Journal of Cell Science 103, 889-895 (1992)
Printed in Great Britain © The Company of Biologists Limited 1992

COMMENTARY

Histone H1 and transcription: still an enigma?

JORDANKA ZLATANOVA* and KENSAL VAN HOLDE†

Department of Biochemistry and Biophysics, Oregon State University, Corvallis, Oregon 97331-6503, USA

*On sabbatical leave from the Institute of Genetics, Bulgarian Academy of Sciences, 1113 Sofia, Bulgaria
†Author for correspondence

Introduction

For many years, the role of lysine-rich histones in the regulation of transcription has been a puzzle. This class of histones, which includes H1 and its many variants, is associated exclusively with the linker DNA between nucleosomes: for brevity, we shall generally refer to the whole class as H1, unless a distinction is important. Early recognition that linker histones stabilized the condensed 30 nm fibre structure of chromatin led to speculation that such proteins might be missing from transcriptionally active regions (see van Holde, 1988; Zlatanova, 1990, for reviews). However, the inadequacy of hitherto available techniques for analysing *local* chromatin structure has left the question unresolved. Only now are methods becoming available that may allow unambiguous answers and permit more sophisticated questions to be posed.

Linker histone stoichiometry and transcription

Earlier studies indicated that chromatin of most cell types contained about one molecule of linker histone per nucleosome, consistent with the presence of one primary binding site in the nucleosome. However, stoichiometries higher than unity have been reported for chromatins highly inactive in transcription: 1.3 for chicken erythrocyte chromatin (Bates and Thomas, 1981) and about 2 for the dry maize embryo (Ivanov and Zlatanova, 1989). Interestingly, the increased H1 content in the inactive dry embryo chromatin was quickly reduced to one molecule per nucleosome upon the transcriptional activation of the genome occurring early during germination (Ivanov and Zlatanova, 1989). These data are consistent with the recent work of Segers et al. (1991), who find that binding of a second linker histone molecule to the nucleosome is possible; however, the second site is of lower affinity; binding to this site is intrinsically different and results in aggregation.

That the relationship between high levels of H1 and repression of transcription is stronger than a mere correlation is indicated by recent in vitro studies by Laybourn and Kadonaga (1991). When H1 was incorporated into reconstituted chromatin at 0.5-1.0 molecule per nucleosome, RNA synthesis was dramatically reduced when compared with chromatin containing only nucleosomal cores; further increasing the content of H1 to 1.5 molecules per nucleosome led to a complete inhibition of transcription (see below).

Complementary data that have long been used to support the idea that H1 depletion promotes transcription come from the general failure to isolate a typical histone H1 from yeast chromatin, most of which is in an at least potentially active state as judged by its sensitivity to DNase I digestion (Grunstein, 1990). Although H1-like proteins have recently been reported in yeast (Srebreva et al., 1987), their properties might be quite different from those of the linker histones of higher organisms. Moreover, the yeast H1 may be present in low amounts and be localized predominantly or exclusively in the heterochromatic regions of the genome or at telomeres.

Initial attempts to assay the presence or absence of H1 in transcriptionally active chromatin regions

Although the data cited above are suggestive, they do not address the really important specific question: are linker histones present, absent, depleted, or differentially bound on those regions of the genome that are actively being transcribed, or on those flanking regions that regulate transcription?

Past attempts to answer such questions depended in large part on rather awkward fractionation schemes, which attempted to separate “active” from “inactive” chromatin (see Rose and Garrard, 1984; Rocha et al., 1984; Xu et al., 1986; for examples). Aside from uncertainty as to the real basis of separation, these experiments suffered from the disadvantage that nothing more than an enrichment in”active” or “inactive” sequences could be obtained. Furthermore, it was soon realized that some properties of H1 in chromatin such as high sensitivity to proteolysis, an easy release from DNA upon nuclease digestion because of its location on the linker DNA, and the ability to redistribute at moderate ionic strength might compromise experimental data obtained on

Key words: chromatin, histone H1, transcription, cross-linking.

