

# Multiple modes of regulation of the human Ino80 SNF2 ATPase by subunits of the INO80 chromatin-remodeling complex

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## SIGNIFICANCE

SNF2 family ATPases are ATP-dependent motors with roles in diverse nuclear processes. Regulation of the catalytic activity of SNF2 family ATPases via their associated proteins is a key element determining their functions. Thus, illuminating the mechanisms of action of SNF2 regulatory proteins is a priority of current studies. Here, we show subunits of the human INO80 chromatin-remodeling complex act by different mechanisms: Ies2 activates INO80 catalytic activity, while Ies6 and Arp5 function in nucleosome recognition.

**Keywords:** INO80 complex, enzyme activity

## ABSTRACT

SNF2 family ATPases are ATP-dependent motors that often function in multisubunit complexes to regulate chromatin structure. Although the central role of SNF2 ATPases in chromatin biology is well established, mechanisms by which their catalytic activities are regulated by additional subunits of chromatin-remodeling complexes are less well understood. Here we present evidence that the human Inositol auxotrophy 80 (Ino80) SNF2 ATPase is subject to regulation at multiple levels in the INO80 chromatin-remodeling complex. The zinc finger histidine triad domain-containing protein Ies2 (Ino Eighty Subunit 2) functions as a potent activator of the intrinsic catalytic activity of the Ino80 ATPase, whereas the YL-1 family Ies6 (Ino Eighty Subunit 6) and actin-related Arp5 proteins function together to promote binding of the Ino80 ATPase to nucleosomes. These findings support the idea that both substrate recognition and the intrinsic catalytic activities of SNF2 ATPases have evolved as important sites for their regulation.

The evolutionarily conserved Ino80 SNF2 family ATPase is the catalytic subunit of the multisubunit INO80 chromatin-remodeling complex (INO80) with functions in transcription, DNA replication, and DNA repair (1–8). Although it is well established that SNF2 family ATPases have evolved to play critical roles as ATP-dependent motors that drive many types of chromatin transactions, in most cases how their intrinsic catalytic activities are regulated by their diverse array of associated proteins is poorly understood.

The INO80 complex was first identified in *Saccharomyces cerevisiae*, where it was shown to be composed of roughly 15 subunits (1, 9). Subsequent purification of the human INO80 complex revealed that it shares with its *S. cerevisiae* counterpart a set of subunits including the Ino80 SNF2 ATPase, the AAA+ ATPases Tip49a and Tip49b, actin-related proteins Arp4, Arp5, and Arp8, and the Ies2 and Ies6 proteins (2, 10–12). The human INO80 complex lacks orthologs of the remaining subunits of the *S. cerevisiae* INO80 complex and contains instead several apparently metazoan-specific subunits including the deubiquitinating enzyme Uch37, the gene altered in glioma (GLI)–Kruppel family zinc finger transcription factor YY1, the forkhead-associated domain (FHA) containing Mcrs1, nuclear factor related to kB (Nfrkb), and the Amida, Ino80D (FLJ20309), and Ino80E (FLJ90652) proteins.

The INO80 complex is capable of regulating chromatin structure in at least two ways. First, it catalyzes ATP-dependent sliding of histone octamers along DNA (1, 2). Second, it catalyzes the replacement of histone H2AZ/histone H2AB dimers in nucleosomes with histone H2A/histone H2B dimers in a reaction that in yeast has been shown to contribute to genome-wide histone H2AZ localization (13).

