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Histone H1 in modulation of chromatin transcriptional activity*

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Histones are the major nuclear proteins responsible for packaging DNA into chromatin. Recent genetic and biochemical evidence indicates that the association of histones with DNA profoundly influences processes such as recombination, replication and transcription. As regards transcription there has been rapid progress within the last 3 - 4 years in understanding the role of nucleosomal core histones in determining the accessibility of *cis* regulatory elements to *trans* acting transcriptional activators [1].

Histone H1, while not directly involved in the formation of nucleosomes, plays a key role in the induction of higher order chromatin structures. The basic tails of H1 bind strongly and in a cooperative manner to internucleosomal linker DNA enabling rapid formation and stabilization of the more compact 30 nm chromatin fiber. The latter is a formidable obstacle to both mRNA chain initiation and elongation. It has to be destabilized or partially disrupted for the transcription to proceede [2]. In the course of our studies we attempted to elucidate some of the ways the cell deals with the repressive effect of H1 on transcription. We studied a particular case of histone H1 involvement in regulation of the transcription of 5 S rRNA genes in *Xenopus laevis* as well as a more general phenomenon of the cell-cycle dependent postsynthetic modification of H1 by phosphorylation [3, 4].

The genome of the frog *X. laevis* contains two large families (oocyte and somatic) of class III genes for 5 S RNA, the smallest of the RNAs occurring in the ribosome. The oocyte 5 S RNA gene family consists of approximately 20000 repeating units. Each repeating unit contains a 120-base pair functional 5 S RNA gene sequence and a closely related "pseudogene" commencing 73 base pairs from the 3′ end of the gene. The heterogeneous (360 - 570 base pairs) 5′ flank of the oocyte 5 S RNA gene, as well as a short spacer dividing it from the pseudogene, is highly enriched in A+T. The somatic 5 S RNA gene family consists of about 400 repeating units. Each unit contains a 120-base pair somatic 5 S RNA gene flanked by a 5′ sequence of about 600 base pairs and a 3′ sequence of 80 base pairs. There is no pseudogene. In contrast to the oocyte-type repeating unit the flanking spacers of somatic 5 S RNA genes in *X. laevis* are G+C rich.

Unlike their flanking sequences, the oocyte-type and somatic-type 5 S DNA coding sequences are very similar, differing in only a few positions out of 120 base pairs. The two families of 5 S RNA genes are differentially regulated during *Xenopus* development. Both oocyte and somatic 5 S RNA genes are actively transcribed in oocytes. During embryogenesis the oocyte 5 S RNA genes are progressively inactivated and are finally switched off completely at about the post-midblastula transition stage of development. In contrast to the oocyte genes, the somatic 5 S RNA genes remain active throughout the whole development period as well as in the mature somatic cells [5].

There is evidence that the selective repression of oocyte 5 S RNA genes depends on changes

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occurring at the chromatin level and is brought about by histone H1. Removal of H1 from somatic chromatin resulted in activation of the silent oocyte-type genes so that both oocyte and somatic 5 S RNAs were synthesized in the *in vitro* transcription system. Readdition of H1 restored the selective repression of the oocyte genes while allowing the transcription of the somatic-type genes [6].

There has been considerable interest in defining the molecular basis for the selective repression of oocyte 5 S RNA genes occurring during development. To gain more information on the mechanism underlying this phenomenon, we have analyzed transcription *in vitro* (using transcription extract derived from HeLa cells) of reconstituted complexes of H1 with plasmid DNAs bearing inserts of somatic 5 S RNA genes together with their flanking sequences [3]. We have used two types of cloned template genes. The "native" templates consisted of plasmids in which were inserted oocyte and somatic 5 S RNA repeating units where the 120 base pair gene (Fig. 1B) was flanked by the native sequences. "Switched" templates were also used in which the flanks were switched between the two types of genes (Fig. 1A).

