *10*). Following incubation with Isw1b, the cleavage at the +70 location is reduced, and new cleavage sites indicating predominant new locations at +22 and +27 are detected. These position nucleosomes close to the end of the fragment, consistent with the increased mobility following electrophoresis (Fig. 1*B*). Nucleosomes are also redistributed to these positions following thermal equilibration (Fig. 2*A*, *lanes 10* and *11*). However, the positions following redistribution by Isw1b exhibit a subtle bias for the locations closest to the end of the fragment. The movement of nucleosomes to positions closely related to those used during thermal redistribution reactions is consistent with previous studies of NURF (16) and recombinant *Drosophila* ISWI protein (4). In contrast, Isw1a relocates this nucleosome to a series of more centrally located positions at +39, +42, +47, and +58 (Fig. 2*A*, *lanes 5–8*). This cluster of locations is too close together to be resolved by native gel electrophoresis (Fig. 1*A*). Isw1a moves nucleosomes to a similar distribution of locations when the nucleosome is initially located at the end of the DNA fragment (Fig. 2*B*, *lanes 1–3*). The enzymes Chd1 and Isw2 also move nucleosomes to the same distribution of locations (Fig. 2*B*, *lanes 4–9*).

The finding that the Isw1 protein when associated with different accessory subunits in the Isw1a and Isw1b complexes moves nucleosomes with different directionalities illustrates that it is not the catalytic subunit alone that is responsible for this. While the locations to which nucleosomes are moved by Isw1b are clearly related to the most favorable locations available on these fragments, the mechanism for selecting the sites used by Isw1a, Chd1, and Isw2 is less clear. Nethertheless, the observation that these enzymes use the same subset of locations suggests that sequence or structural properties of the DNA fragment may contribute to the selection of sites to which nucleosomes are repositioned.

*Isw1a Moves Nucleosomes to a Subset of Locations No Closer than 15 bp from a DNA End*—To investigate what underlies the selection of these more central positions, nucleosome repositioning by Isw1a was investigated on a series of nucleosomes with successively shorter DNA

[2] The abbreviations used are: TAP, tandem affinity purification; MMTV, murine mammary tumor virus; ATPγS, adenosine 5′-*O*-(thiotriphosphate).

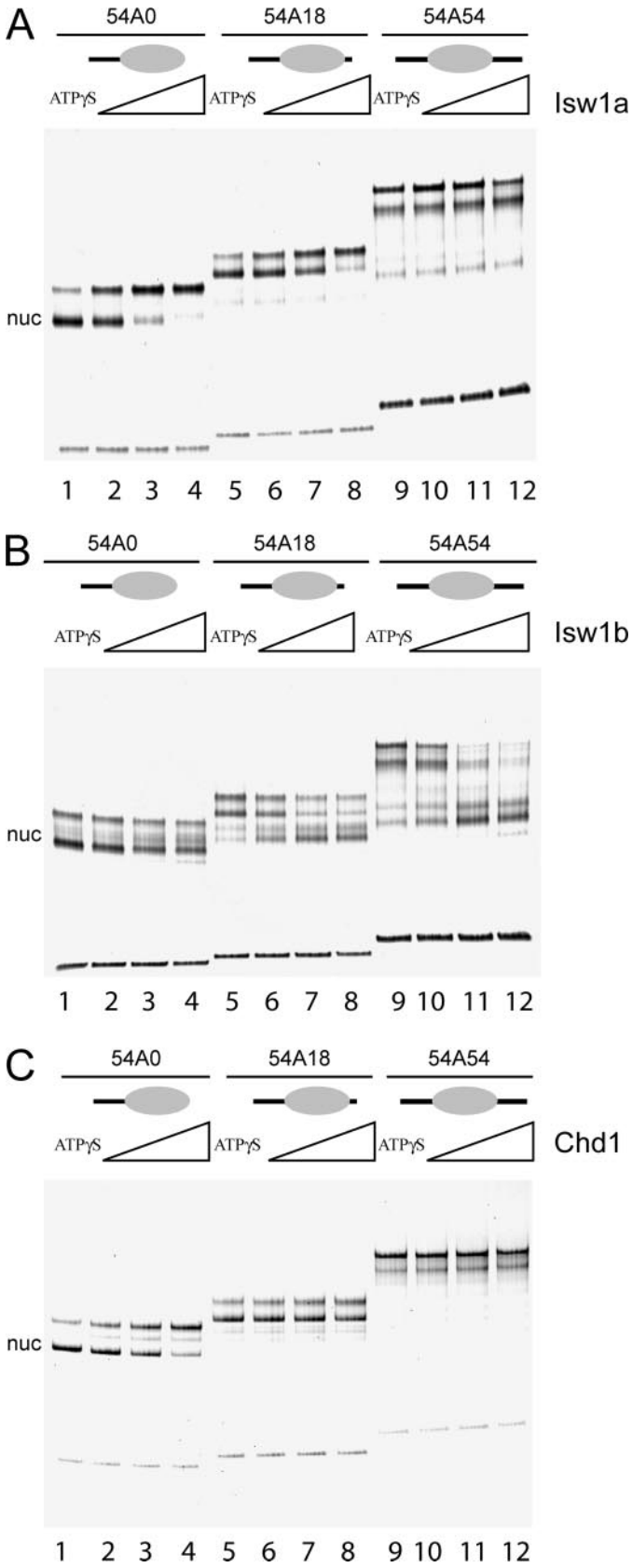


FIGURE 1. **Native gel analysis of nucleosome repositioning catalyzed by Isw1a, Isw1b, and Chd1.** Nucleosomes were assembled onto DNA fragments such that they were initially located at one end of a DNA fragment (54A0 nucleosomes), off center (54A18 nucleosomes), or central (54A54 nucleosomes) prior to incubation with Isw1a (*A*), Isw1b (*B*), or Chd1 (*C*). Isw1a and Chd1 were more efficient at moving nucleosomes from terminal to more central positions, whereas Isw1b moves nucleosomes with the opposite polarity. Approximately 1 pmol of nucleosomes were incubated with Isw1a and Isw1b at 40, 200, and 1000 fmol and 45, 90, and 180 fmol Chd1 for 30 min at 30 °C. All reactions contained 1 mM ATP except those labeled ATPγS, which contained 1 mM ATPγS and the maximum quantity of remodeler.

extensions. Nucleosomes were redistributed predominantly to the +39, +42, and +47 locations on the template with a 54-bp extension (Fig. 2*B*, *lanes 1–3*). As the length of the DNA extension is reduced to 44 and 38 bp, there is a progressive decrease in the proportion of nucleosomes accumulating at the +39 and +42 locations, while nucleosomes are still relocated to the +47 position efficiently (Fig. 3, *lanes 4–9*). It is also notable that the proportion of nucleosomes accumulating at the +58 location increases as the length of the linker DNA is reduced. For the fragments with 34- and 31-bp extension nucleosomes that have relocated 12 bp to the +58 location are the major new species observed following remodeling. It is possible that this 12-bp movement represents a minimal distance that the mechanical action of the Isw1a ATPase motor can move a nucleosome. However, it is also possible that this is the first location that is sufficiently stable to be detected.

