the α side of the saccharide in native T4L predicts that the anomeric configuration of the substrate be inverted in the product. This is difficult to prove because the appropriate substrate for T4L is complex and difficult to synthesize. In contrast, HEWL is known to retain anomeric configuration (*23*). Therefore, the mechanism for T4L shown in Fig. 3B is necessarily different from that commonly accepted for HEWL (*1*, *4*, *5*).

Thus, the mutant lysozyme T26E could be an example of glycosidases that cleave with overall retention of configuration by a double displacement mechanism (*21*, *22*). At the same time, the presence of protein-substrate interactions that stabilize a sugar ring conformation similar to an oxocarbonium ion–like transition state can be taken as evidence that the mechanism of action of the mutant and of the wild-type T4L itself include elements similar to those originally postulated by Phillips for HEWL (*1*, *4*, *5*).

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Chromosome Condensation in *Xenopus* Mitotic Extracts Without Histone H1

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The contribution of histone H1 to mitotic chromosome condensation was examined with the use of a cell-free extract from *Xenopus* eggs, which transforms condensed sperm nuclei into metaphase chromosomes. When H1 was removed from the extract, the resultant metaphase chromosomes were indistinguishable from those formed in complete extract. Nucleosomal spacing was the same for both. Thus, H1 is not required for the structural reorganization that leads to condensed metaphase chromosomes in this egg extract.

During mitosis, genomic DNA is packaged into condensed chromosomes to facilitate its accurate segregation to daughter cells. Concomitant with mitotic chromosome condensation, histone H1 is highly phosphorylated (*1*), presumably by cdc2 kinase that triggers the transition from interphase to mitosis (*2*) and is partially localized on condensed chromosomes (*3*). Histone H1 helps to compact the 10-nm-diameter chromatin filament into a 30-nm fiber (*4*). Thus, H1 and its phosphorylation are thought to cause mitotic chromosome condensation (*5*). To examine the contribution of H1 to mitotic chromosome condensation, we used a cell-free system with amphibian (*Xenopus laevis*) egg extracts, in which sperm chromatin lacking H1 is remodeled to somatic chromatin and then transformed into condensed metaphase chromosomes (*6*).

Unfertilized eggs of *Xenopus* are arrested at the second meiotic metaphase. Cytoplasmic extracts prepared from these eggs can induce nuclear membrane breakdown, chromosome condensation, and spindle formation (*6–9*). When sperm nuclei deprived of the plasma and nuclear membranes are incubated in the mitotic extract, they decondense in a few minutes (*9*, *10*). During this time, sperm-specific basic proteins are selectively removed from sperm DNA and replaced by somatic-type core histones and H1, which are absent from sperm nuclei but stored in the extract (*10–12*). Both of these processes are mediated by nucleoplasmin (*11–13*). The decondensed chromatin is then transformed into condensed metaphase chromosomes after incubation for a further 90 min (*8*, *9*). This condensation is thought to be similar to that imposed on somatic interphase nuclei when incubated in the egg extract (*6*).

Although the type of H1 commonly found in somatic cells is not found in amphibian eggs (*14*, *15*), a subtype termed H1X is found in the nuclei of eggs and early embryos up to the late blastula stage of anuran amphibians (*10*). This subtype is encoded by the B4 mRNA, whose sequence is similar to those of other subtypes of H1 (*16*).

We used antibodies to H1X to immunodeplete the egg extracts (*17*) (Fig. 1A). In both H1X-depleted and normal extracts, sperm nuclei were similarly transformed

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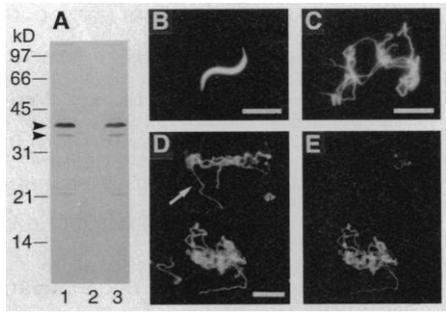

Fig. 1. Immunodepletion of histone H1 from a *Xenopus* egg extract and chromosome condensation in the extracts. (**A**) An egg extract was treated with an anti-H1X serum or a pre-immune serum. Equal volumes of the H1X-depleted (lane 2), mock-depleted (lane 3), and nontreated extracts diluted to equalize its protein concentration (lane 1) were subjected to 15% SDS–polyacrylamide gel electrophoresis (PAGE) and processed for immunoblotting with the antibody to H1X. The *Xenopus* H1X includes some variants indicated by arrowheads (*10, 16*). (**B**) Demembranated sperm nuclei were transformed into (**C**) condensed metaphase chromosomes in a 90-min incubation with the H1X-depleted extract. These chromosomes were mixed with chromosomes formed in a nondepleted extract, fixed on a polylysine–coated glass slide, and then processed for double-staining with (**D**) DAPI and (**E**) immunofluorescence with anti-H1X antibody. H1X-negative (arrow) and positive chromosomes are indistinguishable in appearance (D). Samples (B to D) were stained with DAPI. Bars represent 10 μm.

into condensed metaphase chromosomes (Fig. 1, B to D). However, chromosomes lacking H1X were more easily broken into fragments by vigorous pipetting than were control chromosomes. The absence of H1X was confirmed by immunostaining (Fig. 1E) and SDS-PAGE of acid-extracted chromosomal proteins (*18*) (Fig. 2A). The latter analysis confirms the absence of other H1 subtypes (*19*). The absence of H1X did not seem to affect the association of other chromatin proteins with DNA (Fig. 2B).

