Ubiquitin-Activating/Conjugating Activity of $TAF_{II}250$, a Mediator of Activation of Gene Expression in *Drosophila*

Anh-Dung Pham and Frank Sauer*

Ubiquitination of histones has been linked to the complex processes that regulate the activation of eukaryotic transcription. However, the cellular factors that interpose this histone modification during the processes of transcriptional activation are not well characterized. A biochemical approach identified the *Drosophila* coactivator $TAF_{II}250$, the central subunit within the general transcription factor TFIID, as a histone-specific ubiquitin-activating/conjugating enzyme (ubac). $TAF_{II}250$ mediates monoubiquitination of histone H1 in vitro. Point mutations within the putative ubac domain of $TAF_{II}250$ abolished H1-specific ubiquitination in vitro. In the *Drosophila* embryo, inactivation of the $TAF_{II}250$ ubac activity reduces the cellular level of monoubiquitinated histone H1 and the expression of genes targeted by the maternal activator Dorsal. Thus, coactivator-mediated ubiquitination of proteins within the transactivation pathway may contribute to the processes directing activation of eukaryotic transcription.

Transcriptional activation is initiated by the binding of activator proteins to the enhancer region of their target genes and culminates in the recruitment of the general RNA polymerase II transcription machinery to core promoters of eukaryotic genes (*1*). However, within chromatin, the association of chromosomal DNA with histones (H1, H2A, H2B, H3, and H4) to form nucleosomes can inhibit the interaction of transcription factors and the general transcription machinery with target genes and, hence, transcriptional activation (*2*). To overcome this "nucleosome barrier," transcription factors can recruit coactivators, which by prosttranslational modification of histones establish transcriptionally competent chromatin structures (*3*). Coactivator-mediated acetylation and methylation of histones has been intimately connected with activation of transcription (*4*, *5*).

One other noteworthy posttranslational modification is the conjugation of histones with ubiquitin (*6*). Polyubiquitination represents a mark on proteins that identifies them for degradation and requires the involvement of three enzymes: (i) ubiquitin-activating enzymes (E1), which mediate the adenosine triphosphate (ATP)–dependent conjugation of E1 with ubiquitin via a covalent thioester linkage; (ii) ubiquitin-conjugating enzymes (E2), which mediate the transfer of ubiquitin from E1 to E2, conjugate ubiquitin via thioester bonds and, (iii) together with ubiquitin-protein ligase (E3), link ubiquitin to target proteins via isopeptide bonds (*7*). Polyubiquitination requires all three enzymes, whereas monoubiquitination of proteins requires E1 and E2 activities only (*7*). Unlike polyubiquitination, monoubiquitination of histones has been correlated with activation of gene expression (*3*, *6*, *8*, *9*). However, the functional connections between histone ubiquitination and activation of gene expression remain unknown. Thus, as a first step toward understanding the role of histone ubiquitination for transcriptional regulation, we sought to identify enzymes that ubiquitinate histones in *Drosophila* embryonic nuclear extract (*10*) using an activity gel assay (*11*, *12*).

Nuclear extract was separated in SDS-polyacrylamide gels containing histones. After electrophoresis (SDS-PAGE), gel-bound proteins were subsequently denatured, renatured, and, to monitor enzymatic activities, incubated with ^{32}P-labeled ubiquitin (*13*, *14*). By using this assay, we identified a protein with a molecular mass of approximately 200 kD that mediates ubiquitination of histones (Fig. 1A). To purify the identified activity from nuclear extract, we used heparin-Sepharose, S300 gel filtration, and MonoQ chromatography (*15*). Gel assays revealed that the 200-kD protein was present in the 0.4 M KCl heparin-Sepharose eluate, the void-volume of S300 gel filtration and the 0.2 M KCl eluate of MonoQ chromatography [Web fig. 1 (*16*)]. This fractionation pattern resembles the pattern described for the general transcription factor TFIID (*15*). TFIID is composed of the TATA box–binding protein (TBP) and at least 10 different TBP-associated factors (*17*). To investigate the functional relation, if any, between the 200-kD protein and TFIID, we immunopurified TFIID from the 0.2 M KCl MonoQ fraction using antibodies to TBP (*18*). Gel assays revealed that the 200-kD activity is present in immunoprecipitates obtained with antibodies to TBP and suggest that the 200-kD protein either interacts with *Drosophila* TFIID or is an integral component of the TBA-TAF_{II} complex (Fig. 1B). As the largest TAF_{II}-subunit ($TAF_{II}250$) within the TFIID-complex has a molecular mass of about 200 kD (*17*), the precipitated activity may correspond