In a recent study dissecting mechanism(s) by which the human INO80 complex catalyzes chromatin remodeling, we found that it is composed of at least three modules that assemble with three distinct domains of the Ino80 protein (14) (Fig. 1A). One module is composed of an Ino80 N-terminal domain and all of the metazoan-specific subunits except YY1. A second, the helicase–SANT-associated (HSA) module, is composed of the Ino80 HSA domain, the actin-related Arp4/Baf53a and Arp8 proteins, and YY1; and a third, which we refer to as the SNF2 module, is composed of the Ino80 SNF2 ATPase domain, the AAA+ ATPases Tip49a and Tip49b, the Ies2 and Ies6 proteins, and Arp5. Through purification and assay of INO80 subassemblies containing different combinations of these modules, we demonstrated that maximal ATP-dependent nucleosome sliding by the human INO80 complex is catalyzed by a core complex, referred to as INO80 $\Delta$ N, composed of both the HSA and SNF2 modules but lacking the Ino80 N-terminal domain and its associated metazoan-specific subunits. This study did not, however, shed light on functions of individual subunits of the INO80 complex in nucleosome remodeling.

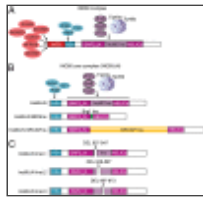


Fig. 1.

Ino80 mutants used in this study. (A) Diagram showing modular assembly of the complete INO80 complex. (B) Ino80ΔN and derivatives. In Ino80ΔN BRGIns and Ino80ΔN SRCAPIns, the Ino80 insertion region, residues 824–1099 of ...

In this report, we explore the mechanism(s) by which individual subunits of the human INO80 complex regulate the catalytic activity of the Ino80 SNF2 ATPase to bring about nucleosome remodeling. We identify distinct functions for the Ies2, Ies6, and Arp5 proteins in regulation of the intrinsic catalytic activity of the Ino80 SNF2 ATPase and in targeting of the INO80 complex to nucleosomes. Our findings argue *i)* that the zinc finger histidine triad (HIT) domain-containing Ies2 protein functions as a molecular switch that potently activates Ino80 DNA-dependent ATPase activity and *ii)* that Ies6 and Arp5 appear not to affect Ino80's ability to catalyze ATP hydrolysis but instead promote binding of the INO80 complex to nucleosomes to help initiate the remodeling reaction. Taken together, our findings provide insights into how ATP-dependent SNF2 family motors are regulated in the context of multisubunit chromatin-remodeling enzymes.

## RESULTS

To investigate mechanism(s) underlying assembly of the catalytically active INO80ΔN core complex and the functions of its subunits, we sought *i)* to isolate mutant core complexes lacking one or more subunits and *ii)* to compare their abilities to catalyze DNA- or nucleosome-dependent ATP hydrolysis and ATP-dependent nucleosome remodeling and to bind to nucleosomes. Because we and others have shown previously that the Ino80 ATPase acts as a scaffold upon which the remaining subunits assemble (9, 14, 15), we reasoned that it might be possible to identify variants of Ino80ΔN that would support assembly of complexes containing only a subset of the INO80 core subunits. To accomplish this, we generated a series of HEK293 cell lines stably expressing N-terminally FLAG epitope-tagged Ino80ΔN ATPase (F-Ino80ΔN) variants (Fig. 1). In addition, we used RNAi to deplete particular INO80 core subunits from cells expressing F-Ino80ΔN. Complexes containing F-INO80ΔN or its variants were then purified by anti-FLAG immunoaffinity chromatography and characterized biochemically.

**Insertion Region of the INO80 SNF2-like ATPase Domain Directs Assembly of the Catalytically Active INO80ΔN Core Complex.** SNF2-like ATPases share a catalytic core composed of conserved SNF2N and helicase superfamily carboxyl-terminal (HELICc) domains separated by a variable length insertion (reviewed in ref. 16). Ino80 belongs to a family of related SNF2-like ATPases, which also includes human Snf2-related cAMP-responsive element binding protein activator protein (SRCAP) and its yeast ortholog Swi2/snf2-related 1 (Swr1) and p400/Domino. Ino80 family ATPases are characterized by the presence of a long insertion sequence separating SNF2N and HELICc, whereas most other SNF2-like ATPases, including the human SWI/switch/sucrose nonfermentable (SNF) catalytic subunits Brg1 or Brm, have much shorter insertions.