"fractionated" chromatin. Thus, the general conclusion from these studies - that active genes were highly depleted in linker histones - has come to be regarded with some suspicion.

In 1984 Weintraub reported an analysis of electrophoretically separated supranucleosomal particles obtained upon mild micrococcal nuclease treatment of nuclei (Weintraub, 1984). Both the particles enriched in the active genes and those harboring the inactive ones contained H1 and H5, and Weintraub suggested that the two types of particles differed not in stoichiometry, but rather in the mode of linker histone interaction with the template: in the inactive particles the linker histones held the oligonucleosomes together, whereas in the active ones they did not. Recently, however, Grigoryev et al. (1991) have questioned the significance of these experiments by demonstrating that the assembly of the inactive particles was artifactual, occurring during the electrophoretic analysis itself. They do, however, confirm Weintraub's result that H1 and H5 are present in both fractions, albeit somewhat reduced in the "active" particles.

New approaches, new insights

Recently, immunochemical and crosslinking techniques have been developed which permit much more discriminating analysis. Antibodies to H1 histones can interact specifically with these proteins in situ. In this way, Srebreva and Zlatanova (1992) studied the effect of specific anti-histone-H1 antibodies on in vitro transcription in isolated nuclei. The incorporation of radioactive precursors into RNA was compared for control nuclei and nuclei that had been preincubated with specific anti-H1 antibody populations; the antibodies significantly and reproducibly inhibited the transcriptional activity. Control experiments excluded the possibility that the observed inhibition was due to some long-distance effect that binding of the antibodies might have on chromatin structure. The results indicated that at least some H1 was intimately associated with chromatin regions actively involved in transcription. Similar conclusions were derived from immunoelectron microscopy studies of the active Balbiani ring genes in the salivary glands of *Chironomus tentans*. With the use of specific antibodies, H1 was identified both on fully expressed templates and on repressed genes in a 30 nm fibre conformation (Ericsson et al., 1990). A monoclonal antibody recognizing an epitope in the C-terminal tail of histone H1 was shown to bind to bands, interbands and puffs, i.e. to all regions of polytene chromosomes, irrespective of their transcriptional status (Hill et al., 1989). Although experiments of this kind do not quantitate the H1 content of active genes, they clearly demonstrate that complete depletion of H1 is not necessary for transcription.

The second technique to provide new insights involves crosslinking of histones to DNA, followed by cleavage of the DNA, separation of the fragments, and hybridization to sequence-specific probes. A variety of methods for crosslinking, cleavage, and analysis have been used (Table 1), but most share the potential to identify contacts of particular histones with specific sequences. The pioneering studies with respect to H1 appear to be those of Karpov et al. (1984), who crosslinked histones to DNA by the dimethyl sulphate technique of Mirzabekov (see Mirzabekov et al., 1989). Electrophoretic analysis of the resultant complexes indicated the absence of H1 on the active *hsp70* genes in *Drosophila*. However, this crosslinking proceeds mainly via the histidines found in the globular domains of the histone molecules. Recent modifications of the crosslinking procedure allowed the creation instead of crosslinks to lysines found in high concentration in the terminal domains (Nacheva et al., 1989). In this case the crosslinking was quantitatively similar for active and inactive *hsp70* chromatin. These results argue that the interaction of the globular domain of H1 with DNA is altered upon gene activation but the histone still remains associated with DNA via its tails. The same approach was recently extended to the ribosomal genes of the same organism (Belikov et al., 1990). The conclusion that, when transcribed, these genes were partially depleted of histones, including H1, was based on chemical crosslinking primarily through the histidines. This approach was, however, complemented with UV crosslinking, which is of relatively low chemical specificity with respect to the amino acid side-chains, with results similar to those obtained from the chemical crosslinking.