Overall, it was found that reducing the length of the DNA extension caused nucleosomes to be moved to locations progressively closer to the starting position. It is also notable that the efficiency of redistribution decreases as the length of linker DNA is reduced, with a greater proportion of nucleosomes remaining at their original location on the shorter fragments.

The nearest to a DNA end that we have observed Isw1a relocate nucleosomes is 15 bp (Fig. 3, *lanes 7–9*). However, the range over which DNA ends influence positioning varies depending on the context. For example, the +47 location is disfavored despite being 31 bp from a DNA end in the presence of a 34 or 54 bp downstream linker (supplemental Fig. 2, *lanes 6–13*).

*Isw1a Repositions Nucleosomes to Thermodynamically Favorable Locations*—In addition to the exclusion of nucleosomes from regions close to the DNA ends, the data in Figs. 2 and 3 show that Isw1a, Isw2, and Chd1 move nucleosomes to discrete locations that are not located at the geometric center of the DNA fragment. We next sought to investigate what underlies the selection of these more central locations. We first characterized the positions to which nucleosomes relocate during thermal incubation in more detail. Nucleosomes were assembled onto series of DNA fragments designed to form nucleosomes with progressively shorter DNA extensions. On nucleosomes with 48 and 44 bp extensions we observed nucleosomes being redistributed to the positions at +22 and +27 as reported previously (4) (Fig. 4, *A* and *B*, *lanes 2–6*). To determine the location of less favorable positions, thermal redistribution was carried out on shorter fragments where these most favorable locations were no longer present. Reduction of the DNA extensions to 38 and 36 bp revealed new locations at +31, +39, +42, and +47 (Fig. 4, *A* and *B*, *lanes 8–13*). It is notable that of these less strongly preferred positions, the +39, +42, and +47 locations were also observed during Isw1a-driven redistribution. This supports the hypothesis that the positioning properties of the DNA contribute to the sites selected by Isw1a. In fact, the positions observed following redistribution by Isw1a appear to result from rearrangement between favorable locations with the exception that locations too close to a DNA ends are excluded.

A prediction of this hypothesis would be that if DNA fragments were designed on which the most highly preferred +22 and +27 locations were sufficiently far from DNA ends, then they would be occupied following redistribution by Isw1a. Fig. 4*C* shows that this is indeed true. Nucleosomes initially located predominantly at the +39

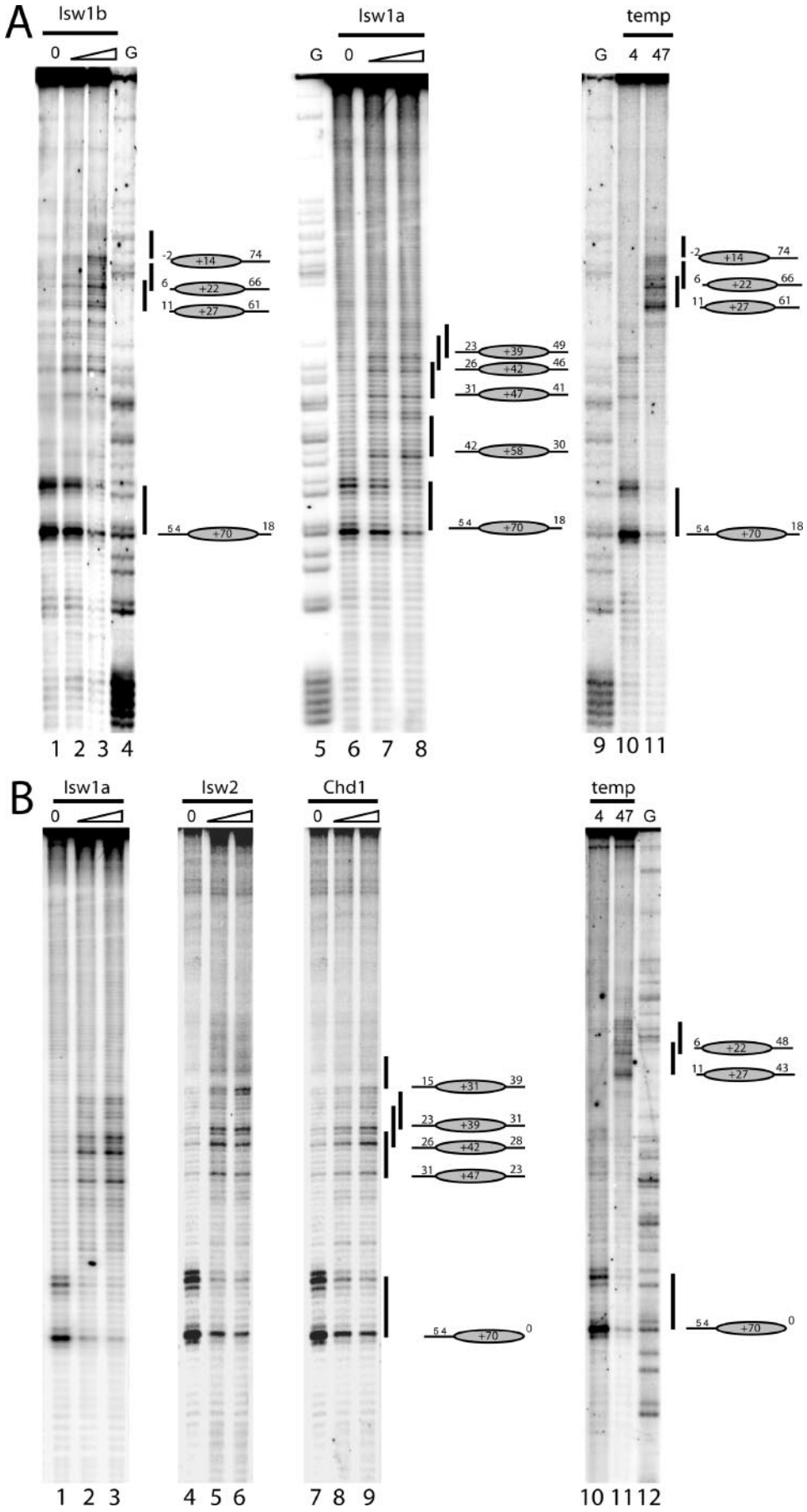


FIGURE 2. **High resolution mapping of nucleosome repositioning by Isw1a, Isw1b, Chd1, and temperature.** To precisely determine the positions to which nucleosomes were relocated following assembly on the off center (54A18; *A*) or end positioned (54A0; *B*) fragments, repositioning assays were carried out using histone octamers derivatised with nucleosome mapping reagent (see "Experimental Procedures"). Nucleosomes were initially assembled predominantly at the +70 location (70 bp upstream of the MMTV transcription start site). Following incubation of the 54A18 fragment with Isw1b (37.5 fmol (*lane 2*) and 75 fmol (*lane 3*)) or at high temperature (47 °C, 1 h), nucleosomes were redistributed predominantly to the +22 and +27 locations. In contrast following incubations with Isw1a (160 fmol (*lane 7*) and 320 fmol (*lane 8*)) nucleosomes were moved to the +39, +42, +47, and +52 positions. The 54A0 nucleosome was relocated by Isw1a (160 fmol (*lane 2*) and 320 fmol (*lane 3*)), Isw2 (1.6 fmol (*lane 5*) and 3.2 fmol (*lane 6*)), and Chd1 (3.6 fmol (*lane 8*) an 7.2 fmol (*lane 9*)) to positions at +39, +42, and +47. In addition Isw2 caused an increase in nucleosomes located at the +39 location (*lane 6*). In contrast incubation of this nucleosome at 47 °C for 1 h resulted in its relocation to the +22 and +27 sites. G tracks of the same DNA molecules were used to confirm the start positions of the nucleosomes and are shown in the *lane* marked *G*.

