and incubated with 50 ng of ^{32}P-labeled ubiquitin (6000 cpm/ng) in 0.2 M HEMG, with or without ATP and DTT, for 30 min at 25°C. Resin-bound proteins were washed with 0.2 M HEMG, resuspended in SDS-PAGE sample buffer with or without 2% β-mercapthoethanol, and separated by SDS-PAGE. Proteins were detected by Coomassie blue staining and autoradiography.

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25. In situ hybridization was performed as described (*26*). The following stocks were used: *dl*^{1} (BL-3236), *TAF250*$^{S\text{-}625}$, and *TAF250*$^{XS\text{-}2232}$ (*23*). *dl*/+ embryos were obtained by mating *dl*/+ flies. Homozygous mutant *TAF250*$^{XS\text{-}2232}$ or *TAF250*$^{S\text{-}625}$ embryos were obtained by mating *TAF250*$^{XS\text{-}2232}$/TM3[*ftzlacZ*] or *TAF250*$^{S\text{-}625}$/TM3[*ftzlacZ*] flies. *dl*/+; *TAF250*$^{XS\text{-}2232}$ or *dl*/+; *TAF250*$^{S\text{-}625}$ embryos were obtained by crossing *dl*/+; *TAF250*$^{XS\text{-}2232}$/TM3[*ftzlacZ*] or *dl*/+; *TAF250*$^{S\text{-}625}$/TM3[*ftzlacZ*] flies. Mutant embryos were identified by the absence of *ftzlacZ* staining. Embryos shown in Fig. 3 are representative examples of four different embryo collections, and mutant phenotypes shown were observed in more than 60% of mutant embryos.
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28. Histone-1 was purified from *Drosophila* nuclei (*10*) obtained from 0- to 3-hour-old embryos using hydroxyapatite (*24*), eluted with 0.65 M NaCl, TCA-precipitated, separated by SDS-PAGE, transferred to nitrocellulose, and detected by Western blot using antibodies to H1 and ubiquitin.
29. Regions within TAF$_{II}$250-M share up to 45% similarity with E1 and E2 enzymes (*22*). Amino acids 1015 to 1120 of dTAF$_{II}$250, which include the residues whose mutation causes loss of ubac activity, share 38% homology with the human E1 enzyme UBA3. Amino acids 1080 to 1137 share 41% similarity with human E2 enzymes.
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Active Remodeling of Somatic Nuclei in Egg Cytoplasm by the Nucleosomal ATPase ISWI

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Cloning by the transplantation of somatic nuclei into unfertilized eggs requires a dramatic remodeling of chromosomal architecture. Many proteins are specifically lost from nuclei, and others are taken up from the egg cytoplasm. Recreating this exchange in vitro, we identified the chromatin-remodeling nucleosomal adenosine triphosphatase (ATPase) ISWI as a key molecule in this process. ISWI actively erases the TATA binding protein from association with the nuclear matrix. Defining the biochemistry of global nuclear remodeling may facilitate the efficiency of cloning and other dedifferentiation events that establish new stem cell lineages.

Successful cloning of frogs and several mammalian species by nuclear transplantation has been reported (*1–3*), establishing the remarkable reversibility of the genetic and epigenetic programs that define cell differentiation. Even highly differentiated somatic nuclei can dedifferentiate in egg cytoplasm to acquire the totipotency essential for supporting normal development to reproductive adulthood. However, the molecular mechanisms of this global remodeling are ill defined. Nuclear transplantation into *Xenopus* eggs offers an unparalleled experimental system for studying the molecular mechanisms of the remodeling of nuclear structure and function by biochemical approaches. During the remodeling process, somatic nuclei transplanted into *Xenopus* eggs lose >85% of radiolabeled protein concomitant with substantial uptake of proteins from the egg cytoplasm (*4*). This protein exchange is likely to be mechanistically involved in the nuclear remodeling. Here, we provide a molecular characterization of this exchange using *Xenopus* egg extract and identify an energy-dependent machine that erases the differentiated state.