To assess the contribution of the Ino80 insertion region to assembly of the INO80 core complex, we compared the composition of complexes containing F-Ino80ΔN to that of complexes containing F-Ino80ΔN BRGIns, in which the ~250 amino acid Ino80 insertion was replaced by the 24 amino acid insertion from Brg1 (Fig. 1B and Fig. S1A). We also prepared complexes containing a second mutant, F-Ino80ΔN BRGInsEQ, which carries a DEAD/H (Asp-Glu-Ala-Asp/His) box Glu→Gln (EQ) point mutation that abolishes the ATPase and nucleosome-sliding activities of the INO80ΔN complex (14). Each complex was analyzed by SDS/PAGE (Fig. 2A) and Western blotting (Fig. 2B). Whereas subunits from all three modules copurified with F-Ino80ΔN as expected, only HSA module subunits copurified with F-Ino80ΔN BRGIns or F-Ino80ΔN BRGInsEQ, (compare Fig. 2A, lanes 2 and 3, with Fig. 2A, lane 1; and Fig. 2B, lanes 4 and 5, with Fig. 2B, lane 2). Thus, Ies2, Ies6, Arp5, Tip49a, and Tip49b assemble with the Ino80 ATPase in a way that depends on the presence of the Ino80 insertion.

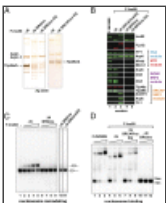


Fig. 2.

Requirement for the Ino80 insertion region. Purified chimeric INO80 complexes (purified through FLAG-tagged Ino80ΔN, Ino80ΔN BRGIns (F-Ino80ΔN BRGIns), or Ino80ΔN SRCAPIns (F-Ino80ΔN SRCAPIns) were subjected to ...

We also generated mutants F-Ino80ΔN SRCAPIns and F-Ino80ΔN SRCAPInsEQ to test the consequence of replacing the Ino80 insertion with the 1,162 amino acid insertion region from the related SNF2-like ATPase SRCAP (Fig. 1B). SRCAP/SWR1 complexes include a set of subunits that are associated with the SRCAP/Swr1 SNF2-like ATPase domain and are strikingly similar to those of INO80 core subunits. Among these are *i)* AAA+ ATPases Tip49a and Tip49b (Rvb1/2 in yeast); *ii)* actin-related protein Arp6; *iii)* ZnHIT1 (Swc6 in yeast), which shares a Zn-HIT (Zinc and histidine

triad) domain with metazoan Ies2; and *iv*) YL1 (Swc2 in yeast), which shares a YL1\_C domain with Ies6. Consistent with our evidence for a role of the Ino80 insertion in assembly of the INO80 core complex, Wu et al. (17) showed that deletion of the insertion from the Swr1 ATPase domain led to loss of Rvb1/2, Arp6, Swc6, and Swc2, as well as an apparently fungal-specific subunit, Swc3. We were unable to isolate cell lines stably expressing F-Ino80 $\Delta$ N SRCAPins, suggesting its expression might be toxic to cells. However, a catalytically inactive variant F-Ino80 $\Delta$ N SRCAPinsEQ could be stably expressed in HEK293 cells (Fig. 2 A and B). Like F-Ino80 $\Delta$ N-BRGins, it copurified with HSA module subunits (Fig. 2B, lane 3) and with the shared SNF2 module subunits Tip49a and Tip49b. In addition, it copurified with SRCAP subunits Arp6, ZnHIT1, and YL1, indicating that the insertion region is sufficient to specify the assembly of SRCAP-specific subunits into the complex.