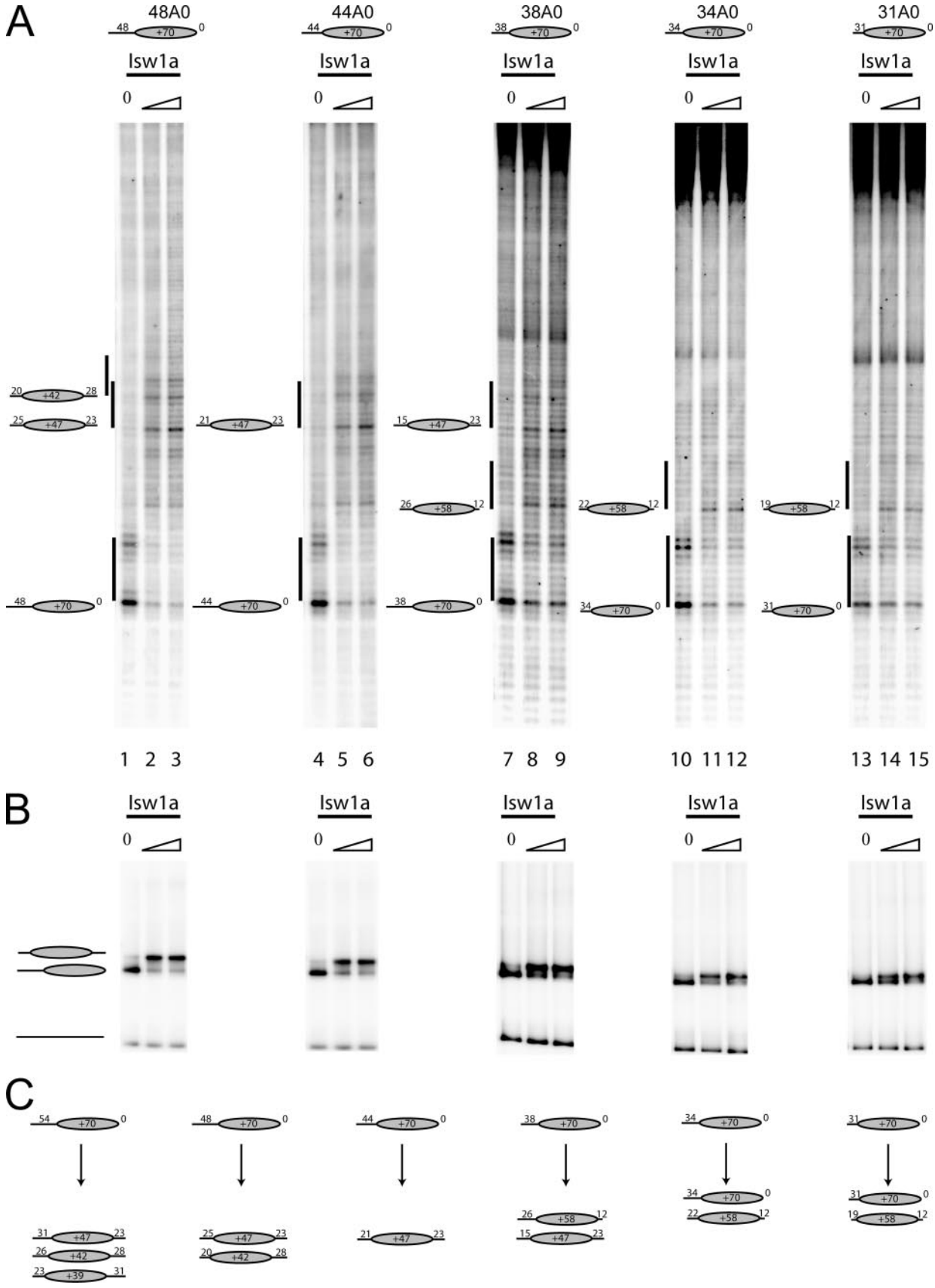


FIGURE 3. **Isw1a moves nucleosomes to a subset of locations no closer than 15 bp from a DNA end.** *A*, to systematically investigate the positions to which nucleosomes are redistributed by Isw1a, remodeling reactions were performed on a series of fragments with successively shorter DNA extensions. The amounts of Isw1a used were 36 fmol (*lanes 14*, *17*, *20*, and *21*), 107 fmol (*lanes 11*, *15*, *18*, *21*, and *25*), 320 fmol (*lane 12*). 10% of each remodeling reaction was loaded on a 5% native gel (*B*). The positions to which nucleosomes were redistributed are illustrated schematically in *C*.

