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The H1A histone variant is an *in vivo* repressor of oocyte-type 5S gene transcription in *Xenopus laevis* embryos

(early development/ribozyme)

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ABSTRACT **Previous *in vitro* transcription studies have pointed to the importance of histone H1 for repression of oocyte-type 5S genes of *Xenopus laevis*. It has been previously reported that in development up to the early gastrula stage, *Xenopus* embryos contain a large pool of the maternal histone H1 variant H1M but are virtually devoid of histone H1A, H1B, and H1C proteins. At the early gastrula stage, there is an increase in H1A protein synthesis and H1A becomes the predominant H1 histone variant. Concomitant with the significant appearance of H1A protein in chromatin, oocyte 5S transcription is repressed. Here it is shown that there appears to be a direct link between H1A accumulation and inhibition of oocyte-type 5S RNA synthesis. Inhibition of H1A synthesis by a ribozyme targeted to H1A mRNA leads to the continued expression of oocyte 5S genes. H1A is proposed to inhibit major oocyte 5S gene transcription by sealing the nucleosome that is positioned over the major oocyte 5S coding sequences and by driving major oocyte 5S gene chromatin into a higher-order structure in which histone H1A molecules interact cooperatively.**

Early embryonic development of *Xenopus laevis* is characterized by 12 rapid cycles of cell division after fertilization. During oocyte maturation and breakdown of the oocyte nucleus, RNA synthesis ceases and resumes again at the midblastula transition (MBT). At this point, synthesis of low levels of both somatic- and oocyte-type 5S RNA resumes, but in later gastrulation, transcription of the oocyte-type 5S genes is largely repressed, whereas somatic-type 5S RNA persists (1). The observed 50-fold discrimination of oocyte-type 5S genes at MBT is established in the egg (2). The final state of differential 5S RNA gene expression represents a further downregulation of oocyte-type 5S RNA genes to a level 1000-fold lower than the level of somatic-type 5S RNA (3). However, it must be considered that oocyte-type 5S RNAs are unstable after MBT, since they are not integrated into ribosomes (4).

During the same developmental period, there is also a differential expression of histone H1 variants (5). In oogenesis, a maternal H1 variant (H1M) accumulates to ≈1 ng per egg. H1M persists during early development and, thereafter, gradually decreases over the next few days of development (5, 6). The first significant appearance of the H1A histone variant translated from stored mRNA (7) coincides with MBT. After general underrepresentation of histone H1 during the blastula stage, an increase in H1A synthesis at the beginning of gastrulation leads to the accumulation of H1A to >10 ng per embryo. Histones H1B and H1C are not detected during this period (5). Hence, there exists a close temporal correlation between increasingly large pools of H1A histone variant protein and the decrease of oocyte 5S RNA synthesis in the early gastrula embryo.

To determine whether the H1A histone variant is a repressor of oocyte 5S RNA synthesis, H1A mRNA translation in the developing embryo was inhibited. In control embryos, oocyte 5S RNA production was repressed, whereas embryos largely deficient in histone H1A not only failed to shut down oocyte 5S RNA synthesis but actually enhanced production of this RNA species. This result suggests that the main cause of differential 5S gene activity is probably not limiting transcription factors but rather the distinct arrangement of nucleosomes on the major oocyte 5S RNA gene cluster that makes it particularly sensitive to H1A-mediated inhibition.

METHODS

T7 Expression Vector of H1A mRNA. The histone H1A genomic DNA clone X1h3 was generously provided by G. H. Thomsen (Rockefeller University, New York). A fragment of X1h3 beginning at nucleotide −60 upstream of the initiation ATG and ending at the *Hin*dIII site downstream of the termination codon was prepared by PCR whereby an *Xba* I site was introduced at the 5′ end via a PCR primer. The *Xba* I–*Hin*dIII fragment was cloned into *Xba* I/*Hin*dIII-digested pAD-CMV2, a vector containing a T7 promotor, which was kindly provided by A. Himmler (Bender, Vienna).