Recently, a number of laboratories have used a combination of protein/DNA crosslinking procedures and immunofractionation to isolate specific protein/DNA complexes, which were then analysed for the presence of active or inactive genes by DNA-DNA hybridization. Thus, Kamakaka and Thomas (1990) using a UV crosslinking procedure showed that none of the sequences studied (active or inactive) were H1-free, although some partial depletion (about 20-40%) was observed in active gene chromatin.

Attractive as such techniques are, their results can be affected by a number of parameters, which must be carefully examined if quantitation is intended. It is often assumed that the intensity of the hybridization signal at the final experimental step reflects the relative concentration of the corresponding sequence in the immunoprecipitate, and that this, in turn, reflects the linker histone content in the mixture of DNA fragments analysed. This assumption, however, is valid only if the efficiency of crosslinking of all H1 (or H5) molecules is the same in both the active and inactive genes, i.e. is independent both of the mode of binding of the histone to the DNA and of the higher-order structure. Neither is probably the case (see, for example, Nacheva et al., 1989). Another complication arises from the fact that the level of hybridization will be the same, irrespective of the number of histone molecules cross-linked to a particular fragment. In the experiments of Kamakaka and Thomas (1990), for example, a low percentage of linker histones were crosslinked - about 2.5%, which corresponds to one molecule bound per every 10 000 bp. It would probably be reasonable to expect that each fragment contains one histone molecule but, unfortunately, the paper lacks the information necessary to make such an evaluation: the average length of the restriction fragments is not given, nor is the possibility discussed that the restriction enzyme might produce fragments of different lengths in the active and inactive chromatin because of differences in protein pro-

Table 1. *Quantitation of the amount of H1 in different genes or gene regions from crosslinking experiments*

Genes	Method	Amount of H1*	Remarks	References
pol II genes				
Drosophila hsp70 gene	DMS† crosslinking, 2D electrophoresis	Promoter: no H1 before or after activation; coding region: removal of most of H1 upon activation; 3′ end: removal of some H1	Crosslinking primarily via the globular domains	Karpov et al. (1984)
Drosophila hsp70 gene	DMS crosslinking, 2D electrophoresis	‡Promoter: no H1 before or after activation ‡coding region: H1 amount similar for active and inactive chromatin	When crosslinking is performed via the globular domains, results identical to those of Karpov et al. (1984) ‡results of crosslinking via terminal regions as well	Nacheva et al. (1989)
Chicken β-globin, ovalbumin and lysozyme genes	Formaldehyde/DMS or UV crosslinking, immunoprecipitation or 2D electrophoresis	50% less H1 and H5 in transcribed β-globin gene than in inactive ovalbumin and lysozyme genes	The depletion of H1/H5 in active β-globin gene is observed even when the histone tails are cross-linked, in contrast to *Drosophila hsp70* gene (Nacheva et al., 1989)	Postnikov et al. (1991)
Chicken β-globin, histone H5, β-actin, ovalbumin, and keratin genes	UV crosslinking, *Pst*I restriction, immunoprecipitation	H1 is present on all genes but somewhat depleted in active genes	The degree of depletion is different for the different types of genes (tissue-specific, housekeeping, etc.) in different tissues	Kamakaka and Thomas (1990)
Tetrahymena thermophila conjugation-specific *ngoA* and *cnjB* genes; actin gene	Formaldehyde fixation, sonication, immunoprecipitation	Inversely proportional to transcriptional activity		Dedon et al. (1991)
Mouse mammary tumour virus promoter	UV crosslinking, *Hae*III restriction, immunoprecipitation, primer extension	45% decrease upon hormone activation	Similar decreases were observed in coding and promoter portions of the fragment; the decrease in H1 crosslinking correlates with the percentage of active templates	Bresnick et al. (1992)
pol I genes				
X. laevis ribosomal genes	Formaldehyde or UV crosslinking, sonication, immunoprecipitation	50%		Dimitrov et al. (1990)
D. melanogaster ribosomal genes	DMS crosslinking, 2D electrophoresis; UV crosslinking, *Alu*I restriction, 2D electrophoresis	Transcr. start site: 5-10% promoter: 30-40% coding region: 45-75%	DMS crosslinking primarily via the globular domains	Belikov et al. (1990)
Tetrahymena thermophila 26 S rRNA gene	Formaldehyde fixation, sonication, immunoprecipitation	Inversely proportional to transcriptional activity		Dedon et al. (1991)