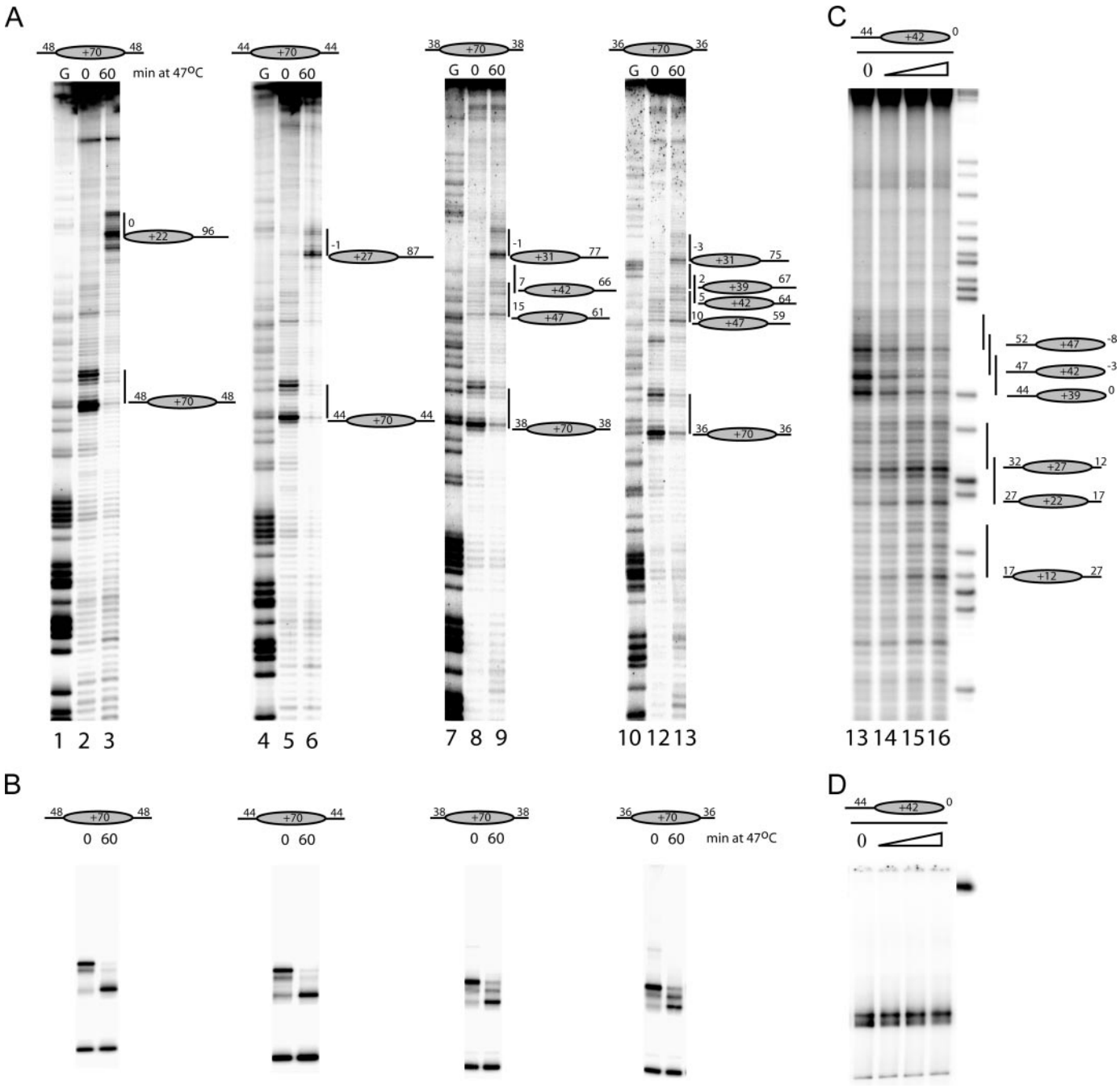


FIGURE 4. **Isw1a relocates nucleosomes to thermodynamically favorable locations.** Thermal redistribution of histone octamers with symmetrical linkers of 36, 38, 44, or 48 bp was analyzed by site-directed mapping (*A*) and native gels (*B*). Nucleosomes with shorter linker DNAs were observed to move to the +39, +42, and +47 locations that are also used by Chd1, Isw2, and Isw1a (see Fig. 2). Nucleosomes deposited predominantly at the +42 location are found to be repositioned to the thermally favored locations at +27 and +22 by Isw1a using site-directed mapping (*C*) and native gel electrophoresis (*D*). Amounts of Isw1a used were 0, 36, 107, or 320 fmol (*lanes 13–16*). Note that DNA is labeled at the upstream end in *C* but on the downstream side in *A*.

position with a 44-bp DNA extension are redistributed to the highly favorable positions at +27 and +22 (Fig. 4*C*, *lanes 13–16*). Similarly it would be anticipated that the bias against an unfavoured location would be reduced by increasing the linker DNA that flanks it. This is illustrated for the +70 location. Although nucleosomes are deposited during assembly at this location, it is not favored following redistribution on a DNA fragment with an asymmetric extension. However, extension of linker DNA on the downstream side of this location such that it is placed more centrally progressively increases occupancy at this site (supplemental Fig. 2).

*Chromatin Remodeling Enzymes Display a Preference for Interacting with Linker DNA That Correlates with the Orientation in Which They Reposition Nucleosomes*—It has previously been proposed that a factor contributing to the preference of the Isw2 remodeling enzyme for the repositioning of nucleosomes to central locations could be the preference of this enzyme for interaction with nucleosomes containing additional linker DNA (30). If this model is generally applicable to other remodeling enzymes that move nucleosomes to more central locations, then it would be anticipated that any enzyme displaying a preference for the relocation of nucleosomes to