T7 Expression Vector of H1A mRNA-Targeted Ribozyme-Pre-tRNA^{Tyr}. The wild-type oocyte tyrosine tRNA gene (*TyrC*) (8) was kindly provided by S. Clarkson (University of Geneva). A 258-bp *Hha* I fragment containing the gene had been cloned into a *Hin*dIII/*Bam*III-digested pBR327 plasmid. The modified gene (*TyrM*) was cloned as a PCR-generated 170-bp fragment into a *Eco*RI/*Sal* I-digested pAALM vector. The complete sequence of the tDNA^{Tyr} fragment is shown in Fig. 2. The pAALM plasmid was constructed by M. Cotten (Institute for Molecular Pathology, Vienna) who cloned a T7 promoter from pSPT18 into pSP64. Ribozymes were cloned into the *Apa* I site of the modified intron sequence. The ribozyme targeted to H1A mRNA contains 11 complementary bases (underlined) on each side of the ribozyme 5′-CUUUUUAGCGGCUGAUGAGUCCGUGAGGACGAAACUCUUGGUGGC-3′.

***In Vitro* Transcription of T7 Plasmids.** Capped RNAs were *in vitro*-transcribed from linearized T7 plasmids by standard procedures (9). Transcripts were purified on a denaturing 6% polyacrylamide gel. Bands were located by short autoradiography (1 min) for radioactively labeled transcripts or by UV-shadowing for unlabeled transcripts and excised with a sterile scalpel blade. Gel slices were transferred to an Eppendorf tube containing 400 μl of extraction buffer and 8 μl of phenol. After overnight incubation at room temperature on a vertical shaker, the mixture was extracted twice with phenol/chloroform and ethanol-precipitated.

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Abbreviation: MBT, midblastula transition.

Injection of *Xenopus laevis* Embryos. *In vitro*-fertilized eggs were dejellied with 2% (wt/vol) cysteine hydrochloride in 0.1× Barth medium (pH 8.0). Ficoll was added to 5% (wt/vol) to the 0.1× Barth medium to help fertilized eggs overcome injection injury. Microinjection was delayed until at least 1 h after fertilization. In some cases, embryos were injected at the two-cell stage when they can best resist injury. Injection volumes were 20–50 nl per embryo. Staging was performed at room temperature (22–24°C) according to the tables of Nieuwkoop and Faber (10). Staged embryos were stored at −80°C after removal of the medium.

Preparation of Protein Extracts and Immunoblot Analysis. Total protein extracts were used for analysis. Embryos were homogenized on ice in an Eppendorf tube with 10 vol of protein extraction buffer [50 mM Tris·HCl/0.5 M urea/2% (vol/vol) Nonidet P-40/1 mM phenylmethylsulfonyl fluoride/5% (vol/vol) mercaptoethanol, pH 7.5] by repeated pipeting through a yellow Gilson tip. After centrifugating for 2 min at room temperature in a microcentrifuge, the pellet was reextracted in the same buffer, and supernatants were combined and stored at −20°C. Extracts were separated on SDS 15% polyacrylamide gels and blotted onto 0.1-μm (pore size) Schleicher & Schuell nitrocellulose membranes. Blots were immunostained and labeled bands were detected with ^{125}I-labeled protein A. Affinity-purified H1A-specific polyclonal peptide antibodies were used (described in detail in ref. 5). Analysis was by autoradiography on x-ray film and with a PhosphorImager (Molecular Dynamics).

Preparation and Analysis of RNA Extracts. Total RNA from *Xenopus* embryos was prepared as described by Busby and Reeder (11). Embryos were homogenized in equivalent TNES buffer [100 mM Tris·HCl/300 mM NaCl/10 mM EDTA/2% (wt/vol) SDS, pH 7.5] at 50 μl per embryo by repeated pipeting in a 1-ml Eppendorf tube on ice. Proteinase K was added to 250 μg/ml and the suspension was incubated at 50°C for 30 min. After digestion the samples were extracted twice with phenol/chloroform and once with chloroform, and the RNA was precipitated with 2 vol of ethanol. The protocol for semi-denaturing PAGE was adapted from Wakefield and Gurdon (3). Stacking gels were omitted. Gels contained 4 M urea and 15% polyacrylamide; running buffer was TBE (0.09 M Tris–borate/0.002 M EDTA at pH 8.3). It is important to use fresh buffer to obtain maximum resolution. Dried RNA samples were dissolved in 4 M urea/1 mM EDTA, with xylene/cyanole dye. Gels were 0.35 mm thick, poured between 20 × 40 cm plates, and electrophoresed at 10 mA (constant current) for 25 h. Oocyte 5S RNA migrates faster than somatic 5S RNA; splitting into doublets and triplets varies from gel to gel. This phenomenon also occurs with transcripts from cloned genes and does not interfere with identification (3).