We used micrococcal nuclease to analyze the formation and spacing of nucleosomes (*20*). Extensive digestion of the control chromosomes that contained H1X revealed a protected DNA fragment of approximately 170 base pairs (bp) (Fig. 3A). This fragment is derived from the combination of the nucleosome monomer and H1 (*21*). The 146-bp fragment (Fig. 3A) corresponds to the nucleosome monomer alone (*21*). Digestion of the H1X-free chromosomes yielded the 146-bp fragment exclusively (Fig. 3A); no fragments longer than 146 bp were detectable after less digestion (*20*). In spite of the lack of H1 in the H1X-free chromosomes, the nucleosomes were spaced approximately 200 bp apart, as found in the control chromosomes (Fig. 3B). Thus, it seems that H1 is not essential for any of the structural reorganization of sperm chromatin into metaphase chromosomes in the egg extract.

Together with the apparent lack of H1 in yeast cells (*22*), our results suggest that H1 is not required for mitotic chromosome condensation. Thus, condensation of chromatin into metaphase chromosomes must be caused by chromatin components other than H1. One of the candidates for such factors is Type II DNA topoisomerase, which is now the only chromatin component that has been demonstrated to function in the formation of metaphase chromosomes (*9, 23*).

However, our results do not exclude H1 from the process of mitotic chromosome condensation. The fragility of metaphase chromosomes formed without H1X may indicate that H1 binding increases the mechanical stability of condensed chromosomes through its stabilizing effect on the 30-nm-thick fiber of coiled nucleosomes (*4*). Moreover, H1 termini contain DNA binding Ser Pro Lys Lys sequences (*24*), which can be phosphorylated by mitotic cdc2 kinase (*2, 25*). Because the phosphorylation of these sites is thought to weaken their association to linker DNA (*24*), the subsequent decrease in H1-DNA interaction may allow access to chromatin of putative factors responsible for chromosome condensation (*26*).

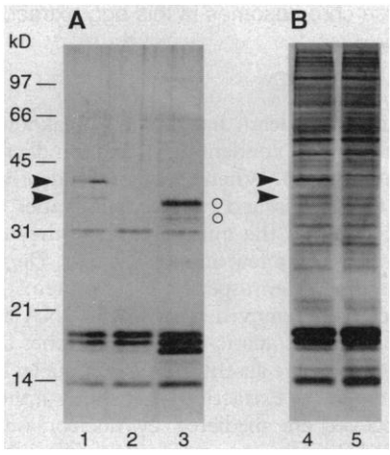

Fig. 2. Protein compositions of *Xenopus* metaphase chromosomes with and without H1X. Chromatin proteins of metaphase chromosomes formed in mock-depleted (lanes 1 and 4) and H1X-depleted (lanes 2 and 5) extracts were extracted with either (**A**) 0.4 N H_2SO_4 or (**B**) an SDS-PAGE sample buffer and electrophoresed on 15% SDS-polyacrylamide gels. Lane 3 is acid-extracted proteins from erythrocyte nuclei. H1X is indicated by arrowheads. H1 subtypes and core histones of erythrocyte nuclei are indicated by open and closed circles, respectively. In the extract, and in chromosomes formed in it, H2A is mostly replaced by H2A.X, an H2A variant that comigrates with H3 (*15, 27*). The band at 31 kD is thought to be high-mobility group A because of its electrophoretic mobility (*28*). This protein was shown by quantitative analyses to be lesser in amount, although more prominent, than the faster migrating H1X variant (*29*). The gels were silver-stained.

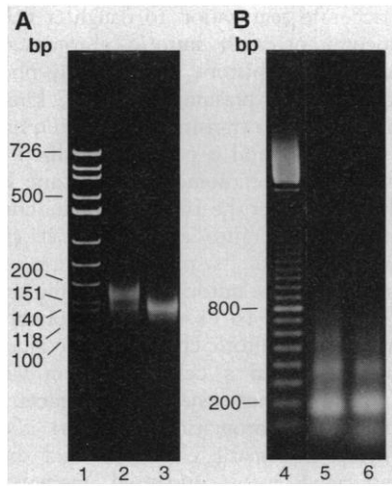

Fig. 3. Micrococcal nuclease digestion of *Xenopus* metaphase chromosomes with and without H1X. Metaphase chromosomes formed in mock-depleted (lanes 2 and 5) and H1X-depleted (lanes 3 and 6) extracts were treated with micrococcal nuclease under (**A**) extensive and (**B**) mild digestion conditions (*20*), and their products were electrophoresed on 7.5% polyacrylamide (extensive digestion) and 1.5% agarose (mild digestion) gels. Lanes 1 and 4 are ϕX174/*Hinf I* digest and 100-bp ladder markers, respectively. The gels were stained with ethidium bromide.