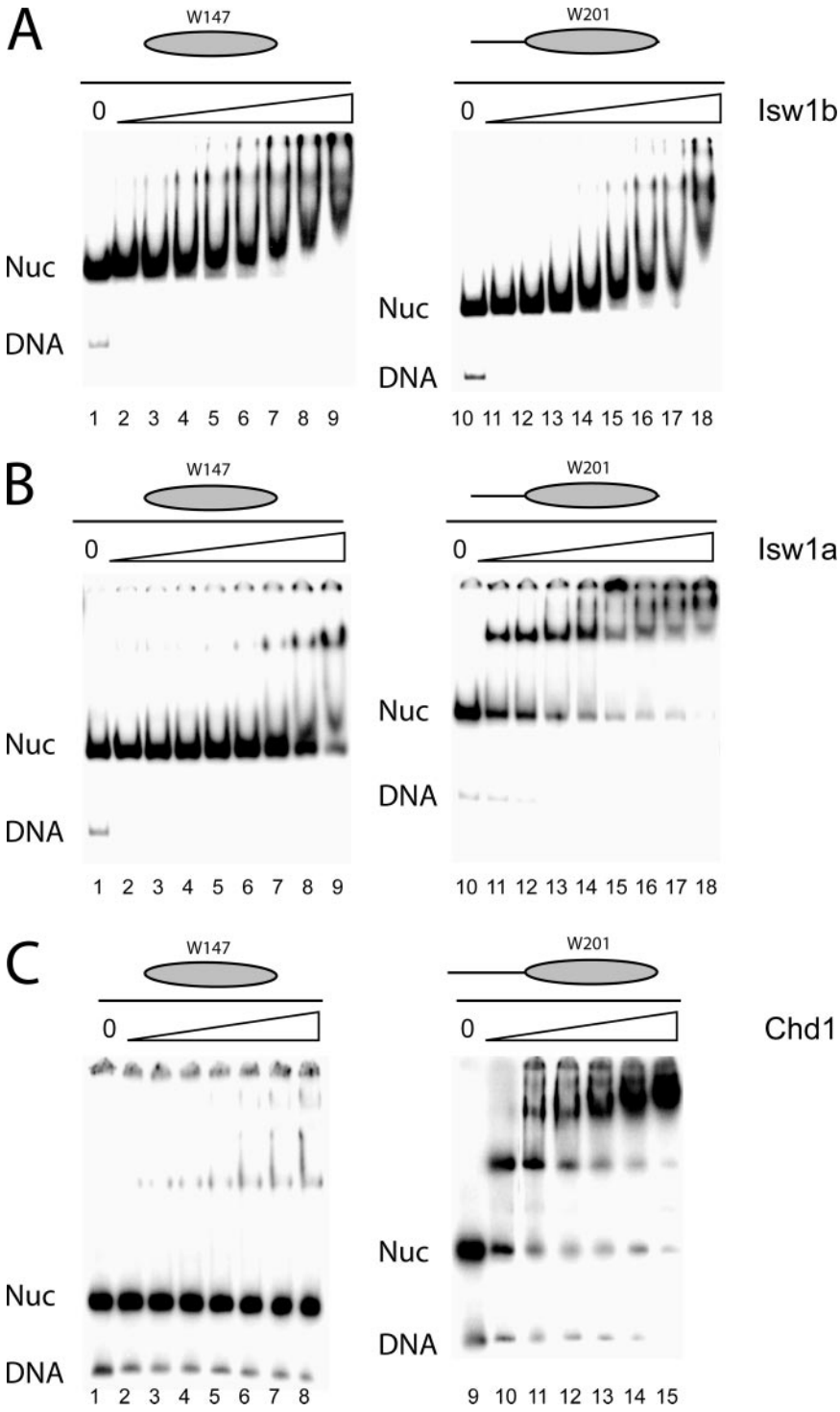


FIGURE 5. **Remodeling enzymes that relocate nucleosomes (*Nuc*) to more central locations preferentially bind nucleosomes bearing linker DNA.** Nucleosomes were assembled onto 147-bp DNA (*W147*) or with 54-bp linker DNA on one side (*W201*). The binding of Isw1b (*A*), Isw1a (*B*), and Chd1 (*C*) to these templates was investigated by native gel electrophoresis. While Isw1b bound to both templates similarly, Isw1a and Chd1 preferentially engage with nucleosome bearing linker DNA. Remodeler concentrations were varied between 0.74 and 12.8 nM for Isw1a, between 2.6 and 45 nM for Isw1b, and between 1.4 and 24 nM for Chd1.

more central locations would also display this property. To extend the repertoire of remodeling enzymes for which this type of investigation has been performed, we investigated the binding of Isw1a, Isw1b, and Chd1 to nucleosomes with minimal and extended linker DNA. Isw1a and Chd1, which both preferentially relocate nucleosomes to more central locations, were both found to display a preference for interacting with nucleosomes containing linker DNA. In contrast Isw1b, which can move nucleosomes to DNA ends, was able to interact with nucleosomes in a way that was not influenced by the presence of linker DNA (Fig. 5). These observations provide additional support for the model proposed by Bartholomew and co-workers (18) in which the preference of the remodeling enzymes for nucleosomes bearing linker DNA facilitates the removal of nucleosomes from DNA ends.

## DISCUSSION

We have characterized nucleosome redistribution by the Isw1a, Isw1b, Isw2, and Chd1 remodeling complexes. Although they share the same Isw1p catalytic subunit, the Isw1a and Isw1b complexes redistribute nucleosomes with different apparent directionalities: the Isw1a complex removes nucleosomes from locations within

about 15 bp from DNA ends whereas Isw1b does not. We also find that the Chd1 protein is able to move nucleosomes and does so with the same directionality as Isw1a and Isw2. Importantly, we find that the inherent nucleosome positioning properties of the underlying DNA play an important role in determining the destinations to which nucleosomes are moved by all of these enzymes. This is consistent with previous studies suggesting that enzymes that do not remove nucleosomes from positions close to DNA ends move nucleosomes to thermodynamically favorable locations (4, 16). However, for enzymes that move nucleosomes with the opposite directionality this is not the case. Instead, it has been reported that the most favorable locations are not selected (18, 30). We believe that the reason for confusion on this issue is that the outcome of remodeling reactions performed using enzymes such as Isw2 involves a compromise between the selection of thermodynamically favored locations and the inability to move nucleosomes to positions close to DNA ends. This means that while the positions selected are favorable in comparison with the surrounding locations, they need not necessarily represent the most stable nucleosome positioning sequence on a given DNA fragment.

To illustrate how enzymes such as Isw2 select more central locations, we generated a plot illustrating the location and relative preference for the different locations along MMTV DNA that we have detected (Fig. 6*A*). Remodeling enzymes might cause redistribution between these sites according to a number of different schemes. For example, an enzyme able to rapidly associate and dissociate from nucleosomes along an idealised DNA with no preferential nucleosome positioning properties would redistribute nucleosomes along the DNA fragment uniformly as shown in Fig. 6*B*, *trace 1*. However, certain DNA motor proteins have been observed to undergo a prolonged dissociation and reassociation reaction upon reaching a DNA end (31). Such behavior would result in an idealised distribution similar to that shown in Fig. 6*B*, *trace 2*. Superposing this with the nucleosome positional preference shown in Fig. 6*A* results in a distribution of nucleosomes with a bias toward DNA ends (Fig. 6*C*). This is very similar to the pattern of redistribution observed by Isw1b (Fig. 2*A*) and previously observed for NURF and ISWI (4, 16). Note that while the enzyme itself exhibits no preference for either end of the DNA, the structural properties of the DNA fragment result in the preferential accumulation of nucleosomes at the end with more favorable nucleosome positioning sequences.

In contrast, the inability to move nucleosomes closer than about 15 bp from DNA ends gives rise to an idealized distribution illustrated in Fig. 6*B*, *trace 3*. However, the 15-bp exclusion limit we found for Isw1a is a lower limit, and DNA ends were observed to reduce occupancy at otherwise favorable locations over distances of at least 34 bp (data not shown). Such behavior is represented by the idealised distribution in Fig. 6*B*, *trace 4*. If this trace is scaled to fit a DNA fragment with a 54-bp extension on one side, it can be superposed with the nucleosome positional preferences shown in Fig. 6*A* to provide a means of modeling the outcome of Isw1a redistribution on this fragment (Fig. 6*D*). The modeled outcome fits well with that obtained experimentally (Fig. 2*B*, *lanes 1–3*). If a similar process is performed on a DNA fragment with a shorter extension, then the idealized trace has to be adjusted to meet the new dimensions of the DNA fragment (supplemental Fig. 3*C*), but again, the outcome of superposition is in good agreement with the experimental data. In fact this holds true for all fragments we have studied (supplemental Fig. 3). This correlation strengthens support for the hypothesis that redistribution between thermally favorable locations that do not encroach upon DNA ends underlies the repositioning of nucleosomes by Isw1a. However, it is important to point out that this model remains qualitative because we have not quantitatively measured either the free energy of nucleosome positioning (32) or the energetic penalty associated with Isw1a moving nucleosomes close to DNA ends. Nonetheless, it provides a basis for understanding the outcome of remodeling carried out by enzymes such as Isw1a.