The ATP-dependent nucleosome-remodeling activities of INO80 and INO80 $\Delta$ N core complexes catalyze the movement of positioned mononucleosomes from a lateral to a more central position of a DNA fragment, resulting in a decrease in the electrophoretic mobility of the mononucleosomes on native polyacrylamide gels (2, 14). As shown in Fig. 2C, INO80 $\Delta$ N BRGins complexes exhibited substantially reduced nucleosome-remodeling activity; in addition, nucleosome-dependent ATPase activity of these complexes was greatly reduced. A substantial fraction of the residual remodeling activity is likely due to contaminant(s) in the immunopurified complexes, because some remodeling activity was also evident in reactions performed with complexes containing the ATPase-dead mutant Ino80 $\Delta$ N BRGinsEQ (Fig. 2C; compare lanes 10 and 11).

To begin to explore mechanism(s) by which the insertion region and/or the SNF2 module subunits contribute to nucleosome remodeling and nucleosome-dependent ATPase activity, we compared the nucleosome-binding activities of complete INO80 complexes or complexes containing Ino80 $\Delta$ N and Ino80 $\Delta$ N variants by monitoring the formation of stable INO80–nucleosome intermediates in EMSAs similar to those used previously to study nucleosome binding by the yeast INO80 (18) and Rpd3S complexes (19) (Fig. 2D). Binding of these complexes to mononucleosomes resulted in the emergence of slowly migrating “shifted” bands. The shifted species formed in the presence of complete INO80 complexes migrate more slowly than those formed in the presence of the smaller INO80 $\Delta$ N complexes, consistent with the idea that they correspond to mononucleosomes stably bound by INO80 or INO80 $\Delta$ N (Fig. 2D, lanes 1–6). Notably, complete INO80 complexes and INO80 $\Delta$ N complexes, which lack the metazoan-specific Ino80 amino-terminal domain (NTD) and associated subunits, bound with similar efficiency to nucleosomes, indicating the N-terminal metazoan-specific module is dispensable not only for nucleosome remodeling and ATP hydrolysis, but also for nucleosome binding in these assays. In contrast, complexes containing Ino80 $\Delta$ N BRGins exhibited no significant nucleosome-binding activity (Fig. 2D, lanes 10–12). Because INO80 $\Delta$ N BRGins complexes lack the SNF2-like ATPase domain-binding subunits Ies2, Ies6, Arp5, and Tip49a/b but retain the HSA module subunits, this observation is consistent with the possibility that the insertion domain and/or some or all SNF2 module subunits are required for optimal nucleosome binding, which cannot be recapitulated by an INO80 subcomplex that contains only the HSA module subunits YY1, Arp8, and Arp4.

**Assembly of Ies2, Ies6, and Arp5 into the INO80 $\Delta$ N Core Complex.** Having defined the Ino80 insertion region as critical for assembly of Ies2, Ies6, Arp5, and the AAA+ ATPases Tip49a and Tip49b into the INO80 core complex, we sought to identify smaller regions within the Ino80 insertion that might be required for binding of a single subunit or set of subunits. Like the Ino80 $\Delta$ N BRGins mutant, two additional mutants with small deletions at the N-terminal end of the insertion (Ino80 $\Delta$ N–Ins $\Delta$ 1, missing residues 837–841, and Ino80 $\Delta$ N–Ins $\Delta$ 2, missing residues 849–897) assembled into complexes that included the HSA module subunits but lacked Ies2, Ies6, Arp5, and the AAA+ ATPases (Fig. 3A, lanes 3 and 4). In contrast, an additional mutant, Ino80 $\Delta$ N–Ins $\Delta$ 3 (missing residues 967–973), was able to assemble with the AAA+ ATPases, Arp5, and Ies6, but bound only 15–20% as much Ies2 as Ino80 $\Delta$ N (Fig. 3A, lane 5). Consistent with this observation, complexes purified from F-Ino80 $\Delta$ N–expressing cells that had been treated with siRNA targeting Ies2 (F-Ino80 $\Delta$ N:siIes2 cells) were substantially depleted of Ies2 but retained other subunits, indicating that the other SNF2 module subunits can assemble into the complex independently of Ies2 (Fig. 3B, lanes 9–11).