*When percentage is given, comparison is with inactive chromatin.
†DMS, dimethyl sulphate.

tection of cleavage sites, etc. The interpretation of the results in this case is also complicated, as noted by the authors themselves, by the use of different hybridization probes (cDNA or genomic sequences) for the different genes. And last, but not least, the quantitative interpretation of the results can be meaningful only if data are available on the proportion of templates actually being transcribed in the particular system. Because of methodological difficulties in obtaining such data, only a few papers approach this issue.

Results similar to those of Kamakaka and Thomas (1990) were obtained by Postnikov et al. (1991) with the help of both UV and formaldehyde/dimethyl sulphate crosslinking. These studies were directed toward the active β-globin gene and inactive lysozyme and ovalbumin genes in chicken embryonic erythrocytes, and utilized probes to the transcribed regions of all three genes, plus a probe to the 5′ flanking region of the β-globin gene. The results clearly indicate a depletion of about 50 % in the H1/H5 content of the globin gene and its 5′ flank as compared to the ovalbumin and lysozyme genes, even when crosslinking methods that should bind linker histone tails to DNA were employed. Thus, the results appear to be inconsistent with earlier quantitative data on *Drosophila hsp70* genes (Nacheva et al., 1989, see above). Postnikov et al. (1991) suggest several possible reasons for the descrepancy, none of which can be definitely confirmed at this point.

In an important recent application of this technique, Bresnick et al. (1992) have shown that transcriptional activation

of the mouse mammary tumour virus promoter by steroid hormone involves the release of specific H1 molecules from linkers situated around the promoter-coding region boundary. This is, to our knowledge, the first study to reveal the precise location of H1 release, and to associate H1 release with specific activation events. Importantly, the decrease in H1 content correlates with the percentage of templates being transcribed, which would imply that active templates are totally H1-depleted in the particular gene region analysed.

A variation of the crosslinking/immunofractionation approach has been used to estimate the linker histone content of actively transcribed ribosomal genes in *Xenopus laevis* embryos. Reduced binding of H1 was documented for the spacer enhancer and promoter regions of the active genes (Dimitrov et al., 1991). An inverse correlation between the level of immunoprecipitation with H1 antiserum and transcriptional activity was also evident from an analysis of four different genes, one of them ribosomal, in *Tetrahymena* during logarithmic growth, starvation and conjugation (Dedon et al., 1991). In this study, the fragmentation of chromatin before the immunoprecipitation step was accomplished by sonication to an average fragment size of about 600 bp, which, at the crosslinking efficiency of 10-20% would secure on the average one molecule of H1 per immunoprecipitated fragment.

In summary of results to date: it now seems well established that linker histones are present, but reduced in amount in actively transcribed genes and generally in their 5′ flanking regions as well. H1 may be completely absent from those portions of the promoter regions that contain DNase I-hypersensitive regions (see, for example, Nacheva et al., 1989; Postnikov et al., 1991) or totally depleted from some well-defined gene regions upon activation (Bresnick et al., 1992). In addition, the mode of binding may be different for that fraction remaining bound.