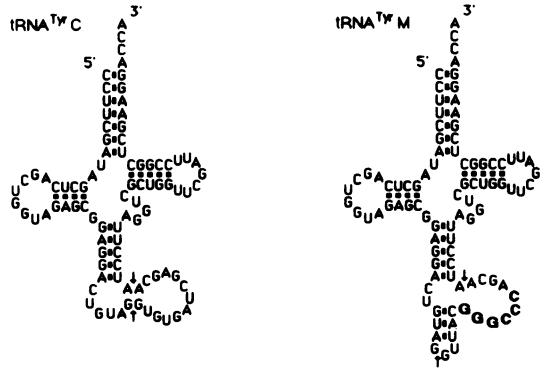
RESULTS

Antisense and Ribozyme Strategies in *Xenopus laevis* Embryos. In this paper the possible direct involvement of histone H1A in developmental regulation of 5S RNA genes in *Xenopus laevis* was investigated by determining consequence(s) of inhibiting histone H1A synthesis on 5S gene transcription. The extremely high sensitivity of *Xenopus* embryos to the slightest perturbation represents a central problem for an antisense strategy. Antisense oligodeoxynucleotides have proved to be of limited value in *Xenopus* embryos because they exhibit toxic effects at subeffective doses (12, 13). In preliminary experiments, antisense oligodeoxynucleotides directed against histone H1A mRNA largely disrupted RNA synthesis and some of the newly synthesized major U small nuclear RNA species were missing from analytical gels. Early development was severely perturbed. When thioate-modified oligodeoxynucleotides were used at low doses (<5 ng per embryo), no cleavage was initiated and fertilized eggs started to decay rapidly (unpublished results). These problems are enhanced by the fact that unmodified and modified oligodeoxynucleotides are very unstable in embryos.

As an alternative to oligodeoxynucleotides, the utility of ribozymes for a knock-out of H1A mRNA was investigated. Ribozyme-containing tRNA molecules were previously shown to cleave their target RNAs *in vivo* (14). Therefore, ribozyme sequences were presented in the context of the intron-containing tyrosine tRNA that occurs naturally in *Xenopus laevis* embryos (18). This construct should not be toxic to the embryo. To minimize perturbations in pre-tRNA structure, the intron was chosen as a site for introduction of a "hammerhead" ribozyme (15) targeted to H1A mRNA.

The natural intervening sequence of the oocyte tyrosine tRNA (*TyrC*) gene was modified by a PCR-based approach using the overlap extension principle (Fig. 1). Note that the modified tRNA^{Tyr} gene has an intron loop with an *Apa* I site for easy insertion of additional sequences and that the anticodon loop has been extended to strengthen its secondary structure. The reason for making the latter modification was that in yeast, where tRNA introns can be as large as 60 nucleotides, complementarities between intron and anticodon triplets occur (16). Such complementarities might be important for structural stabilization of native configuration of the pre-tRNA^{Tyr} of an artificially enlarged intron. Such a ribozyme construct should be physiologically stable in and acceptable to the developing embryo.

The insertion of ribozyme sequences into the intron leaves the "cardinal" nucleotides (17) untouched and splicing of the intron should not be affected. Therefore, one issue was to what extent ribozyme-modified pre-tRNA^{Tyr} persisted in the living system, since maturation of pre-tRNA, if it occurred, would lead to destruction of the ribozyme sequences. To assess this potential problem, ribozyme-containing and unmodified tRNA^{Tyr} genes were injected into *Xenopus* oocyte nuclei with cloned 5S RNA genes as internal control, and transcripts generated in the oocyte nucleus were studied over an extended period. In these injection experiments (results not shown), it was found that 5′ and 3′ processing of the ribozyme-tRNA^{Tyr} was not changed compared to the unmodified gene and that the intron splicing event was the rate-limiting step in the processing pathway. Hence, as for endogenously expressed *Xenopus* oocyte-type tRNA^{Tyr} (8), unspliced but 5′- and 3′-processed pre-tRNA^{Tyr} accumulated