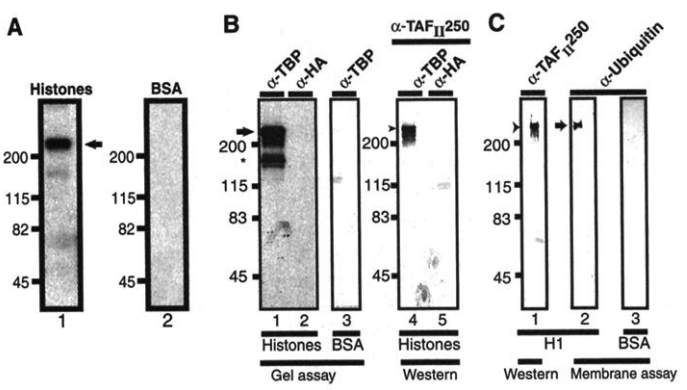

Fig. 1. A 200-kD *Drosophila* protein ubiquitinates. (**A**) Autoradiogram of activity gel assays. *Drosophila* nuclear extract was fractionated by SDS-PAGE using gels supplemented with histones or BSA. The 200-kD activity (arrow) was observed in gels containing histones (lane 1) but not in gels containing BSA (lane 2). (**B**) Proteins in the 0.2 M KCl MonoQ fraction [Web fig. 1 (*16*)] were immunoprecipitated using antibodies to TBP (lanes 1, 3, and 4) or HA (lanes 2 and 5) and were analyzed by SDS-PAGE using gels containing histones (lanes 1, 2, 4, and 5) or BSA (lane 3). Ubac activity (arrow) was detected by autoradiography (lanes 1 to 3). $dTAF_{II}250$ (arrowhead) was detected using antibodies to $dTAF_{II}250$: 2B2 (lanes 4 and 5) or 30H9 (*22*). The asterisk represents an unknown protein that interacts with TFIID and ubiquitinates histones. (**C**) Membrane assays of $dTAF_{II}250$ separated by SDS-PAGE using gels containing H1 (lanes 1 and 2) or BSA (lane 3). Reaction products were detected by Western blot using antibodies to $TAF_{II}250$ (lane 1) or ubiquitin (lanes 2 and 3). The positions of $dTAF_{II}250$ (arrowhead) (lane 1) and the 200-kD protein having ubac activity (arrow) are indicated. The position and size (in kilodaltons) of protein standards are indicated to the left (A to C).

Zentrum für Molekulare Biologie der Universität Heidelberg (ZMBH), Im Neuenheimer Feld 282, 69120 Heidelberg, Germany.

*To whom correspondence should be addressed. E-mail: f.sauer@mail.zmbh.uni-heidelberg.de

to *Drosophila* $TAF_{II}250$ ($dTAF_{II}250$). Western blot analyses of the immunoprecipitates from antibodies to TBP using antibodies to $dTAF_{II}250$ (*19*) revealed that the 200-kD activity coincided with $dTAF_{II}250$, suggesting that $dTAF_{II}250$ may ubiquitinate histones (Fig. 1B).

To support this hypothesis we used membrane assays (*20*). Purified, recombinant $dTAF_{II}250$ [Web note 1 (*16*)] was loaded onto SDS-polyacrylamide gels supplemented with individual histones and separated by SDS-PAGE, transferred onto polyvinylidene difluoride (PVDF) membrane, denatured, renatured, and incubated with ubiquitin. After incubation, proteins were treated with reducing agents to disrupt thioester bonds. On membranes loaded with histone H1 (H1) (Fig. 1C), but not on membranes loaded with H2A, H2B, H3, or H4 [Web fig. 2A (*16*)], antibodies to ubiquitin detected ubiquitin at a position that coincides with $dTAF_{II}250$, suggesting that $dTAF_{II}250$ ubiquitinates H1. The membrane assay [which subjects proteins to boiling in the presence of SDS, size-fractionation by electrophoresis, and denaturation by 7 M guanidine HCl (GuHCl)] and the molecular weight of known enzymes in the ubiquitin pathway (*7*) suggest that membrane-bound $dTAF_{II}250$ most likely does not interact with E1, E2, or E3 enzymes. As monoubiquitination requires at least E1 and E2 activities, our results imply that $TAF_{II}250$ may have intrinsic E1 and E2 activities. The ubiquitin/H1 conjugates detected in the membrane assay resisted reducing agents, suggesting that $dTAF_{II}250$ may mediate a covalent bond between ubiquitin and H1 by means of isopeptide linkages. As this enzymatic reaction is characteristic for E2 enzymes, $dTAF_{II}250$ may have intrinsic E2 activity.