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18. Demembranated sperm (5×10^5) were incubated with 100 μl of egg extracts for 90 min. The mixture was diluted 10-fold with EB, and metaphase chromosomes were sedimented (10,000 *g*, 10 min) onto 2.0 M sucrose in EB through 100 μl of 1.2 M sucrose in EB. Metaphase chromosomes at the 1.2 to 2.0 M interphase were suspended in EB and then pelleted (5000*g*, 10 min). Chromatin proteins were extracted from metaphase chromosomes and erythrocyte nuclei with 0.4 N H_2SO_4 as described (*10*) or are SDS-PAGE sample buffer and analyzed on 15% polyacrylamide gels.
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Role of U6 snRNA in 5′ Splice Site Selection

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Two models describing the interaction between U6 small nuclear RNA (snRNA) and the 5′ splice site of introns have been proposed on the basis of cross-linking experiments. Here it is shown that a conserved sequence present in U6 snRNA forms base pairs with conserved nucleotides at the 5′ splice junction and that this interaction is involved in 5′ splice site choice. These results demonstrate a specific function for U6 snRNA in splicing and suggest that U6 snRNA has a proofreading role during splice site selection. A model is presented in which this new interaction, in concert with previously described interactions between U6 snRNA, U2 snRNA, and the pre–messenger RNA, would position the branch point near the 5′ splice site for the catalysis of the first splicing step.

Splicing of nuclear introns occurs by a two step pathway (*1*). In the first step, the phosphodiester bond at the 5′ splice site is attacked by the 2′-OH of an adenosine residue in the intron, the branch point. This reaction produces a free upstream exon and a lariat intermediate molecule containing both the downstream exon and the intron with its 5′ end covalently linked to the branch nucleotide. The selection of the 5′ splice site phosphodiester bond to be cleaved by this reaction must be highly accurate. Indeed, most errors would introduce frame shifts into the coding part of mRNAs thereby impairing protein production. Accurate splice site choice probably results from the numerous interactions established between the pre-mRNA and splicing factors during spliceosome assembly (*1*, *2*).

The U1, U2, U4, U5, and U6 small nuclear RiboNucleoProteins (snRNPs) are splicing factors that function in spliceosome assembly and splicing catalysis. U1 snRNA forms base pairs with 5′ splice sites, and in some species, with 3′ splice sites (*3*, *4*, *5*), whereas U2 snRNP interacts with the branch point (*6*). U1 snRNA recognizes pre-mRNAs containing introns and commits them to the splicing pathway (*3*). For alternatively spliced pre-mRNA, U1 snRNP will thus select the splice site to be used from the potential 5′ splice sites. However, U1 snRNA is not involved in the selection of the precise 5′ splice site bond to be cleaved (*7*, *8*). The U5 snRNA interacts with exon sequences in the pre-mRNAs, intermediates, and products of the splicing reaction (*9*, *10*) and is one of the factors implicated in the choice of the 5′ phosphodiester bond to be cleaved. However, additional unidentified factors are also postulated to play a role in 5′ cleavage site choice (*3*, *7*, *8*).

Because U6 is the most conserved of the snRNAs involved in splicing, it is assumed that it has an important, but unidentified, function (or functions) in the splicing reaction (*2*). Cross-linking experiments in mammalian and yeast systems indicate that U6 snRNA and the pre-mRNA are in close proximity (*11*, *12*, *13*). Because of limitations of the cross-linking technique, it has been unclear whether U6 snRNA forms base pairs with the 5′ splice site, or if the cross-linking results from the proximity of the two RNAs in the spliceosome (*12*, *13*). After these studies, two mutually exclusive models proposed that base pairing occurs between U6 snRNA and the 5′ splice site (*12*, *13*). In the first model (*12*), a region of yeast U6 flanking C48 would form base pairs with intron sequences flanking G5 (Fig. 1, A and B). This model suggests an interaction between conserved sequences in U6 and at the 5′ end of introns. Furthermore, several U6 nucleotides near C48 are critical for U6 snRNP function (*14*, *15*). In a second proposal (*13*), U6 snRNA C43 and flanking nucleotides would form base pairs around intron position 5 (Fig. 1C). Nucleotide C43 and some flanking nucleotides are neither evolutionarily conserved nor essential for U6 function (*14*, *15*). Interactions predicted by both models would extend only over a few nucleotides.

Yeast introns mutated at position 5 display two phenotypes (*16*). (i) They have a reduced splicing efficiency, which is reflected by an increase in pre-mRNA levels in

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