Histone H1 as a repressor: possible mechanisms of action

Earlier functional studies (reviewed by Zlatanova, 1990) suggest that histone H1 inhibits transcription and that the different subtypes of the histone (i.e. H1 versus H5) differ in the degree of inhibition imparted. In more recent studies chromatin reconstituted in vitro from defined DNA fragments, core histones and purified H1 have been used to approach this problem directly. Worcel's group studied transcription from minichromosomes assembled on the *Xenopus* 5 S rRNA gene in the absence (Shimamura et al., 1988) and in the presence (Shimamura et al., 1989) of histone H1. The gene could be repressed in the absence of H1 at high nucleosomal densities (one nucleosome per 160 bp). Importantly, repression could be achieved at lower nucleosomal densities (one per 215 bp) when H1 was present. Gentle removal of H1 led to activation whereas repression could be restored by adding H1 back. The inactivation by H1 could be prevented if the assembly of the transcription complex was allowed to proceed onto the H1-depleted minichromosomes before H1 addition. The recent studies in Kadonaga's group (Croston et al., 1991; Laybourn and Kadonaga, 1991) also uneqivocally support the notion that H1 is a transcriptional repressor (see below).

The mechanism by which H1 represses transcription is still not known. The first and most obvious possibility is through its involvement in the formation and maintenance of the higher-order structure. However, a decondensation of chromatin or at least some loosening of the higher-order structure might be achieved even by partial removal of H1 (e.g. see Kamakaka and Thomas, 1990) if H1-H1 interactions, thought to maintain the 30 nm chromatin fibre are cooperative as is the case with H1 bound to free DNA (for a review on the cooperativity issue, see Zlatanova and Yaneva, 1991a).

There are clearly other possible mechnisms by which H1 could act as a transcriptional repressor. Even if chromatin were opened up, and not condensed into 30 nm fibres, the presence of H1 molecules at or near start sites could block initiation; alternatively, the presence of H1 molecules in coding regions could block the opening or displacement of nucleosomes that is needed for passage of polymerase (see van Holde et al., 1992). Direct evidence for the multiple roles of H1 in transcriptional regulation comes from recent studies by Laybourn and Kadonaga (1991). Using in vitro reconstituted transcription systems, they found that blockage of the initiation site by nucleosomes resulted in repression of transcription as compared to naked DNA and this repression could not be counteracted by the sequence-specific transcription factors Sp1 and GAL4-VP16. The incorporation of H1 into the nucleosomal template at the physiological level of 0.5-1 molecule per nucleosome resulted in a more drastic inhibition of transcription, which, however, could be counteracted by these *trans*-factors. Thus, transcriptional repression mediated by the nucleosomal cores and that by H1 are evidently of distinct molecular natures, since the transactivating factors can overcome the repression caused by H1 but not that caused by the cores. Further increase of H1 to a level of 1.5 molecules/nucleosome led to a complete inhibition, which could not be rescued by transcription factors. Since the chromatin templates remained soluble under these conditions, the inhibitory effect could not be attributed to aggregation. The results from this and a previous study from the same laboratory (Croston et al., 1991) allow a clear distinction between two modes of action of sequence-specific transcriptional activators: true activation (defined as the increase in transcription by binding of the factors to naked DNA templates) and antirepression (transcriptional stimulation by the factors due to counteraction of the histone-mediated repression). These phenomenological studies obviously need to be extended to the molecular level to see where in the chromatin template H1 is bound, how the antirepression by the transcription factors is exerted, etc. Evidently, important results can be anticipated in this field of study.

The data reviewed above (see also Zlatanova, 1990) imply that linker histones can be regarded as a part of a general repressor mechanism of eukaryotic transcription. Some recent data, however, suggest that histone H1 might also be involved in a finer and more specific control of the transcriptional activity of some individual genes. Participation in such control would require that the histone possess

sequence-specific binding of high affinity to *cis*-acting DNA elements of the H1-regulatable genes. Data in support of this have been summarized and discussed by Zlatanova and Yaneva (1991b) and will be only briefly presented here.