The E1 enzyme requires ATP to conjugate with ubiquitin by means of thioester bonds (*7*). Therefore, to explore whether $dTAF_{II}250$ has E1 activity, we investigated the capability of $dTAF_{II}250$ for conjugating with ubiquitin by means of thioester bonds (*21*). $dTAF_{II}250$ conjugated with ubiquitin in an ATP-dependent manner in the absence, but not in the presence, of reducing agents (Fig. 2A), suggesting that $dTAF_{II}250$ and ubiquitin form a covalent bond by means of a thioester linkage. Thus, $dTAF_{II}250$ may have both E1 and E2 activities and may therefore be a ubac.

To provide supporting evidence that $dTAF_{II}250$ mediates ubiquitination of H1, we used solution assays [Web note 2 (*16*)]. Reactions containing $dTAF_{II}250$, ^{32}P-labeled ubiquitin, H1, and ATP mediated the formation of a 39-kD protein that was recognized by antibodies to both ubiquitin and H1 (Fig. 2, B and C). These results suggest that the 39-kD protein represents a conjugate composed of one ubiquitin moiety (7 kD) and H1 (32 kD). By contrast, $dTAF_{II}250$ did not ubi-

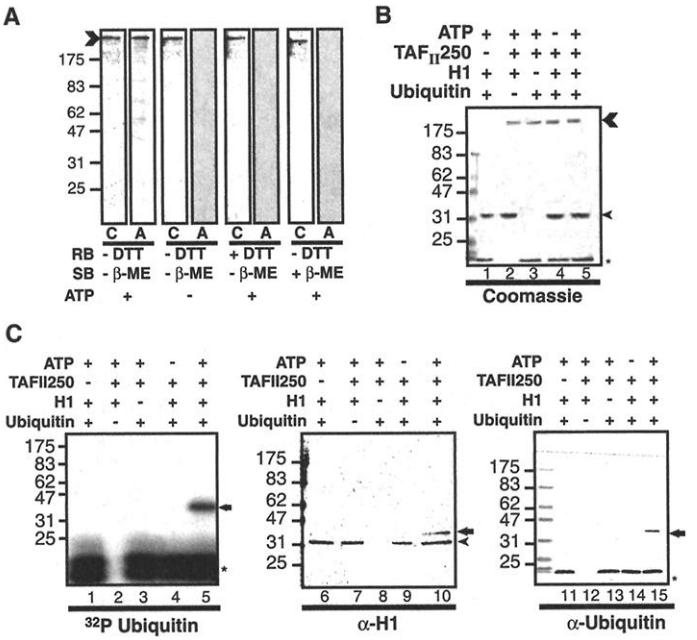

Fig. 2. The *Drosophila* coactivator $dTAF_{II}250$ has H1-specific ubac activity. (**A**) Coomassie blue–stained gel (C) or autoradiogram (A) of solution assays containing $dTAF_{II}250$ (double arrow) and ^{32}P-labeled ubiquitin and ATP, DTT, or β-mercaptoethanol (β-ME) in the reaction (RB) or SDS-PAGE sample buffer (SB). (**B**) Aliquots of solution assays containing H1 (arrowhead), ^{32}P-labeled ubiquitin (asterisk), $dTAF_{II}250$ (double arrowhead), and ATP, as indicated, were separated by SDS-PAGE, and proteins were detected by Coomassie blue staining. (**C**) Aliquots of solution assays described in (B) were separated by SDS-PAGE, and proteins were detected by autoradiography (lanes 1 to 5), antibodies to H1 (lanes 6 to 10) or ubiquitin (lanes 11 to 15). The positions of monoubiquitinated H1 (arrow) and H1 (arrowhead) are indicated. (A to C) The position and size (in kilodaltons) of protein standards are indicated to the left.