For the native templates we have found that the inhibition of transcription by H1 was much more effective for the oocyte than for the somatic genes (Fig. 2) in close resemblance to the situation *in vivo*. When templates with switched flanking sequences were used (oocyte 5 S RNA gene positioned within the somatic-type flanks and *vice versa*) the situation was reversed and the transcription of the somatic gene was inhibited by H1 with far greater efficiency than that of the oocyte gene (Fig. 3). Thus, it is evident that the selective inhibitory effect of H1 in this system depends entirely on the flanking sequence of 5 S RNA genes and not on the coding sequence itself. At physiological H1:DNA ratio (0.6 - 0.8) H1 strongly inhibited the transcription of the gene flanked by the A+T-rich sequences, whereas it did not prevent transcription of the genes flanked by the G+C -rich sequences. This was reflected by strongly preferential binding of H1 to isolated 5 S RNA genes contained within A+T-rich flanks (Fig. 4).

The selective inhibition of the oocyte 5 S RNA gene transcription by H1 in chromatin isolated from *Xenopus* somatic cells [6] was the first, and so far, the only demonstration *in vitro* of the regulation of any specific gene occurring at chromatin level. The reasons for the observed effect were not clear. The results reported here show clearly that one of the underlying reasons may be the design of the oocyte-type flanking sequences. They comprise the internally repeated sequence most of which can be represented as follows [7]:

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    C          AA    C G
CAA GTTTTCAA   TTTGA TTTTCT
    A          GG    A T
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This sequence is 76% A+T, providing a strong H1 binding region in the direct vicinity of the oocyte 5 S RNA gene. The tightly bound H1

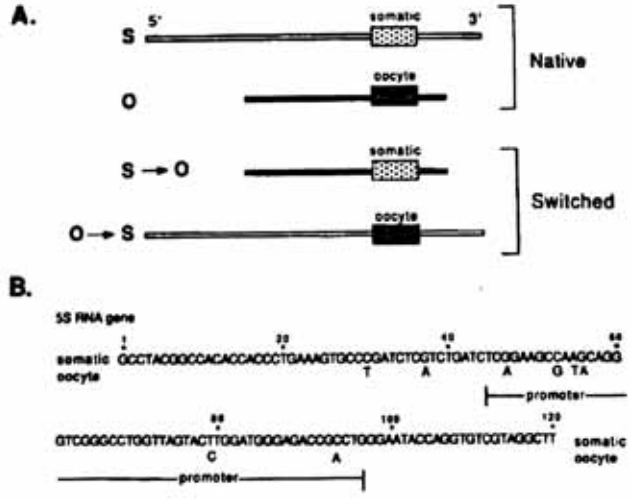


Fig. 1. Structure of templates used in the in vitro transcription experiments.
A. Diagrammatic representation of inserts carrying the *X. laevis* 5 S RNA (S) somatic and (O) oocyte genes. B, sequences of *X. laevis* 5 S RNA genes. Description in the text (Jerzmanowski & Cole [3])