Insert of tRNA- ribintron H1A

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5'     CTTTTT AGCGGCTGAT GAGTCCGTGA GGACGAAACT CTTGGTGGCG GGCC 3'
3' CCGGGAAAAA TCGCCGACTA CTCAGGCACT CCTGCTTTGA GAACCACCGC    5'
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FIG. 1. Intron containing oocyte-type tRNA^{Tyr} of *Xenopus laevis* (tRNA^{TyrC}) (*Upper Left*) and the PCR-modified *Apa* I site containing tRNA^{Tyr} (tRNA^{TyrM}) (*Upper Right*), which is used as a cassette for the introduction of the ribozyme coding sequence (*Lower*) targeted to the H1A mRNA (see Fig. 3).

at an ≈10-fold higher level than mature tRNA^{Tyr}. These findings suggest that because of the slow splicing of the pre-tRNA, ribozyme-containing tRNA should remain available in sufficient quantity and for a protracted enough period to act on its substrate.

Resequencing of the H1A gene (18) revealed a tandem repeat of a 50-bp sequence near the 3′ end of the coding region that had been missed (5). Each repeat sequence contains a GUC motif considered to be the best ribozyme cleavage site (19). Hence, a ribozyme with two target sites in the H1A mRNA could be designed. The hammerhead ribozyme (15) was designed with 11 complementary bases on each side of the cleavage site yielding a 61-nt intron. Intron sequences were inserted into a T7 expression vector (pAALM.rib.tyr) as shown in Fig. 2. *In vitro* transcription with T7 polymerase produced a 240-nt RNA. Its ribozyme activity was demonstrated in an *in vitro* assay (performed according to ref. 14) with *in vitro* transcripts of the H1A gene (results not shown).

Inhibition of Histone H1A by the H1A mRNA-Targeted Ribozyme. *In vitro*-fertilized *Xenopus* eggs go through the first cell division after ≈90 min of development. Microinjection was delayed until at least 1 h after fertilization, since at this stage embryos appeared to suffer less from the aftereffects of microinjection. Fertilized eggs showed great tolerance for high doses of ribozyme–tRNA^{Tyr} and doses up to 50 ng of injected RNA did not result in a change of phenotype. The *Xenopus* egg contains ≈60 pg of stored maternal H1A mRNA. After H1A protein synthesis resumed in the blastula embryo, the H1A mRNA pool slowly decreased to ≈100 pg in the early gastrula embryo (unpublished results). For knockout of H1A mRNA, fertilized eggs were injected with 5–10 ng of the 240-nt *in vitro*-synthesized transcripts of clone pAALM.rib.tyr, representing a 100- to 150-fold molar excess over maternally stored H1A mRNA substrate. Injected embryos passed through cleavage and gastrulation unperturbed and on schedule compared with uninjected fertilized eggs.

Total protein extracts from ribozyme-injected and uninjected control embryos from the same fertilization batch were prepared and H1A protein was examined by Western blot analysis (Fig. 3). The increase in H1A synthesis at beginning of gastrulation (stage 10), ≈9 h after fertilization in normal embryos, was suppressed in ribozyme-injected embryos.

Inhibition of Histone H1A Synthesis Prevents Downregulation of Oocyte-Type 5S RNA Genes in Early Development. After the demonstration that H1A protein synthesis could be largely suppressed in early gastrula embryos, expression of oocyte and somatic 5S RNA genes in control embryos and in embryos lacking H1A was studied. One set of fertilized eggs

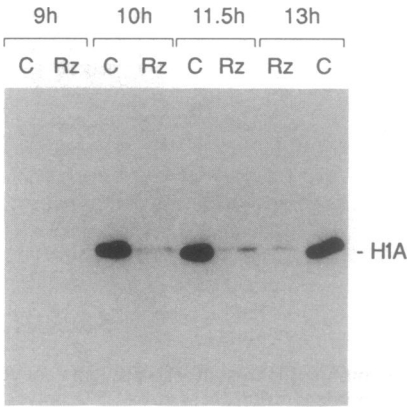

FIG. 3. Ribozyme-mediated inhibition of H1A synthesis in early development of *Xenopus laevis*. Western blot analysis of H1A levels in ribozyme-injected gastrula embryos (lanes Rz) and uninjected controls (lanes C) from the same fertilization. For the early gastrula stage (9 and 10 h), two embryo equivalents of total protein extract were analyzed per lane, whereas for mid- and late-gastrulae stage (10.5 and 13 h of development), just one embryo equivalent was used.