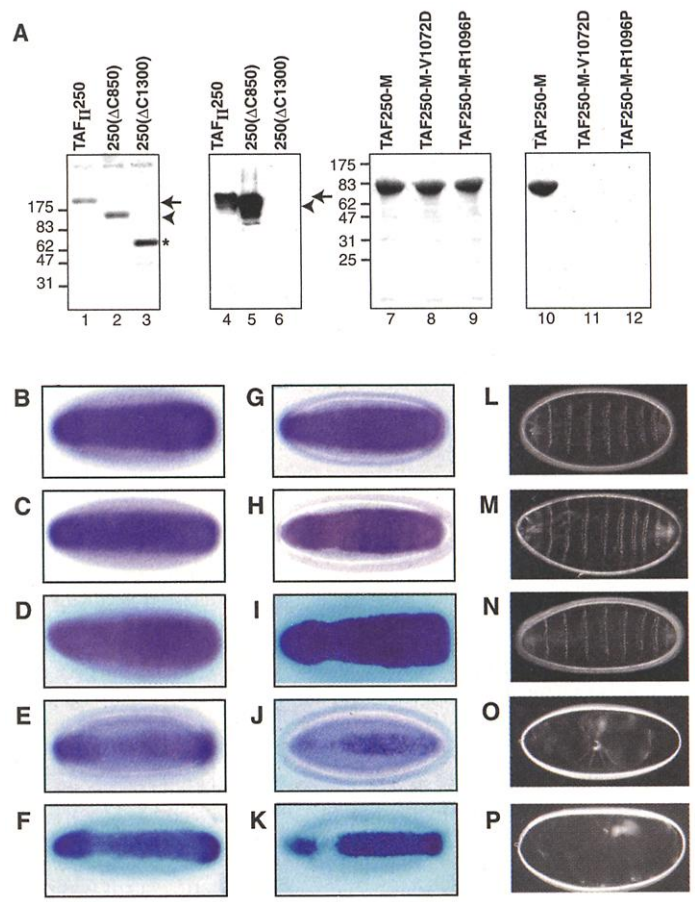

Fig. 3. Point mutations in the ubac domain of $dTAF_{II}250$ abolish H1-specific ubac activity. (**A**) Coomassie blue-stained gel (lanes 1 to 3) or membrane assays (lanes 4 to 6) of $dTAF_{II}250$ (arrow) (lanes 1 and 4), 250(ΔC850) (arrowhead) (lanes 2 and 5), and 250 (ΔC1300) (asterisk) (lanes 3 and 6), and Coomassie blue-stained gel (lanes 7 to 9) and membrane assays (lanes 10 to 12) of TAF250-M (lanes 7 and 10), TAF250-M-V1072D (lanes 8 and 11), and TAF250-M-R1096P (lanes 9 and 12). Proteins were separated in SDS-polyacrylamide gels containing H1 and detected using antibodies to ubiquitin (lanes 4 to 6 and 10 to 12). The position and size (in kilodaltons) of protein standards are indicated to the left. (**B** to **K**) Reduction of gene expression in embryos lacking $dTAF_{II}250$ ubac activity. In situ hybridizations showing *twist* (B to F) or *snail* (G to K) expression in *dl*/+ (B and G), $TAF250^{XS\text{-}2232}$ (C and H), $TAF250^{S\text{-}625}$ (D and I), *dl*/+; $TAF250^{XS\text{-}2232}$ (E and J) and *dl*/+; $TAF250^{S\text{-}625}$ (F and K) embryos. The ventral surface of blastoderm-stage embryos is shown with anterior pointing to the left. (**L** to **P**) Dark-field images of the cuticular body pattern of *dl*/+ (L), $TAF250^{XS\text{-}2232}$ (M), $TAF250^{S\text{-}625}$ (N), *dl*/+; $TAF250^{XS\text{-}2232}$ (O), and *dl*/+; $TAF250^{S\text{-}625}$ (P) embryos.

quitinate other histones [Web fig. 2B (*16*)], H2A/H2B dimers, H3/H4 tetramers, or core nucleosomes (*22*). Thus, dTAF$_{II}$250 mediates monoubiquitination of H1.

To determine the portion of dTAF$_{II}$250 that mediates monoubiquitination of H1, we used dTAF$_{II}$250 mutants truncated at the COOH-terminal [Web fig. 3 and note 1 (*16*)]. Membrane assays indicate that full-length dTAF$_{II}$250 and 250(ΔC850), lacking the 850 amino acids closest to the COOH-terminal, but not 250(ΔC1300), lacking the 1300 amino acids closest to the COOH-terminal, ubiquitinated H1 (Fig. 3A). Thus, the H1-specific ubac activity is likely to reside between amino acids 768 and 1218.