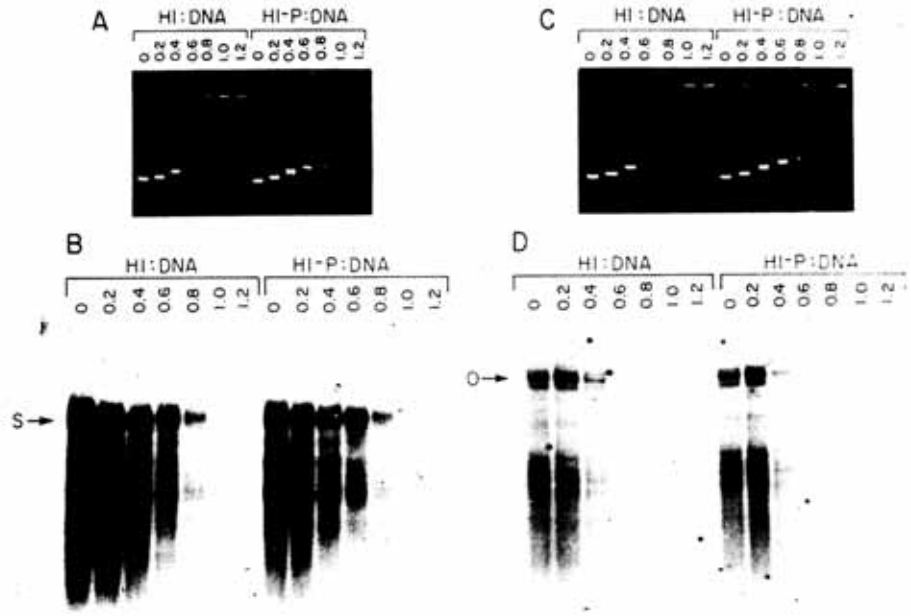
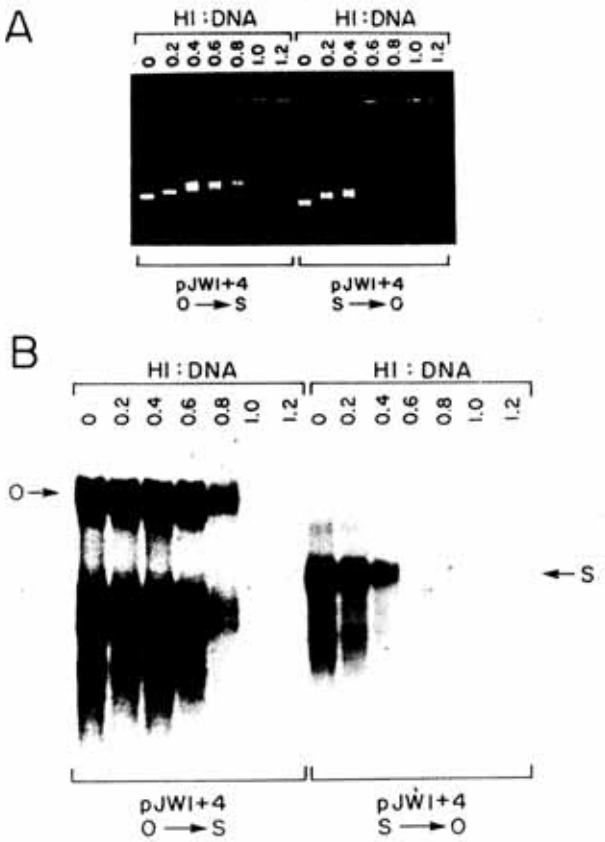


Fig. 2. Effect of histone H1 on transcription of superhelical plasmids carrying 5 S RNA genes flanked by native sequences.

A and C, complexes between H1 and template plasmids. Non-modified (H1) and mitotically phosphorylated (H1-P) H1 were reconstituted at different H1:DNA ratios with plasmids carrying somatic (A) and oocyte (C) 5 S RNA genes. Aliquots of the resulting complexes were analyzed on 1% agarose gels.The ratio (w/w) of H1 or phosphorylated H1 to DNA is indicated above each lane. B and D, transcription of the complexes of H1 and H1-P with templates. Autoradiogram of gel electrophoretic analysis of transcription products from *in vitro* transcription by HeLa cell extract of reconstituted complexes of H1 and H1-P with plasmids carrying somatic (B) and oocyte (D) genes. Arrows indicate the position of somatic (s) and oocyte (o) 5 S RNAs (Jerzmanowski & Cole [3])



could form an effective barrier for transcription either in the absence or presence of nucleosomes. It should be noted here that during the early stages of *Xenopus* development (up to midblastula transition) the adult-type H1 cannot be detected in *Xenopus* chromatin. This period of H1 deficiency almost perfectly correlates with the period of effective synthesis of the oocyte-type 5 S RNA [5, 8].

The experimental system used provided the opportunity to assess the effect of H1 phosphorylation on the inhibitory effect of H1 on transcription. Of particular interest to us was the mitotic-type phosphorylation of H1, which occurs cyclically at mitosis and is a universal phenomenon among eukaryotes as diverse as mammals [9] and slime molds [10]. The mitotic phosphorylation results in maximally phosphorylated H1 (as compared to levels of non-mitotic phosphorylations of H1) and usually

Fig. 3. Effect of histone H1 on transcription of superhelical plasmids carrying 5 S RNA genes with switched flanks.