The first clear demonstration that H1 may show different affinity for different eukaryotic sequences came from studies on a series of restriction fragments from the rat albumin gene. Filter binding experiments showed that only a fragment containing a part of the 5′ flanking region and a portion of the coding sequence was selectively retained on nitrocellulose filters via H1 binding (Berent and Sevall, 1984). Later, three specific interaction sites were localized within a 346 bp fragment at an exon/intron border (Sevall, 1988). Pauli et al. (1989) incubated protein blots containing H1 with a series of radioactively labelled fragments from the human histone H4 gene and reported that H1 specifically interacted with some distal promoter sequences which had been previously identified as "dehancer" elements. Yaneva and Zlatanova (1992) studied this issue in a homologous system; mouse liver histone H1 was allowed to interact with different regions of the mouse α-globin gene under a variety of experimental conditions. Reminiscent of the results with the rat albumin gene (see above), it was found that H1 possessed a clear-cut preference for DNA sequences encompassing the 5′ flanking regions and the first half of the coding sequence. Importantly, this selectivity was mainly expressed under conditions of non-cooperative binding of the histone to DNA. The relevance of these findings to the situation in the chromatin-contained gene has yet to be demonstrated.

An important study of the relationship between the affinity of binding of H1 to regulatory sequences and transcriptional activity in an in vitro system was reported by Jermanowski and Cole (1990). These authors addressed the issue of the possible mechanisms involved in the discriminatory action of histone H1 on the in vivo and in vitro transcription of the *X. laevis* oocyte and somatic 5 S RNA genes (Schlissel and Brown, 1984; Wolffe, 1989; for review, see Zlatanova, 1990). It was found that the inhibition of transcription in an H1/DNA model system was much more effective for the oocyte than for the somatic genes; the reason for this was the greater affinity of H1 for the oocyte-type flanking sequences. With templates with switched flanking sequences (oocyte 5 S RNA gene with somatic flanks and vice versa) the situation was reversed. To our knowledge, this is the first direct demonstration that individual gene transcription can by affected by the affinity of H1 to defined sequences. It remains to be seen, however, whether such a mechanism could work in a chromatin milieu in vitro and in vivo.

Finally, it is possible that H1 may play a role in regulating transcription by participating in the process of nucleosome positioning, thereby either blocking or making accessible *cis*-acting regulatory elements. Bradbury's group have performed a careful study on chromatosome positioning in chromatin assembled in vitro on tandemly repeated nucleosome positioning sequences (Meersseman et al., 1991). Linker histones were found not to override the underlying positioning signals of the DNA sequence that induce regular nucleosome spacing in this system; they were, however, decisive in determining the relative distribution of nucleosome positions between possible alternatives. In a complementary study, Jeong et al. (1991) reported that H5 (or H1) was capable of inducing physiological spacing and extensive ordering of nucleosomes in a sequence-specific way. However, H5 was not found to interact preferentially with the sequence required for the nucleosome positioning and the molecular mechanism of this phenomenon remains to be elucidated. In a study aimed at determining the chromosomal organization of *X. laevis* oocyte and somatic 5 S RNA genes in vivo, Chipev and Wolffe (1992) found evidence that H1 had a role in determining the formation of nucleosomes over the repressed oocyte 5 S DNA repeat. Removal of H1 caused disruption of this chromatin organization and facilitated the transcriptional activation of this gene in a cell-free extract.

It is also possible that H1 binding to specific sequences might influence the gene regulation in chromatin loop domains. Recently, Izaurralde et al. (1989) and Käs et al. (1989) reported that H1 bound to many (but not all) scaffold attachment regions with a remarkable degree of specificity. In this case, however, the preferential binding was not directed by a strict consensus in the regions but resulted from the overall structure and/or conformation of oligo(dA).oligo(dT) tracts. The authors speculate that this type of highly preferential binding of H1 to sequences involved in attachment of the chromatin loops to the matrix may nucleate cooperative assembly of H1 along the SAR (scaffold-associated regions) into the flanking non-SAR DNA, thus creating a uniform (and probably repressed) conformation in the whole domain.