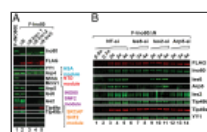


Fig. 3. Isolation of INO80 subcomplexes lacking either Ies2 or Arp5 and Ies6. (A) Complete INO80 complexes (lane 1) or INO80 subcomplexes (lanes 2–5) were purified through the indicated FLAG-tagged protein and subjected to Western blotting. The data shown ...

In an effort to generate mutant INO80 $\Delta$ N core complexes selectively lacking the remaining INO80 SNF2 module subunits, we also used siRNAs to deplete SNF2 module subunits from F-Ino80 $\Delta$ N–expressing cells. Depletion of either Arp5 or Ies6 individually led to loss of more than 90–95% of both proteins from immunopurified complexes (Fig. 3B, lanes 6–8 and 12–14), indicating that stable association of Arp5 and Ies6 with the INO80 $\Delta$ N core complex is interdependent and raising the possibility these subunits may enter the complex as a subassembly. Indeed, we observe that Arp5 and Ies6 form a stable, isolable heterodimer when coexpressed in baculovirus-infected insect cells; notably, Ies6 was the only protein that exhibited substantial Arp5-binding activity in a systematic screen for Arp5-binding partners among INO80 complex subunits (Fig. S2). Consistent with these observations, Fenn (20) reported that human Arp5 and Ies6 from *S. cerevisiae* can form a stable heterodimeric complex.

Our results argue that the AAA+ ATPases enter the INO80 complex independently of Ies2, Arp5, and Ies6, because there was no detectable change in the amount of Tip49a or Tip49b associated with INO $\Delta$ N complexes from cells depleted of Ies2, Arp5, or Ies6 (Fig. 3B). Evidence from a previous

study suggests that the yeast orthologs of Tip49a and Tip49b, Rvb1 and Rvb2, are needed to recruit Arp5 into the yeast INO80 complex in a reaction that depends on ATP (21). Whether this is also true in the case of the human complex remains to be determined, because we could not achieve sufficient knockdown of either of the AAA+ ATPases in our experiments. We note, however, that we were unable to detect a direct physical interaction between Arp5 and the AAA+ ATPases when they were coexpressed in insect cells (Fig. S2), suggesting that such interactions, if they occur, most likely take place only in the context of the larger INO80 complex.

**Ies2 Is an Activator of Ino80 ATPase Activity.** To explore further the contributions of SNF2 module subunits to activities of the INO80 complex, we assayed the mutant INO80 complexes described in the previous section for their abilities to catalyze ATP hydrolysis and nucleosome remodeling. As shown in Fig. 4 A and B, complexes containing Ino80ΔN InsΔ1 and InsΔ2, which lacked all of the SNF2 module subunits, were severely defective in both ATPase and nucleosome-remodeling activities. INO80ΔN InsΔ3 complexes, which include apparently normal amounts of the AAA+ ATPases, Arp5, and Ies6 but contain significantly reduced levels of Ies2, had substantially reduced ATPase and nucleosome-remodeling activities (Fig. 4 A and B), raising the possibility that Ies2 is required for maximal ATPase and nucleosome remodeling. Providing further support for this idea, we observed that addition of recombinant Ies2 to reactions containing INO80ΔN InsΔ3 complexes led to a dramatic increase in nucleosome remodeling, whereas addition of Ies2 to control reactions containing INO80ΔN InsΔ2 complexes had no effect (Fig. 4C). Interestingly, the efficiency of nucleosome remodeling by INO80ΔN core complexes was also stimulated by addition of recombinant Ies2, suggesting that these complexes likely contain substoichiometric amounts of endogenous Ies2.

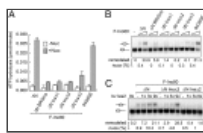


Fig. 4.