While most of the data we have presented has been obtained using Isw1a, we believe that the principles involved are likely to be applicable to other enzymes that act with this apparent directionality. For example, the Isw2 remodeling complex has previously been found to redistribute nucleosomes to positions no closer than 13 bp from DNA ends (30). This is very similar to the limit of 15 bp we have observed for Isw1a (Fig. 3). In the case of Isw2 it has been proposed that preferential binding to nucleosomes containing linker DNA may underlie the exclusion of nucleosomes from locations close to DNA ends (18). The rationale for this is that if a remodeling enzyme requires contact with linker DNA on one side of a nucleosome to move it in that direction, the DNA available will be reduced as the nucleosome approaches the end of the fragment making it difficult to move any further (30) (Fig. 6*E*). Our observation that the Isw1a and Chd1 activities preferentially bind to nucleosomes bearing linker DNA but the Isw1b complex does not (Fig. 5) provides further support for this model and suggest it may be generally applicable. In fact published observations suggest that it applies for ACF and Mi-2 (16, 33). Our observation that positioning sequences also contribute to the process explains why nucleosomes are not always moved to the geometric center of relatively short mononucleosomal DNA fragments.

Interestingly, Bartholomew and co-workers (30) previously observed that while extending linker DNA to 20 bp most significantly improves binding of Isw2 to nucleosomes, additional linker DNA extending to over 60 bp has more subtle effects. The strong requirement for a short length of linker DNA together with an extended region over which more subtle effects are observed is consistent with the broad range over which we observe that DNA ends can influence nucleosome positioning by Isw1a.

We also report here the yeast Chd1 protein can slide nucleosomes and does so with a directionality to move nucleosomes away from DNA ends in a manner that is very similar to the Isw2 and Isw1a complexes. This similarity in action may be related to the fact that these complexes perform partially redundant functions *in vivo* (19).

Although our observations are made using short DNA fragments on which DNA ends are encountered at a far higher frequency than would be expected in a physiological setting, we believe that they reflect important mechanistic differences in the way these complexes function. The behavior of remodeling enzymes as they encounter a DNA end may be relevant to nucleosome remodeling in proximity to other barriers which *in vivo* are most likely to be adjacent nucleosomes or bound transcription factors (Fig. 6*E*). Supporting this, many of the enzymes that move nucleosomes to more central locations have the ability to space arrays of nucleosomes (19, 21, 23). Although some enzymes that have the ability to move nucleosomes to positions adjacent to DNA ends have also been reported to be able to space nucleosomes, they appear to be less efficient in this assay (23). If the variable range over which we observe DNA ends influence nucleosome positioning also applies in the context of a nucleosome spacing reaction, we anticipate that these enzymes would be capable of establishing nucleosome spacings over the range 15 to at least 34 bp.

In biological contexts, movement of nucleosomes away from barriers may act to organize chromatin, while enzymes that move nucleosomes close to barriers may be more disruptive. An example where this may

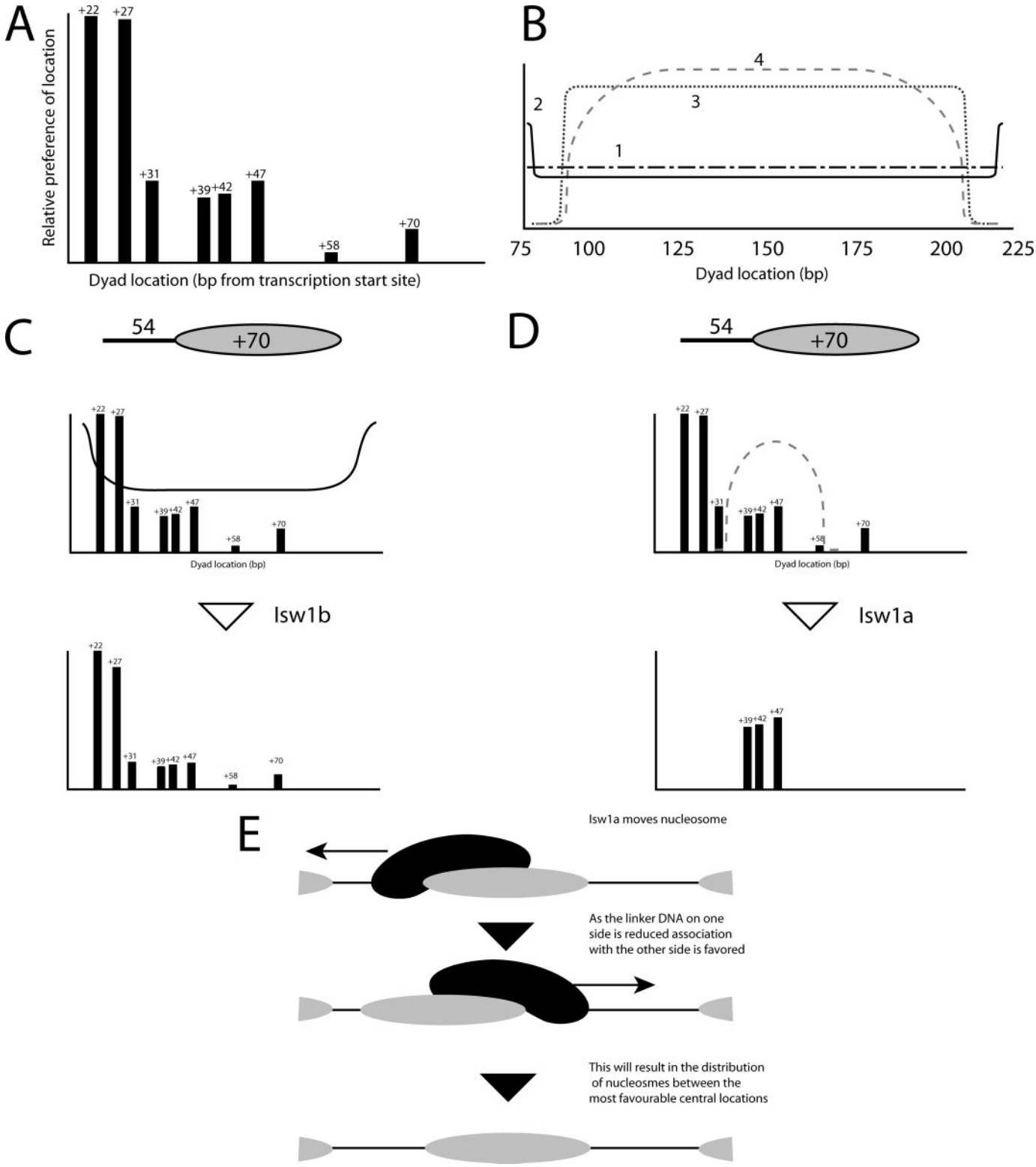


FIGURE 6. **Superpostion of nucleosome positioning preferences with enzyme-specific distribution patterns models the outcome of remodeling reactions on many DNA fragments.** *A*, the hierarchy of favorable nucleosome locations over the MMTV NucA region was estimated from analysis such as that shown in Fig. 4. *B*, idealized nucleosome distribution patterns for a 200-bp DNA fragment with no nucleosome positioning properties. *Trace 1*, remodeler distributes nucleosomes at uniform velocity; *trace 2*, remodeler undergoes a delay at DNA ends; *trace 3*, remodeler is excluded from 15 bp close to DNA ends; *trace 4*, enzyme exhibits progressive bias a against positions from 45 to 15 bp from the DNA end. Superposition of *trace 2* with the positioning preferences for a DNA fragment with upstream extensions of 54 bp results in a close match to the experimental observed repositioning with Isw1b (*C*). Superposition of *trace 4* with the positioning preferences of the same DNA fragment results in a close match to the experimental observed repositioning with Isw1a (*D*). Scaling of *trace B* to fit other DNA fragments used shows that this fit holds true for all of the DNA fragments studied (supplemental Fig. 3). Note that while the distribution patterns of the enzymes are asymmetrical, the positioning properties of the DNA can result in asymmetrical nucleosome distributions. *E* illustrates how a requirement for engagement with linker DNA could result in the redistribution between favorable locations that are central with respect to barriers such as the ends of a short DNA fragment or adjacent nucleosomes.