A. Agarose gel electrophoresis of H1:DNA complexes. B. Transcription of H1:DNA complexes. Descriptions as in Fig. 1 and Fig. 2 (Jerzmanowski & Cole [3])

affects the whole population of H1. Although the enzyme responsible for this phosphorylation, the growth-associated H1 kinase (a homolog of yeast *cdc2/cdc28* gene product), was shown recently to regulate the entry of cells into mitosis (for review, see Dunphy & Newport [11]) the role of mitotic phosphorylation of H1 remains unclear.

We found that the mitotic phosphorylation of H1 invariably decreased the ability of H1 to induce aggregation of H1:DNA complexes and concurrently enhanced the availability of DNA in these complexes for transcription (Fig. 2). *In vivo*, this modification of H1 may increase the availability of chromatin DNA to various protein factors present in the cell at the time of mitosis despite high condensation of chromatin. It would be particularly interesting to examine whether this increased availability had the effect of preparing the DNA for the forthcoming round of replication. It has long been known that, in order to start replication, chromosomes must be "primed" by passing through mitosis [12, 13].

To gain more information on the role of mitotic phosphorylation of H1 we studied the requirements for the effective phosphorylation of HeLa chromatin H1 by homologous cdc2-type kinase *in vitro* [4].

Mitotic cdc2-type kinase was prepared from HeLa cells enriched in the mitotic-stage cells by treatment with vinblastine. The kinase (presumably in the complex with cyclin B) efficiently phosphorylated the isolated H1 in an *in vitro* system. Figure 5 presents two-dimentional maps of tryptic phosphopeptides of the H1b subfraction of HeLa histone H1 phosphorylated *in vivo* in mitotic cells and *in vitro* by the homologous mitotic kinase. The two types of phosphorylation resulted in basically similar although not completely identical maps. In contrast to the high efficiency with which the kinase phosphorylated free H1 phosphorylation of chromatin-bound H1 under identical *in vitro* conditions was completely ineffective (not shown). As a result we have ruled out the possibility of the increased chromatin aggregation and the activity of endogenous phosphatase. However, the displacement of H1 from some the chromatin DNA sites by elevated concentration of NaCl turned out to be sufficient to restore H1 phosphorylation by exogenous kinase (Fig. 6). At 300 mM NaCl H1 is not yet dissociated totally from chromatin but probably it is partly displaced from its tight binding sites. This was confirmed by finding that, *in vitro* only a subset of the sites became phosphorylated at that NaCl concentration by mitotic H1 kinase (Fig. 7).

It seems very probable that the situation *in vitro* mirrors that in the cell and that the chromatin bound H1 is actively displaced just before mitosis, prior to its phosphorylation by the cdc2-type kinase. This would require a factor causing the displacement of chromatin H1 *in vivo*. Probable candidates would be proteins containing distinct tracts of acidic amino acids like nucleoplasmin [14] or HMG 1 and 2 which could potentially displace histones from DNA.

The active displacement of chromatin H1 during mitosis would not only enable its rapid and effective phosphorylation by the cdc2-type kinase but would also, by lifting the H1-conferred inhibition, open up the chromosomal DNA for interactions with regulatory proteins during a crucial period of its mitotic exposure to cytoplasmic environment.

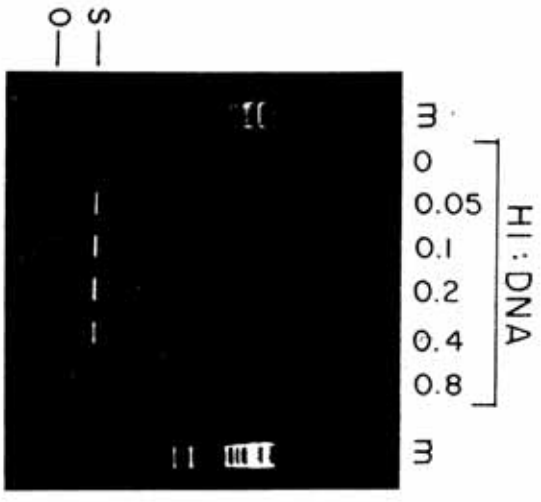


Fig. 4. Competitive binding of H1 to DNA inserts bearing somatic (s) and oocyte (o) 5 S RNA genes. DNA inserts bearing somatic and oocyte 5 S RNA genes surrounded by native flanks were excised from corresponding plasmids, mixed in a 1:1 (w/w) ratio and reconstituted with different amounts of H1. Aliquots of the resulting complexes were analyzed on 1% agarose. The ratio of H1 to DNA is indicated above each lane. Markers, m (Jerzmanowski & Cole [3])