Ies2 regulates Ino80 catalytic activity. (A) Approximately 0.2 pmol of INO80 complex or subcomplexes were assayed as described in ref. 14 for their abilities to hydrolyze ATP with (+Nuc) or without (–Nuc) a saturating concentration (~200 ...

We also observed that INO80ΔN:Ies2-si complexes, which contained less than 10% of the amount of Ies2 present in complexes purified from cells treated with nontargeting siRNA, exhibited substantially reduced DNA- and nucleosome-activated ATPase (Fig. 5A) and nucleosome-remodeling activities (Fig. 5B). Arguing that this reduction in activity was due specifically to loss of Ies2, the nucleosome-remodeling activity of these complexes was greatly enhanced upon addition of recombinant Ies2 purified from *Escherichia coli* or baculovirus-infected insect cells, but not of recombinant Ies6 (Fig. 5C).

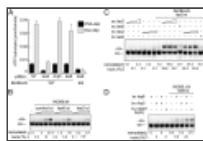


Fig. 5.

Differential roles of Ies2 and of Ies6/Arp5 in INO80 chromatin remodeling. (A) Approximately 0.5 pmol of INO80ΔN or INO80ΔN EQ complexes purified from cells treated with NT siRNA or siRNAs targeting Ies2, Arp5, or Ies6 were assayed for ...

**A Role for Ies6 and Arp5 in Nucleosome Recognition.** Whereas INO80ΔN:Ies2-si complexes had little DNA- or nucleosome-activated ATPase activity, depletion of more than 90% of Ies6 and Arp5 from INO80ΔN core complexes had little effect on ATPase activity at all DNA or nucleosome concentrations tested (Fig. 5A and Fig. S3). Complexes purified from Ies6 siRNA-treated cells that express the catalytically dead Ino80ΔN EQ mutant were inactive (Fig. 5A), arguing that the ATPase activity associated with INO80ΔN:Ies6-si and INO80ΔN:Arp5-si complexes was not due to a contaminating ATPase.

Although Arp5 and Ies6 are largely dispensable for ATP hydrolysis, they are required for optimal nucleosome remodeling by INO80ΔN complexes. As shown in Fig. 5B, the nucleosome-remodeling activity of INO80ΔN:Ies6-si complexes was reduced relative to control, although not as severely as that of complexes depleted of Ies2. In reconstitution experiments, we observed that addition of either recombinant Ies6 or Arp5 to INO80ΔN:Ies6-si complexes led to an increase in nucleosome-remodeling activity, whereas addition of both Ies6 and Arp5 increased remodeling activity further (Fig. 5D).

To test the contributions of Ies6 and/or Arp5 to nucleosome binding, we purified INO80ΔN complexes from a stable cell line expressing F-Ino80ΔN and a shRNA that targets Ies6; as expected, depletion of Ies6 using shRNA led to depletion of both Ies6 and Arp5 from the complex (Fig. 6A). As shown in Fig. 6B, INO80ΔN:shIes6 complexes exhibited reduced nucleosome binding as assayed by EMSA, suggesting that Ies6 and/or Arp5 are needed for optimal binding to nucleosomes in these assays. In contrast, Ies2 does not seem to make a major contribution to nucleosome binding because INO80ΔN InsΔ3 complexes, which contain little Ies2, behave similarly in these assays as do complete INO80ΔN complexes containing all of the SNF2 module subunits (Fig. 6C).

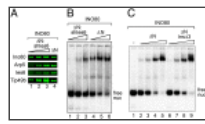


Fig. 6.

Arp5 and Ies6, but not Ies2, contribute to nucleosome recognition. (A) INO80ΔN complexes purified from cells that do (ΔN:shIes6) or do not (ΔN) stably express Ies6 shRNA were analyzed by Western blotting with the indicated antibodies. ...