was injected with ribozyme-pre-tRNA^{Tyr} RNA and [^{32}P]GTP and controls were injected with [^{32}P]GTP only. The labeled RNA species from staged embryos were separated on a semi-denaturing gel by the protocol of Wakefield and Gurdon (3). If electrophoresed at room temperature, the 4 M urea-containing gel system resolves oocyte-type and somatic-type 5S RNA into two distinct fractions, presumably due to slight differences in secondary structure of the RNA molecular species. Besides 5S RNA, U1, U2, and U5 small nuclear RNAs and 7S RNA are the major newly synthesized RNA species of low molecular mass that may serve as internal standards for RNA synthesis. Semi-denaturing gel analysis revealed that the only significant effect of repressing H1A synthesis was the continuation of active expression of oocyte-type 5S RNA in gastrula embryos (Fig. 4). Expression of somatic 5S RNA appeared not to be affected by the decrease of histone H1A synthesis, a situation presaged by *in vitro* transcription data of Wolffe (20).

As described by Wakefield and Gurdon (3), equal levels of accumulated oocyte- and somatic-type 5S RNA at MBT represent a 50-fold lower expression of oocyte-type 5S RNA genes (20,000 copies per haploid genome) compared with somatic-type 5S RNA genes (400 copies per haploid genome). The persistence of oocyte-type 5S RNA is the more signifi-

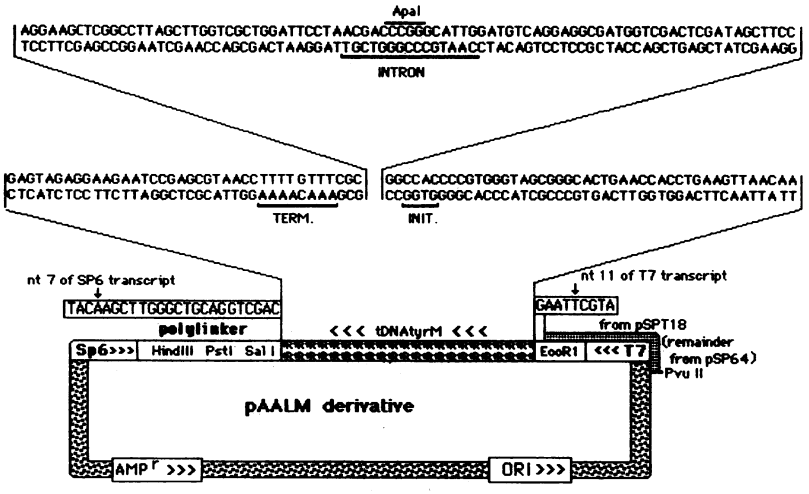

FIG. 2. Structure of the T7 expression vector containing the modified tRNA^{Tyr} gene with the *Apa* I site for easy introduction of ribozyme-coding sequences. TERM., termination site; INIT., initiation site; AMP^r, ampicillin resistance gene; ORI, origin.