Are H1 levels in the nucleus under dynamic regulation?

If H1 binding plays any of the roles suggested above in regulating transcription, it seems likely that its level in the nuclear milieu should be under regulation. Suggestive evidence is found in the fact that the cytoplasm of mammalian cells, both proliferating and quiescent, contains a relatively large pool of H1 (Zlatanova et al., 1990). The fact that some H1, as well as some HMG1,2 (see references quoted by Zlatanova et al., 1990), is stored in the cytoplasm, whereas no such pools can be detected for the core histones, can be interpreted as an indirect indication of the participation of these two chromatin protein groups in the regulation of transcription of genes. The proteins participating in this regulation are stored in the cytoplasm, ready for transfer into the nucleus whenever necessary. Most interestingly, and in accord with this idea, a cytoplasmic receptor for histone H1 has been recently identified (Breeuwer and Goldfarb, 1990). Even though H1 is small enough and hence potentially able to diffuse into the nucleus, it is localized in the cytoplsm by a receptor-mediated process that precludes its free diffusion through the nuclear pores (Breeuwer and Goldfarb, 1990).

Conclusion

In part, the puzzle of the role of linker histones in tran-

scriptionally active genes seems to have been resolved. It is now clear that proteins like H1 are present in transcribing genes. Their amount seems to be reduced, as compared with inactive chromatin, and the mode of binding may be different. These statements seem to be valid for both regulatory and coding regions, with the possible exception of DNase I-hypersensitive regions in promoters, which are devoid of all histones, including H1. However, it should be noted that we have no evidence concerning what happens to linker histones at the precise site of polymerase passage.

It seems paradoxical that at the same time evidence for H1 in active genes is becoming firmer, the case for H1 as a repressor (both in a general sense and at specific sites) has also been strengthened. How these two, seemingly contradictory, results are to be reconciled is unclear. It should be noted, however, that most of the repressor studies have been carried out in vitro, using systems that may lack histone modifications and structural organization found in active cellular chromatin. The fact that we cannot as yet distinguish between a number of possible mechanisms of H1 repression may also contribute to the confusion.

The balance shifts, but enigmas remain.

References

Bates, D. L. and Thomas, J. O. (1981). Histones H1 and H5: one or two molecules per nucleosome? *Nucl. Acids Res.* **9,** 5883-5894.

Belikov, S. V., Dzherbashyajan, A. R., Preobrazhenskaya, O. V., Karpov, V. L. and Mirzabekov, A. D. (1990). Chromatin structure of Drosophila melanogaster ribosomal genes. *FEBS Lett.* **273**, 205-207.

Berent, S. L. and Sevall, J. S. (1984). Histone H1 binding at the 5′ end of the rat albumin gene. *Biochemistry* **23**, 2977-2983.

Breeuwer, M. and Goldfarb, D. S. (1990). Facilitated nuclear transport of histone H1 and other small nucleophilic proteins. *Cell* **60**, 999-1008.

Bresnick, E. H., Bustin, M., Marsaud, V., Richard-Foy, H. and Hager, G. L. (1992). The transcriptionally-active MMTV promoter is depleted of histone H1. *Nucl. Acids Res.* **20**, 273-278.

Chipev, C. C., and Wolffe, A. P. (1992). Chromosomal organization of Xenopus laevis oocyte and somatic 5 S RNA genes in vivo. *Mol. Cell. Biol.* **12**, 45-55.

Croston, G. E., Kerrigan, L. A., Lira, L. M., Marshak, D. R. and Kadonaga, J. T. (1991). Sequence-specific antirepression of histone H1-mediated inhibition of basal RNA polymerase II transcription. *Science* **251**, 643-649.

Dedon, P. C., Soults, J. A., Allis, C. D. and Gorovsky, M. A. (1991). Formaldehyde cross-linking and immunoprecipitation demonstrate developmental changes in H1 association with transcriptionally active genes. *Mol. Cell. Biol.* **11**, 1729-1733.