## DISCUSSION

SNF2 family ATPases play critical roles as ATP-dependent motors in diverse nuclear processes from transcription to DNA replication and repair. Over time it has become clear that regulation of the catalytic activity of SNF2 family ATPases via the action of their associated proteins is a key element in determining their functional specification. As a consequence, illuminating the mechanisms of action of SNF2 regulatory proteins is a top priority of current studies. Here, we present evidence that the human Ino80 SNF2 ATPase is subject to regulation at multiple levels in the context of the human INO80 chromatin-remodeling complex. Our findings identify the Ies2 protein as a potent activator of Ino80 catalytic activity and bring to light an important role for the Ies6 and Arp5 proteins in nucleosome recognition and binding. These observations may provide a mechanistic basis for previous evidence that Ies2, Arp5, and Ies6 contribute to various Ino80-dependent nuclear transactions in fungi and in higher eukaryotes (4, 10, 22–25).

How might Ies2 affect catalysis? A definitive understanding of the mechanism(s) by which it might do so awaits high resolution information about how Ies2 (and other subunits) affect the conformation of the Ino80 catalytic site; however, it is noteworthy that the Ino80 insertion region, required for interaction of Ies2 and other SNF2 module subunits with the INO80 complex, is predicted based on sequence alignments to be located within or in very close proximity to a domain that in the zebrafish Rad54 structure has been termed helical domain 2 (HD2) (26, 27). HD2 is one of two helical domains situated on each of the two RecA-like helicase lobes of SNF2 family members and is positioned on one side of a predicted DNA-binding site (Fig. S1 B and C), where it could induce distortions in duplex DNA and/or influence the relative orientation of the RecA-like lobes, thereby influencing catalytic capacity (26, 27). Indeed, we have identified a negative regulatory element that lies within the predicted HD2 region of Ino80 and may correspond to the imitation switch (ISWI) NegC domain described by Cairns and coworkers (28). Similar to ISWI NegC, we observe that deletion of this Ino80 region leads to a dramatic increase in Ino80 ATPase activity without significantly affecting nucleosome-remodeling activity, suggesting an uncoupling of ATPase and remodeling (Fig. S4).

Our identification of roles for Ies6 and Arp5 in nucleosome recognition is consistent with previous results that have led to the proposal that actin-related proteins might participate directly in nucleosome recognition. Actin-related proteins, including the HSA module subunits Arp4 and Arp8, have been reported to be capable of binding directly to histones (9, 29–31). In addition, Ies6 includes a conserved domain shared with the SRCAP complex subunit YL1 and its yeast counterpart Swc2; the Swc2 YL1 domain has been shown to bind the histone variant H2AZ (17). Nevertheless, it is not yet known whether Arp5 and/or Ies6 enhance nucleosome binding by the INO80 complex via direct contacts with DNA and/or nucleosomes or by some alternative mechanism, for example, by altering the conformation of the Ino80 SNF2 ATPase domain so that it may more effectively engage its substrate.

Although Ies6 and Arp5 are required for optimal substrate recognition by the INO80 complex, it is clear that additional component(s) of the complex also participate in this process. Arguing that the HSA module contributes to nucleosome and/or DNA recognition, yeast INO80 complexes lacking the HSA module subunits Arp4, Arp8, and actin neither bind DNA nor support nucleosome remodeling (9), whereas an isolated HSA module composed of the yeast SNF2 HSA domain bound to Arp7, Arp9, and Rtt102 can bind either free or nucleosomal DNA (32). Further, we observed only a partial loss of nucleosome-binding activity in Arp5- and Ies6-deficient complexes purified from cells treated with Ies6 shRNA. Although we cannot rule out the possibility that the remaining nucleosome-binding activity can be attributed to the presence of residual Arp5 and Ies6, it is noteworthy that yeast INO80 complexes purified from an *arp5* deletion strain and therefore completely devoid of Arp5 exhibited only an ~50% reduction in their ability to bind DNA (9). Finally, we observed that complexes lacking the Ino80 insertion region and all SNF2 module subunits, but retaining the HSA module, had no detectable nucleosome-binding activity. Thus, similar to the HSA domain-containing RSC (Remodels the Structure of Chromatin)-remodeling complex (33, 34), it seems likely that substrate recognition by the INO80 complex is accomplished through the combined action of multiple binding surfaces, including those that depend upon the HSA module, Arp5 and Ies6, and, most likely, additional nucleosome- and/or DNA-binding interfaces within the SNF2 module.