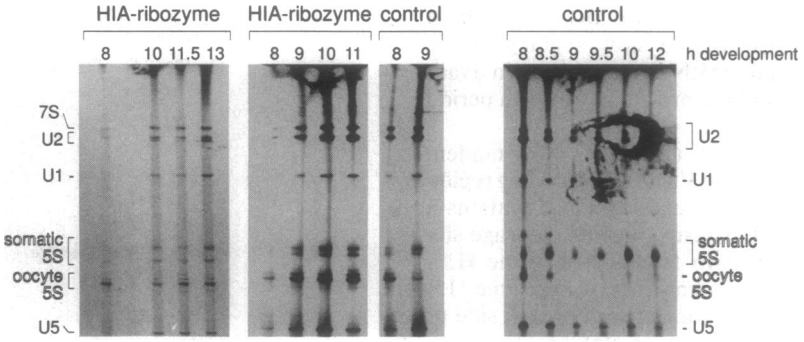

FIG. 4. Inhibition of H1A synthesis prevents oocyte 5S RNA repression. Analysis of total RNA extracts from ribozyme-injected and control embryos by semi-denaturing (4 M urea/15% polyacrylamide) gel electrophoresis. Fertilized eggs were injected with 5–10 ng of *in vitro*-transcribed ribozyme tRNA and 0.1 μCi of [α-^{32}P]GTP (1 Ci = 37 GBq), and controls were injected with [α-^{32}P]GTP only. Developmental stages were determined according to Nieuwkoop and Faber (10), but respective times of development in hours are indicated to account for the rapid changes in the transcriptional pattern occurring in the late blastula (stage 9) embryo (8–9 h). Total RNA of one embryo equivalent (*Left*) and three embryo equivalents (*Center* and *Right*) was analyzed. Transcripts were identified by their mobility as described by Wakefield and Gurdon (3). Note the quick inhibition of oocyte 5S RNA transcription between 8 and 9 h in controls, whereas in the H1A-inhibited embryos accumulated pools of (unstable) oocyte 5S RNA predominate over (stable) somatic 5S RNA.

cant, since oocyte 5S RNA transcripts expressed after MBT are very unstable (4). In spite of this, a significant accumulation of oocyte-type 5S RNA was seen, resulting in at least a 2-fold higher pool of this RNA over somatic 5S RNA. This suggests that inhibition of histone H1A synthesis actually results in the enhancement of oocyte 5S RNA synthesis in ribozyme-treated embryos. However, quantitation of the effect was not possible due to the instability of oocyte 5S RNAs.

To exclude the possibility that the effects on 5S RNA synthesis were due to nonspecific effects associated with injecting pre-tRNA^{Tyr}, an identical construct that differed from the above H1A mRNA-targeted ribozyme-pre-tRNA^{Tyr} by targeting the ribozyme to an irrelevant viral sequence was injected into fertilized eggs, and the labeled RNA was analyzed as before. The RNA pattern of embryos injected with the virus-targeted ribozyme-pre-tRNA^{Tyr} was indistinguishable from that of [^{32}P]GTP-injected control embryos (results not shown).

DISCUSSION

Differential regulation of somatic and oocyte 5S RNA genes has been proposed to be a consequence of differential stabilities of transcription complexes assembled on somatic and oocyte 5S RNA genes against a background of progressively limiting amounts (per cell) of transcription factors during early embryonic development (1, 21). This report shows that after inhibition of histone H1A production, oocyte 5S transcription is enhanced compared to somatic 5S transcription at a time when transcription factors were assumed to be the limiting factor. Therefore, there must be a sufficiency of transcription factors beyond early gastrulation when oocyte 5S genes are normally silenced. Thus, the concept of limiting transcription factors as the main cause for the decrease of oocyte 5S gene transcription is not supported by my findings. The only caveat is that inhibition of H1A synthesis might fortuitously stimulate synthesis of otherwise limiting transcription factors. In the view of Wolffe and Brown (21), repression of oocyte 5S genes by nucleosomes and histone H1 would be opportunistic in that whatever DNA region was not assembled into active transcriptional complexes would be compacted into repressed chromatin. This work suggests a more active role for somatic H1A.

A role for histone H1 in repressing oocyte 5S genes was recognized early (22). Thus, inactive oocyte 5S genes in the chromatin of somatic tissues were shown to lack specific transcription factors and to be prevented from binding factors by virtue of being complexed in a chromatin structure dependent on histone H1. Furthermore, Wolffe (20), using soluble extracts of *Xenopus laevis* oocyte nuclei and chromatin deficient in H1, demonstrated that addition of H1 results in the dominant and selective repression of oocyte 5S RNA gene transcription *in vitro*. The proposal was made that changes in chromatin structure could have a dominant role in regulating class III genes during *Xenopus* embryogenesis. Later, histone H1 was shown to have a role in determining organization of nucleosomes on the oocyte 5S DNA repeat and in repressing *in vitro* transcription of oocyte 5S RNA genes (23). The loss of H1 specificity when using naked genomic DNA or chromatin depleted of H1 by salt (which allows sliding of the nucleosomes) as template suggested that differential interaction of H1 with oocyte 5S genes cannot be explained simply on the basis of H1–DNA interaction but that some aspect of nucleosomal arrangement is involved in establishing a repressed state (20).