hold true is provided by the *Saccharomyces cerevisiae MET16* promoter (34). Here, Isw1a is involved in establishing nucleosome positioning that is refractory to transcription initiation. When expression is induced, the repositioning of a nucleosome by Isw1b appears to play an important role in regulating the amount of RNA polymerase II that is able to enter productive elongation. Further studies will be required to establish whether remodeling enzymes that remove nucleosomes from barriers generally act to organize chromatin.

Our observations suggest that the inherent nucleosome positioning properties of DNA fragments play a role in determining the outcome of remodeling reactions. This means that the underlying structural properties of DNA are likely to establish a context that plays an important role in determining the outcome of remodeling reactions carried out by ISWI and Chd1 complexes. The observation that nucleosomes are shuttled between relatively favorable locations also indicates that, like many classical enzymes, these remodelers act to accelerate the otherwise slow redistribution of their substrate between energetically favored states.

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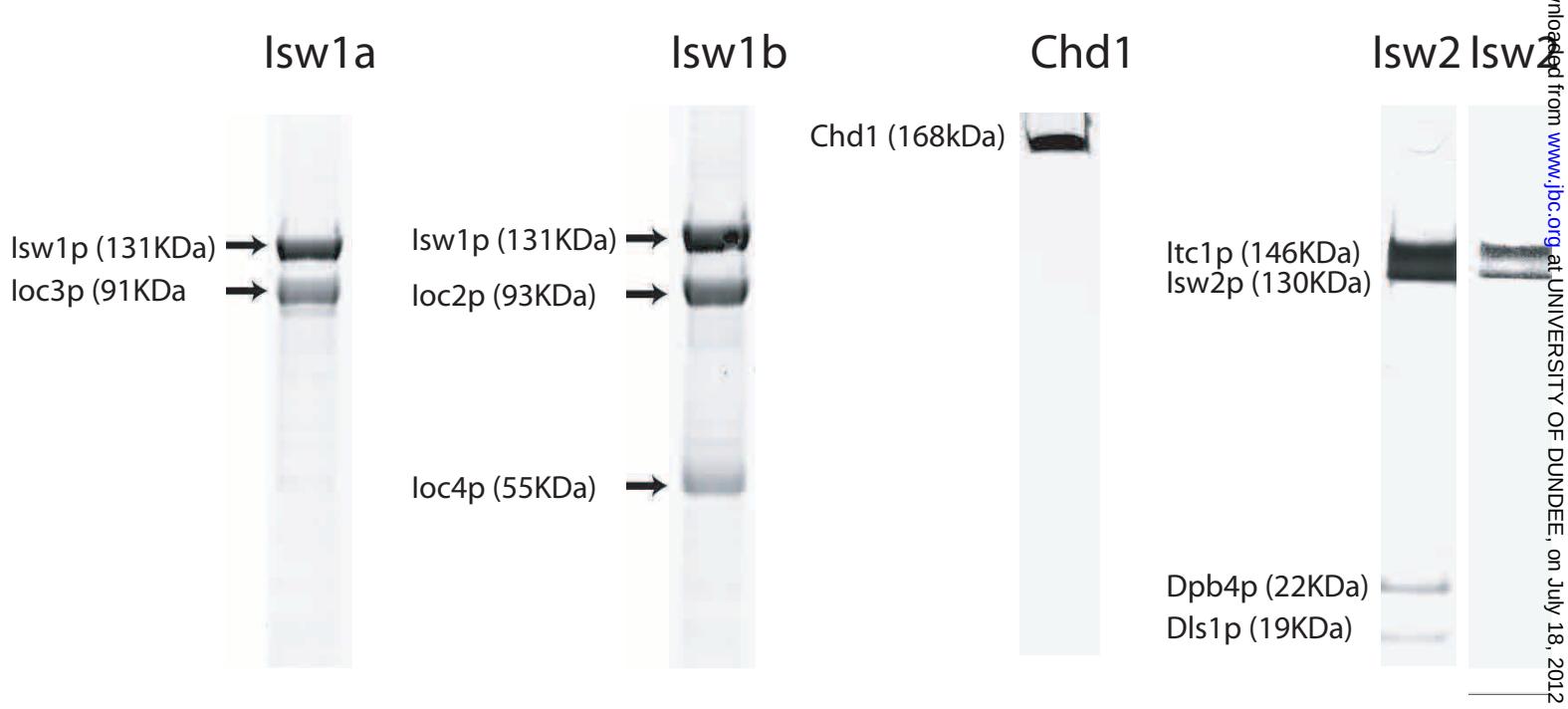

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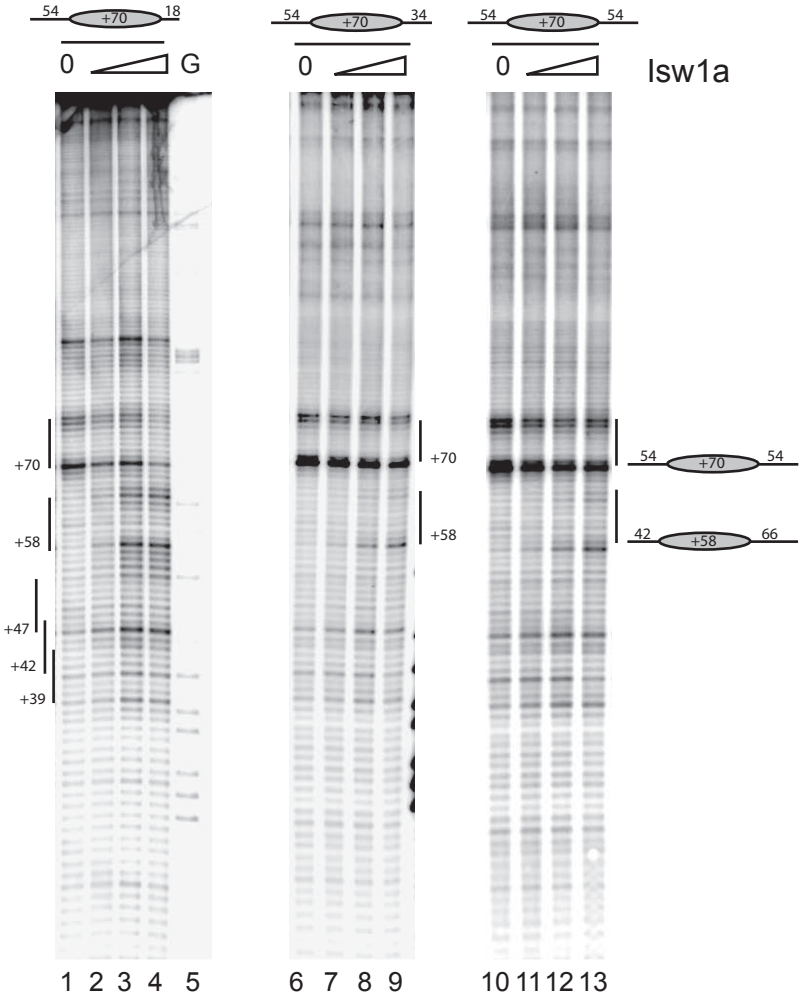