Most recently, two *Drosophila TAF250* alleles, *TAF250$^{XS-2232}$* and *TAF250^{S-625}*, have been described, which contain single–amino acid point mutations that reside within the putative dTAF$_{II}$250 ubac domain (*23*). *TAF250$^{XS-2232}$* contains a valine-1072 to aspartic acid change, and *TAF250^{S-625}* an arginine-1096 to proline change [Web fig. 3 (*16*), (*23*)]. To investigate the effect of these mutations on dTAF$_{II}$250 ubac activity, the middle region of TAF$_{II}$250 containing amino acids 612 to 1140 (TAF250-M), TAF$_{II}$250-M-V1072D containing the (V^{1072} to D) mutation, and TAF$_{II}$250-M-R1096P containing the (R^{1096} to P) mutation (*23*), were subjected to membrane assays. Although TAF250-M ubiquitinated H1, the mutants did not (Fig. 3A). Wild-type and mutant TAF$_{II}$250-M proteins have histone acetyltransferase activity (*23*). The TAF250-M proteins used for the membrane assays acetylated histones (*22*), suggesting that the lack of ubac activity seen with TAF250-M-V1072D and TAF$_{II}$250-M-R1096P is most likely not due to a general functional inactivity of the mutant proteins.

In *Drosophila*, TFIID mediates transcriptional activation by the maternal activator Dorsal (*24*). Dorsal activates the expression of the mesoderm-determining genes *twist (twi)* and *snail (sna)*, which are transcribed in 20 and 18 of the ventral-most cells of cellularizing embryos, respectively (*24*). To investigate the functional relevance of TAF$_{II}$250 ubac activity for Dorsal-dependent transcriptional activation in vivo, we used in situ hybridization (*25*) to monitor *twi* and *sna* expression in *Drosophila* embryos containing reduced levels of Dorsal and expressing TAF$_{II}$250$^{XS-2232}$ or TAF$_{II}$250^{S-625}, which lack ubac activity in vitro. Both *twi* and *sna* expression were severely reduced in *dl*-sensitized, *TAF250$^{XS-2232}$* embryos (Fig. 3, E and J) and *dl*-sensitized, *TAF250^{S-625}* embryos (Fig. 3, F and K) but not in control embryos (Fig. 3, B to D and G to I). Weak *twi* mRNA levels were detectable in 10 to 12 cells (Fig. 3, E and F), and *sna* expression was restricted to 4 to 12 ventral-most cells and disrupted by gaps (Fig. 3, J and K). Analyses of cuticular preparations (*26*) revealed that *dl*-sensitized *TAF250$^{XS-2232}$* mutants (Fig. 3O) or *dl*-sensitized *TAF250^{S-625}* mutants (Fig. 3P), but not control embryos (Fig. 3, L to N) exhibited a dorsalized and twisted body pattern. These results indicate that Dorsal-dependent activation of transcription is impaired in embryos lacking TAF$_{II}$250 ubac activity.

To investigate whether H1 may represent a target for dTAF$_{II}$250 ubac activity in *Drosophila*, we purified H1 from nuclei prepared from 0- to 3-hour-old wild-type and TAF$_{II}$250 mutant embryos (*27*, *28*). Western blot analyses indicate that antibodies to both H1 and ubiquitin detected a monoubiquitin/H1 conjugate (Fig. 4). This result indicates that at least a fraction of H1 present in early *Drosophila* embryos is monoubiquitinated. Moreover, Western blot analyses indicate that compared with wild-type embryos, mutant embryos that lack TAF$_{II}$250 ubac activity contained a significantly reduced level of monoubiquitinated H1 (Fig. 4). These results suggest that dTAF$_{II}$250 ubac activities may contribute to monoubiquitination of H1 in *Drosophila*.

How coactivators convert activation signals from activation domains of transcription factors into enhanced levels of mRNA synthesis lies at the heart of transcriptional regulation. Our results suggest that one coactivator, TAF$_{II}$250, may use intrinsic ubiquitin-activating/conjugating activities to mediate activation of transcription. Multiple-alignment analysis and comparison with protein database sequences revealed that TAF250-M exhibits similarities to E1 and E2 enzymes (*29*). Thus, our result that TAF$_{II}$250 mediates monoubiquitination of H1 in vitro is in agreement with other results suggesting that E1 and E2 activities are sufficient to mediate monoubiquitination of proteins (*7*). As point mutations that abrogate TAF$_{II}$250 ubac activity in vitro also reduce gene expression in the *Drosophila* embryo, TAF$_{II}$250 ubac activity may play an important role for the activation of gene expression in *Drosophila*. Although the in vivo targets of TAF$_{II}$250 ubac activity remain unknown, our results that H1 is monoubiquitinated in *Drosophila* and that the level of monoubiquitinated H1 is significantly reduced in embryos lacking dTAF$_{II}$250 ubac activity imply that H1 may represent one in vivo target of dTAF$_{II}$250. Thus, ubiquitination of H1 or other proteins within the transcription machinery, or both, by TAF$_{II}$250 may constitute an important coactivator function of TAF$_{II}$250 and, hence, may allow TFIID to direct events during the processes of transcriptional activation.