In view of the *in vivo* results, the question that must be asked is, when histone H1A abounds, which features allow the oocyte-type 5S gene to be silenced and the somatic 5S genes to be active. The disparate chromatin structure of major oocyte and somatic 5S gene clusters and the differential association of these genes with transcription factors could certainly be involved. Indeed, in erythrocytes, the oocyte 5S gene sequence is occupied by a positioned nucleosome (23), and as a consequence, the repressed oocyte 5S coding sequences are protected from micrococcal nuclease attack, whereas nucleosomes on the also repressed somatic gene cluster are not positioned relative to sequence and the 5S coding sequences are, thus, accessible to nuclease attack. If this situation also prevails in embryos, one would predict that during chromatin assembly, in presence of H1A and weakly interacting transcription factors, oocyte-type 5S genes would associate with a positioned nucleosome. When H1A is present it may compete effectively with weakly binding transcription factors and may "seal" the nucleosome in its position and perhaps drive the gene cluster with its positioned nucleosomes into a solenoid-like 30-nm fiber in which H1 molecules interact cooperatively in a head-to-tail fashion (24). In contrast, the somatic gene cluster, because of its arrangement of nucleosomes that are not positioned with regard to DNA sequences (23), should permit binding of transcription factors and resist assembly into a repressed chromatin structure as suggested by Wolffe and Brown (21). In line with this argument is the recent work of Scidel and Peck (25) who report a preferential and more rapid formation of transcrip-

tion complexes on the somatic 5S gene than on the major oocyte 5S genes.

The predisposition of major oocyte 5S genes for positioning of nucleosomes might be caused by the notably A+T-rich spacer regions of various lengths that are not found in somatic 5S gene clusters. Jerzmanowski and Cole (26) reported that major oocyte-type spacer regions represent preferential binding sites for histone H1 *in vitro* and suggested a dominant role of spacer sequences in differential regulation of major oocyte- and somatic-type 5S RNA genes.

It was already stressed that the *in vivo* data in this work allow only a qualitative evaluation, due to the instability of oocyte 5S RNA transcripts. A further limitation is that there is no differentiation between major and trace oocyte 5S RNA genes. The same applies to *in vivo* results of Andrews and Brown (27) who report a high transient responsiveness of oocyte 5S RNA gene transcription to overexpression of transcription factor TFIIIA in *Xenopus* embryos. Trace oocyte 5S genes are present at 1300 copies per haploid genome and show significant differences to major oocyte-type genes in both sequence and repeat structure (28). They exhibit lower affinity to transcription factor TFIIIA than major oocyte- and somatic-type genes but are equally and actively transcribed as somatic 5S genes in oocyte S150 extracts where major oocyte-type genes are significantly discriminated (29). The observed discrimination of major oocyte 5S RNA genes despite the presence of nonlimiting amounts of transcription factors *in vitro* might be explained by an interaction with the maternal histone variant H1M that is present in oocyte and egg extracts. Further, it is clear that *in vitro* assays using cloned genes cannot be extended to include effects of a natural chromatin environment. However, the available evidence tentatively suggests that the trace oocyte 5S genes are regulated in a distinct way and are not subject to histone H1-mediated repression.

Here it is proposed that the H1A protein plays a dominant role in shifting the equilibrium in the major oocyte gene cluster into a "closed" chromatin configuration, perhaps even driving the gene cluster into solenoid assembly. The transient expression of oocyte 5S RNA after the midblastula transition appears to be a consequence of the lack of histone H1 in the late blastula embryo. In contrast, the somatic gene cluster, because of its random arrangement of nucleosomes and its stable association with transcription factors, is not subject to an H1-mediated formation of repressed chromatin.

Thus the *in vivo* biological function of the ubiquitous histone variant H1A in the regulation of a specific gene has been shown. It remains to be determined whether genes other than oocyte 5S genes are subject to H1A regulation. Nevertheless, it is noteworthy that embryos that lack H1A protein during the early gastrula stage develop normally at least up to swimming tadpole stage at which point observation was terminated.

Note Added in Proof. Recently, Wolffe's group, using a virtually identical ribozyme construct, has confirmed the above observation of a dominant role of histone H1 in mediating the differential expression of the oocyte and somatic 5S genes (30).

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