To simplify nuclear remodeling assays by eliminating nuclear transport steps, we permeabilized the plasma membrane and nuclear envelope of *Xenopus* XTC-2 cells (henceforth called nuclei) with the detergent digitonin (*5*). These epithelial cells were originally derived from the tadpole-stage organisms (*6*) and were in the log phase of growth when used. The permeability of the nuclear envelope was confirmed by the passive entry of fluorescence-labeled immunoglobulin G (150 kD) into the nuclei (*7, 8*). The nuclei were incubated in *Xenopus* egg S phase extract for 2 hours and isolated by centrifugation (*9*). Such remodeled nuclei showed distinct patterns of protein composition depending on energy utilization. TATA binding protein (TBP) represents a class of proteins released from the remodeled nuclei. By immunoblot, TBP was undetectable in the extract (Fig. 1A, Extract lane), indicating that the signal detected in this analysis was of nuclear origin. TBP was released from the nuclei in the presence of an energy regeneration system (ERS) (Fig. 1A, ERS lane). This release was inhibited by either adenosine 5′-triphosphate (ATP) depletion by apyrase (ATPase and adenosine diphosphatase) (Fig. 1A, Apyrase lane) or inclusion of nonhydrolyzable ATP or guanosine 5′-triphosphate analogs (Fig. 1A, AMP-PNP and GMP-PNP lanes). Because the detection limit for TBP in this assay was $<10^4$ nuclei per lane (each lane contains 1×10^5 nuclei), this result indicates that the remodeled nuclei lost >90% of total TBP. Although the DNA of the nuclei replicated in the extract (*9*), the release of TBP did not require chromatin disassembly accompanied with DNA replication because neither aphidicolin nor arabinosylcytosine inhibited the loss of TBP (Fig. 1A, Aphidicolin and AraC lanes) (*9*). The loss of TBP is not due to mitotic chromosome condensation, which was confirmed by microscopic examination of the nuclear envelope under phase contrast. The nuclei enter the S phase on addition of this type of extract. ORC2, a component of the prereplicative complex, represents the second class of proteins that are incorporated into the nuclei from the extract, independent of energy or DNA replication (Fig. 1A). ORC2 accumulation in the nuclei was necessary for detection because this protein was not recovered from centrifugation of the extract alone (Fig. 1A, Extract, pellet lane). Core histones of the remodeled nuclei were not degraded or released in the extract, and histone H2B was used as a loading control for nuclei (*10*).

We extended these observations by analyzing the behavior of other nuclear proteins to determine the selectivity of their redistribution. The levels of linker histone H1, nucleolin, and the general transcription factor TFIIB were diminished in the remodeled nuclei in the presence of ERS (Fig.

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1B). Histone H1 is replaced with the egg-type linker histone B4 by a molecular chaperone, nucleoplasmin (*10*). Nucleolin, located in the dense fibrillar component and granular component of the nucleolus, moderately decreased in the remodeled nuclei with ERS, which is consistent with the disappearance of the nucleoli (Fig. 2, A and B, ERS panels). Similar to ORC2, linker histone B4, nucleoplasmin, nucleosomal ATPase ISWI (see below), and TFIIFα were incorporated into the remodeled nuclei (Fig. 1C). The third class of proteins [including methyl-CpG binding protein 2, topoisomerase II, histone deacetylase (Rpd3 and Sin3), and RNA polymerase II] remained unchanged in abundance in the remodeled nuclei (Fig. 1D); this may reflect their stable association with the nuclear matrix (*11, 12*).

The release of TBP from nuclei was confirmed by immunofluorescence microscopy (Fig. 2A) (*13*). The nuclei clearly retained their integrity, although their chromatin became decondensed (Fig. 2A, ERS and Aphidicolin ERS panels). Nucleoli were greatly diminished in size with ERS (Fig. 2A). The loss of TBP required the extract because the nuclei did not lose TBP in bovine serum albumin (BSA) and buffer B. The passive incorporation of ISWI is also identical, whether monitored by immunoblot or microscopy (Fig. 2B).