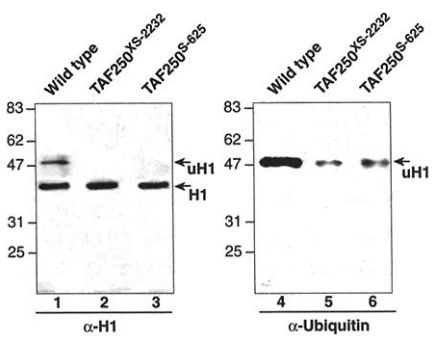

Fig. 4. Histone-1 is ubiquitinated in *Drosophila*. Western blot analysis of H1 isolated from wild-type (lanes 1 and 4), homozygous mutant *TAF$^{XS-2232}$* embryos (lanes 2 and 5), or homozygous mutant *TAF^{S-625}* embryos (lanes 3 and 6). Proteins were detected using antibodies to H1 (lane 1 to 3) or ubiquitin (lanes 4 to 6). The positions of H1 and monoubiquitinated H1 (uH1) are indicated. The position and size (in kilodaltons) of protein standards are indicated to the left.

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12. Activity gel assays were performed as described (*11*). SDS-polyacrylamide gels were supplemented with 1 mg of histones (Sigma) or bovine serum albumin (BSA) per milliliter resolving gel. To monitor ubac activity, gels were incubated with 200 ng of ^{32}P-labeled ubiquitin (6000 cpm/ng) in 0.2 M HEMG (25 mM Hepes, pH 8, 0.1 mM EDTA, 6.25 mM $MgCl_2$, 0.2 M NaCl, 10% glycerol) containing 0.1 M ATP for 1 hour at 25°C. After incubation, gels were washed extensively with 0.2 M HEMG supplemented with 5% trichloroacetic acid (TCA) and 5 mM dithiothreitol (DTT), and reaction products were detected by autoradiography.
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20. Proteins (approximately 2 to 4 μg) were separated in SDS-polyacrylamide gels containing 1 mg of BSA or 0.5 mg of histones per milliliter of resolving gel. After SDS-PAGE proteins were transferred onto PVDF membrane, denatured in 7 M GuHCl, renatured in 0.2 M HEMG (*11*), and incubated with 5 ml of 0.2 M HEMG containing 50 μg/ml ubiquitin (Sigma) and 0.1 M ATP for 1 hour at 25°C. After incubation, membranes were washed with 0.2 M HEMG containing 5 mM DTT. Reaction products were detected using antibodies to ubiquitin (Sigma) and the ECL-Western blotting detection system (Amersham/Pharmacia, Uppsala, Sweden).
21. Recombinant hemagglutinin (HA)-dTAF$_{II}$250 (200 ng) was immobilized to antibodies to HA attached to a resin

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.
30. We thank D. A. Wassarman (NIH) for flies and bacterial expression plasmids; J. Pyrowolakis and S. Jentsch (MPI, Munich, Germany) for the protocol describing expression, purification, and labeling of ubiquitin; M. Scheffner (University of Cologne, Germany) for the GST-ubiquitin expression plasmid; H. Jäckle (MPI, Göttingen, Germany) and R. Paro (ZMBH, Heidelberg, Germany) for critical reading of the manuscript; and S. Müller, J. Rami, and Y. Cully for technical assistance, editorial help, and photowork. This work was supported by DFG (SA791/11).

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Active Remodeling of Somatic Nuclei in Egg Cytoplasm by the Nucleosomal ATPase ISWI

Nobuaki Kikyo,[1] Paul A. Wade,[1] Dmitry Guschin,[2] Hui Ge,[1] Alan P. Wolffe[2]*

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.

[1]Laboratory of Molecular Embryology, Building 18T, Room 106, National Institutes of Health, Bethesda, MD 20892, USA. [2]Sangamo BioSciences, Point Richmond Tech Center, 501 Canal Boulevard, Suite A100, Richmond, CA 94804, USA.

*To whom correspondence should be addressed. E-mail: awolffe@sangamo.com