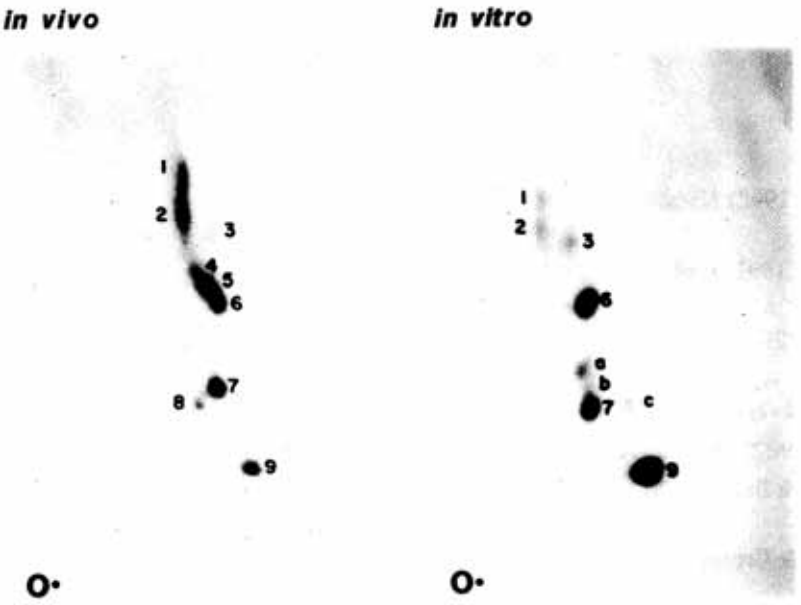


Fig. 5. Two-dimensional phosphopeptide maps of the H1B subfraction of HeLa histone H1.
Left panel: radioactive peptides from H1 phosphorylated *in vivo* in mitotic cells. Right panel: radioactive peptides from free H1 phosphorylated *in vitro* by mitotic H1 kinase. O •, origin (Jerzmanowski & Cole [4])

In summary, the results of our studies provide a possible explanation for the mechanism by which the repressive effect of H1 on transcription can be used for differential regulation of genes. An important new suggestion based on the results of our *in vitro* phosphorylation

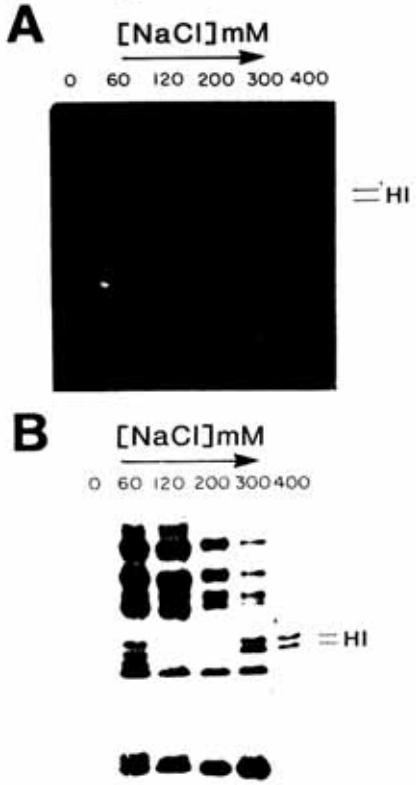


Fig. 6. Phosphorylation in vitro of the chromatin-bound H1 by mitotic kinase at different concentration of NaCl.
A. SDS-gel electrophoresis of extracted basic proteins. B. Autoradiogram of A (Jerzmanowski & Cole [4])

Fig. 7. Two-dimensional phosphopeptide map of the H1B subfraction of HeLa histone H1 phosphorylated in vitro by mitotic H1 kinase in chromatin at 300 mM concentration of NaCl. O•, origin (Jerzmanowski & Cole [4])

experiments is that H1 is actively displaced from chromatin during mitosis.

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