We used TBP as a specific molecular marker for nuclear remodeling. TBP is a fundamental component of the general transcription machinery required for transcription by all three RNA polymerases (*14*). Its loss from somatic nuclei reflects dynamic transitions in nuclear function and structure. Because TBP is undetectable in the egg extract, consistent with previous studies (*15*), somatic nuclei supply >99% of the protein, simplifying interpretation. TBP released from the remodeled nuclei was readily detectable in the supernatant (Fig. 2C, ERS panels), indicating that degradation of TBP did not explain removal from the nuclei. We conclude that *Xenopus* egg extract contains the activity or activities to reorganize nuclear structure and selectively release TBP from chromatin in an energy-dependent manner, leaving TBP and nuclear integrity intact. This activity-directing release of TBP from the nuclear infrastructure is not abundant in the egg extract. Twofold dilution of the extract with BSA results in a loss of the activity (*7*).

We fractionated the activities in the egg that direct the release of TBP from nuclei. Preliminary studies indicated that ISWI-containing fractions released TBP from nuclei. ISWI is a member of the SWI2/SNF2 superfamily and is the common catalytic subunit of three ATP-dependent chromatin-remodeling complexes enriched in *Drosophila* eggs and embryos (*16–18*). These complexes mobilize nucleosomes using the energy derived from ATP hydrolysis, and the ISWI subunit per se is also capable of remodeling chromatin (*19, 20*). ISWI is the most abundant nucleosome-dependent ATPase in *Xenopus* egg, and we predicted that its chromatin-remodeling activities might contribute to the release of TBP from nuclei. We purified ISWI-D complex from *Xenopus* egg extract, which contains two subunits, p195 and ISWI (Fig. 3A) (*21*). ISWI-D is a nucleosomal ATPase comparable to recombinant ISWI (rISWI) from *Drosophila* (Fig.

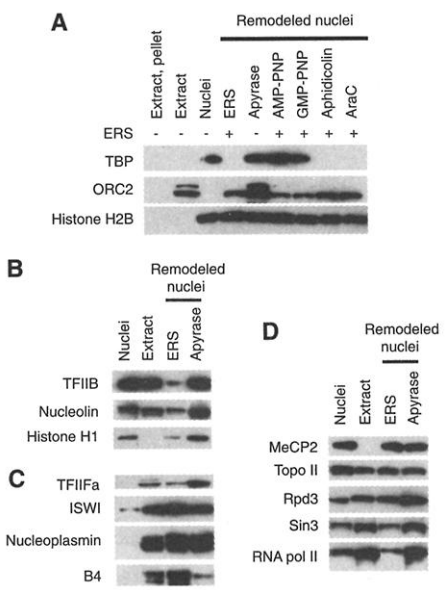

Fig. 1. Active and selective redistribution of proteins during remodeling of somatic nuclei. (**A**) TBP is actively removed from somatic nuclei during remodeling. Lanes contain the following: precipitated material from the egg extract (Extract, pellet), 4 μl of the egg extract (Extract), 1 × 10^5 nuclei (Nuclei), remodeled nuclei isolated from the reaction mix (ERS), nuclear remodeling conducted in the presence of 10 units/ml of apyrase (Apyrase), 2 mM 5′-adenylylimidodiphosphate (AMP-PNP), 2 mM 5′-guanylylimidodiphosphate (GMP-PNP), 150 μM aphidicolin (Aphidicolin), or 200 μM arabinosylcytosine (AraC). (**B**) A subset of nuclear proteins is depleted from the nuclei. (**C**) Selective proteins from the extract accumulate in the nuclei. (**D**) Nuclear proteins that resist redistribution.