## MATERIALS AND METHODS

**Antibodies.** Antibodies used in this study are detailed in the [Supplemental Information](#).

**Purification of INO80 Complexes and Subcomplexes.** HEK293 cell lines stably expressing FLAG–INO80E (FLJ90652), FLAG–Ino80ΔN, and FLAG–Ino80ΔN EQ have been described (2, 14). HEK293 cell lines stably expressing FLAG–Ino80ΔN BRGins and FLAG–Ino80ΔN SRCAPins were generated as described in *SI Materials and Methods* and stably introduced into Flp–In HEK293 cells (14). The INO80ΔN:shIes6 double-stable cell line

was generated by introducing a plasmid encoding shRNA against Ies6/INO80C (SA Biosciences, KH58205N) into HEK293 cells expressing FLAG–Ino80ΔN. Complexes were purified from nuclear extracts by FLAG–agarose chromatography, and their concentrations were estimated as described in refs. 14 and 35. To prepare INO80ΔN complexes from siRNA treated cells, ON-TARGETplus Nontargeting siRNA pool (D-001810-10) or pools targeting Ies2 (ZNHIT4, L-009848-01), Ies6 (C18ORF37, L-019327-01), or Arp5 (ACTR5, L-018395-01) were transfected into HEK293 cells stably expressing FLAG–Ino80ΔN in 40 15-cm dishes seeded with  $5.4 \times 10^6$  cells each. Nuclear extracts were prepared 60 h after siRNA transfection.

**Recombinant Ies2, Ies6, and Arp5.** cDNAs encoding N-terminally myc-tagged human Ies2, Ies6, and Arp5 were introduced into a BacPAK8 derivative and purified from lysates of baculovirus-infected Sf9 cells by anti-c-Myc agarose chromatography using the same buffers used for purification with FLAG–agarose (35). Alternatively, 6-histidine–tagged Ies2 was introduced into a pET-19 derivative and purified from *E. coli* by nickel agarose chromatography according to the manufacturer's instructions.

**Analysis of INO80 Activities.** Nucleosome remodeling and ATPase activities were assayed as described in ref. 14. To measure nucleosome-binding activity, purified INO80 complexes or subcomplexes were incubated with  $\sim 3$  nM nucleosomes, consisting of a mixture of mononucleosomes on a 216-bp  $^{32}$ P-labeled DNA fragment containing a laterally positioned 601 nucleosome positioning sequence and HeLa cell nucleosomes, and 1 mM ultrapure ATP (Affymetrix) in buffer containing 20 mM Hepes–NaOH (pH 7.9), 50 mM NaCl, 5 mM MgCl<sub>2</sub>, 1 mM DTT, 0.1 mM PMSF, 0.1 mg/mL BSA, 5% (vol/vol) glycerol, 0.02% Nonidet P-40, 0.02% Triton X-100, in a final volume of 10  $\mu$ L. After 30 min at 30 °C, reactions were applied to native polyacrylamide gels containing 3.5% Acrylamide/Bis 37.5:1, 1% Glycerol, 0.5 $\times$  TBE (45 mM Tris borate, 1 mM EDTA), 0.01% ammonium persulfate (APS), and 0.001% N,N,N',N'-tetramethylethylenediamine (TEMED). Gels were run at 200 V for 2.5 h in a cold room with buffer circulation, using 0.5 $\times$  TBE as running buffer.

## SUPPLEMENTARY MATERIAL

### Supporting Information:

[Click here to view.](#)

## ACKNOWLEDGMENTS

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## FOOTNOTES

The authors declare no conflict of interest.

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