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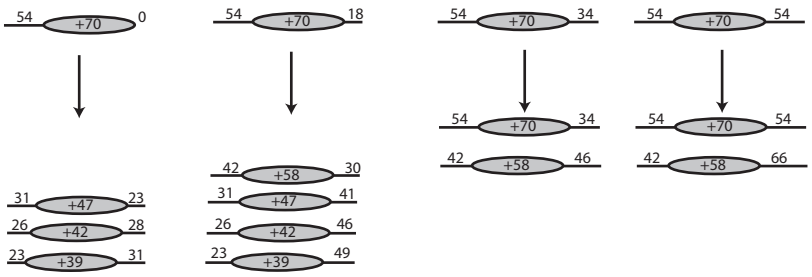

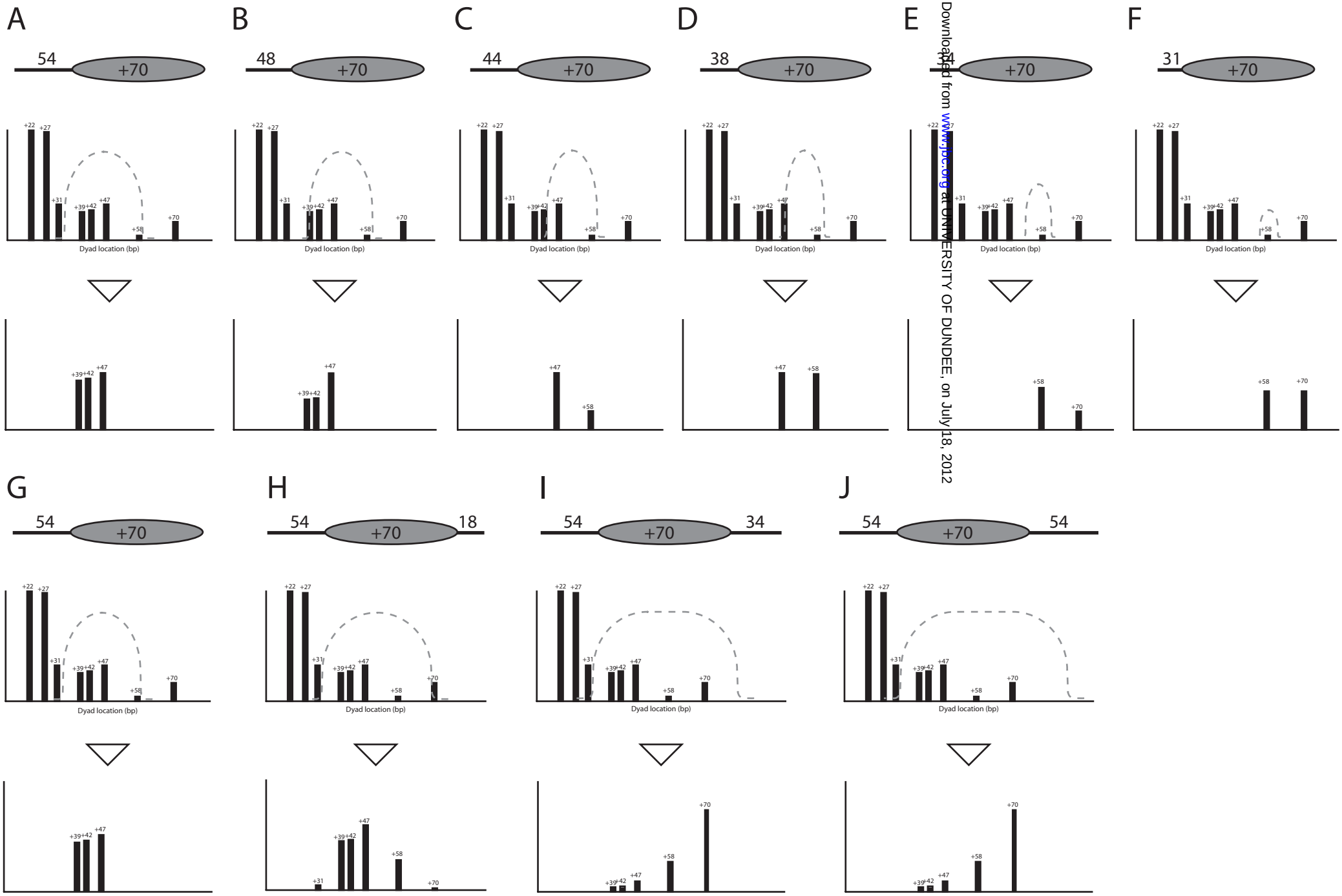

Supplementary Figure 1. SDS polyacrylamide gels indicating the purity of the remodelling enzymes used in this study. The subunits present in each complex together with their molecular weights are indicated. The migration of the bands correlated well with their expected mobility in comparison to molecular weight markers (not shown). The gels for the Isw1a, Isw1b and Chd1 complexes were stained with Sypro Orange. The gel for Isw2 was silver stained. Two exposures are shown so that both the small subunits can be detected and the large subunits distinguished as separate species.

Supplementary Figure 2. The effect of progressive extension of the downstream linker DNA extension on redistribution by Isw1a. Nucleosomes were assembled onto DNA fragments with 54bp upstream extensions and 18 (Lanes 1-5), 34 lanes (6-9) and 54 bp (lanes 10-13) extensions on the downstream side. Repositioning reactions were carried out in the presence of Isw1a 100, 200, 400 fmoles lanes 2, 3 and 4; 36 fmoles, lanes 7 and 11, 107 fmoles lanes 8 and 12, 320 fmoles lanes 9 and 13. The positions to which nucleosomes are relocated are illustrated schematically in (B). The positions for a fragment with no downstream extension (obtained from Figure 2) are also illustrated.

Supplementary Figure 3. Superposition of nucleosome positioning preferences with the idealised Isw1a DNA end exclusion profile matches experimental observations on all DNA fragments used. Superposition of the nucleosome positioning preferences with the idealised Isw1a profile as described for Figure 6D, but scaled to fit each of the DNA fragments studied. In all cases the results fit well with the experimentally obtained data.