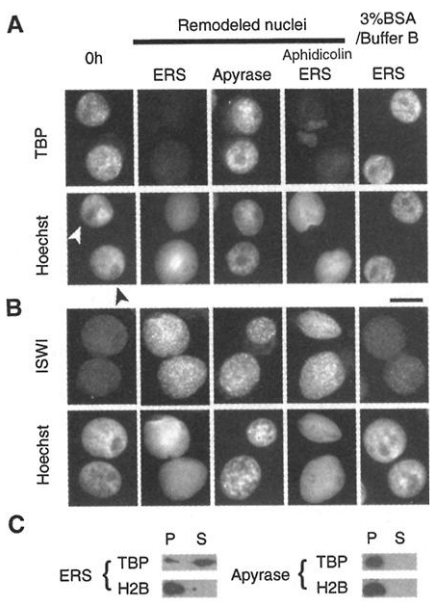

Fig. 2. The redistribution of proteins is accompanied by changes in nuclear structure. Remodeled nuclei were compared with control nuclei before incubation in the egg extract (0h) and nuclei incubated in 3% BSA and buffer B using (**A**) antibody to TBP or (**B**) antibody to ISWI. The bar represents 15 μm. The arrowheads indicate the nucleoli. DNA was counterstained with Hoechst 33342. (**C**) Detection of intact TBP in the supernatant. P, pellets containing remodeled nuclei; S, supernatant.

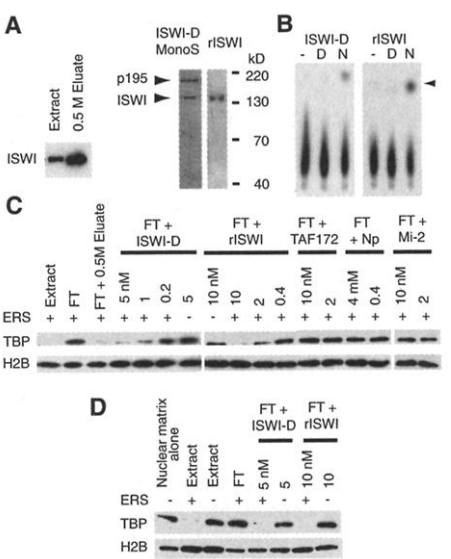

Fig. 3. TBP is released from nuclei by ISWI in a biochemical complementation assay. (**A**) Purification of *Xenopus* ISWI-D. The left panel shows immunoblot analysis of ISWI in the egg extract (Extract) and the 0.5 M NaCl eluate from the BioRex 70 column. The right panel shows SDS–polyacrylamide gel electrophoresis of the purified ISWI-D complex and rISWI. (**B**) ATPase activity of ISWIs. ATPase activity was determined in the presence of buffer (–), DNA (D), or nucleosomes (N). The arrowhead indicates the free phosphate generated by ATP hydrolysis. (**C**) A biochemical complementation assay for the release of TBP from nuclei. FT complemented with various purified proteins at indicated concentrations was compared for the activity to release TBP from nuclei. Np, nucleoplasmin. (**D**) ISWI releases TBP from the nuclear matrix. After incubation in the egg extract, the nuclear matrix protein was analyzed by immunoblot. Each lane contained 4 × 10^5 nuclei.

3B) (*21*). We found that ISWI-D and rISWI are essential for the release of TBP from nuclei. The endogenous activity of the extract to release TBP from nuclei was depleted by applying the extract to SP Sepharose and restored by combining the flow through (FT) and the 0.5 M NaCl eluate from BioRex 70 purification matrix that contained ISWI (Fig. 3A, 0.5 M Eluate lane, and Fig. 3C, FT and FT+0.5 M Eluate lanes) (*22*). Both ISWI-D and rISWI were competent to reconstitute the release of TBP from nuclei when complemented with FT in the presence of ERS (Fig. 3C, FT+ISWI-D and FT+rISWI lanes). A negative control of rISWI, in which Lys^{159} in the adenine nucleotide binding domain was replaced with Arg (*19*), lost nucleosomal ATPase activity and could not reconstitute the release of TBP from nuclei (*7*). Neither ISWI-D nor rISWI could release TBP without FT (*7*). We also tested three other candidate proteins known to modify protein-DNA interactions. TAF172, the human homolog of yeast Mot1, also belongs to the SWI2/SNF2 superfamily and removes TBP from DNA in vitro (*23, 24*). The *Xenopus* Mi-2 complex contains Mi-2 (also a member of the SWI2/SNF2 superfamily), in addition to five other subunits, and remodels chromatin (*25, 26*). Nucleoplasmin remodels sperm chromatin upon fertilization (*10, 27*). None of these proteins could reconstitute the activity (Fig. 3C, FT+TAF172, FT+Np, and FT+Mi-2 lanes) (*28*). Not knowing the percentage of active enzymes, we also tested higher concentrations of these proteins, but the results were the same (*7*). The release of TBP from nuclei by ISWI and FT was confirmed by immunofluorescence study (*9*). Because nucleoplasmin decondenses sperm chromatin (*27*), we tested whether this activity can substitute FT proteins required to release TBP. But the combination of nucleoplasmin and ISWI-D or rISWI did not release TBP from nuclei in the absence of FT (*7*). In conclusion, ISWI can release TBP from nuclei by a specific and active mechanism in the presence of other proteins derived from the egg. Importantly, the inability of TAF172 and the Mi-2 complex to reconstitute the activity underscores the specific contribution of the chromatin-remodeling factor ISWI to this global reorganization of somatic nuclei. ISWI and FT could not release other nuclear proteins listed in Fig. 1B (*7*).

To further understand the mechanism of the TBP release, we first prepared the nuclear matrix from nuclei without incubation in the egg extract by using 0.5% Triton X-100, deoxyribonuclease I, 250 mM ammonium sulfate, and 2 M NaCl (*9*). We found that ~30% of TBP and ~20% of histone H2B were recovered with the nuclear matrix (Fig. 3D, Nuclear matrix alone lane), consistent with a previous report (*29*). About 15% of the DNA remained in the nuclear matrix estimated from recovered radiolabeled DNA (*7*). Thus, most TBP is tightly bound to the nuclear matrix itself or to chromatin associated with the nuclear matrix. Next, the purified nuclear matrix was incubated in the egg extract and analyzed by immunoblot. The whole extract effectively released TBP from the nuclear matrix in the presence of ERS (Fig. 3D, Extract lane). TBP was still attached to the nuclear matrix when incubated in FT alone, and the addition of ISWI-D or rISWI was necessary to release this TBP fraction (Fig. 3D, FT, FT+ISWI-D, and FT+rISWI lanes). These data suggest that ISWI can efficiently release TBP, even when it is tightly bound to the nuclear infrastructure. The mobilization of TBP from the nuclear matrix is consistent with the capacity of ISWI to destabilize even strong interactions such as those between histones and DNA. rISWI- and ISWI-containing complexes can mobilize histone octamers along a linear DNA molecule in an ATP-dependent manner (*19, 20*). We propose that the capacity of ISWI to destabilize chromatin may underlie the dissolution of chromatin and TBP association with the nuclear matrix. Once the nuclear matrix has been disrupted, other activities in the egg might aid in the further displacement of TBP from DNA.

Whether differentiated nuclei might require specific global chromatin-remodeling activities on transplantation into eggs or during the generation of stem cell lineages is currently attracting strong interest (*30*). Transplanted nuclei might lose preexisting chromatin binding proteins passively through dilution during DNA replication in the large volume of *Xenopus* egg and embryo cytoplasm, rendering such activities unnecessary. In contrast, emerging evidence suggests that the reprogramming of epigenetic states can occur in the nuclei of differentiated cells following heterokaryon formation with stem cells, a process in which dilution does not occur (*31*). Additionally, remodeling of local chromatin and DNA methylation states requires the activity of nucleosomal ATPases (*16–20, 32*). These remodeling events result in dramatic changes in the functionality of DNA, leading to alterations in gene activity and cell physiology (*17, 32*). Our results identify ISWI as a major contributor to the active, highly specific, and extraordinarily efficient removal of key regulatory components such as TBP from somatic nuclei in the egg environment. It seems probable that other ISWI complexes and members of the SWI2/SNF2 family of enzymes are involved in comparable nuclear-remodeling events operative in early embryogenesis.

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22. To prepare FT from SP Sepharose (Amersham Pharmacia), we equilibrated the resin with buffer A (*25*) and briefly spun it to deplete the buffer. The egg extract was incubated with this resin for 30 min in a cold room followed by a brief spin to collect FT.
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