Dimitrov, S. I., Stefanovsky, V. Yu., Karagyozov, L., Angelov, D., and Pashev, I. G. (1990). The enhancers and promoters of the Xenopus laevis ribosomal spacer are associated with histones upon active transcription of the ribosomal genes. *Nucl. Acids. Res.* **18**, 6393-6397.

Ericsson, C., Grossbach, U., Bjorkroth, B. and Daneholt, B. (1990). Presence of histone H1 on an active Balbiani ring gene. *Cell* **60**, 73-83.

Grigoryev, S. A., Spirin, K. S. and Krasheninnikov, I. A. (1991). Loosened nucleosome linker folding in transcriptionally active chromatin of chicken embryo erythrocyte nuclei. *Nucl. Acids Res.* **18**, 7397-7406.

Grunstein, M. (1990). Histone function in transcription. *Annu. Rev. Cell Biol*. **6**, 643-678.

Hill, R. G., Watt, F., Wilson, C. M., Fifis, T., Underwood, P. A., Tribbick, G., Geysen, H. M. and Thomas, J. O. (1989). Bands, interbands and puffs in native Drosophila polytene chromosomes are recognized by a monoclonal antibody to an epitope in the carboxy-terminal tail of histone H1. *Chromosoma* **89**, 411-421.

Ivanov, P. V. and Zlatanova, J. S. (1989). Quantitative changes in the histone content of the cytoplasm and the nucleus of germinating maize embryo cells. *Plant Physiol. Biochem.* **27**, 925-930.

Izaurralde, E., Käs, E. and Laemmli, U.K. (1989). Highly preferential nucleation of histone H1 assembly on scaffold-associated regions. *J. Mol. Biol*. **210**, 573-585.

Jeong, S. W., Lauderdale, J. D. and Stein, A. (1991). Chromatin assembly on plasmid DNA in vitro. Apparent spreading of nucleosome alignment from one region of pBR327 by histone H5. *J. Mol. Biol.* **222**, 1131-1147.

Jermanowski, A. and Cole, R. D. (1990). Flanking sequences of Xenopus 5 S RNA genes determine differential inhibition of transcription by H1 histone in vitro. *J. Biol. Chem.* **265**, 10726-10732.

Kamakaka, R. T. and Thomas, J. O. (1990). Chromatin structure of transcriptionally competent and repressed genes. *EMBO J.* **9**, 3997-4006.

Karpov, V. L., Preobrazhenskaya, O. V. and Mirzabekov, A. D. (1984). Chromatin structure of hsp 70 genes, activated by heat shock: selective removal of histones from the coding regions and their absence from the 5′ region. *Cell* **36**, 423-431.

Käs, E., Izaurralde, E. and Laemmli, U.K. (1989). Specific inhibition of DNA binding to nuclear scaffolds and histone H1 by distamycin. The role of oligo(dA).oligo(dT) tracts. *J. Mol. Biol.* **210**, 587-599.

Laybourn, P. J. and Kadonaga, J. T. (1991). Role of nucleosomal cores and histone H1 in regulation of transcription by RNA polymerase II. *Science* **254**, 238-245.

Meersseman, G., Pennings, S. and Bradbury, E. M. (1991). Chromatosome positioning on assembled long chromatin. Linker histones affect nucleosome placement on 5 S rDNA. *J. Mol. Biol.* **220**, 89-100.

Mirzabekov, A. D., Bavykin, S. G., Belyavsky, A. V., Karpov, V. L., Preobrazhenskaya, O. V., Shick, V. V. and Ebralidse, K. K. (1989). Mapping DNA-protein interactions by cross-linking. *Meth. Enzymol.* **170**, 386-408.

Nacheva, G. A., Guschin, D. Y., Preobrazhenskaya, O. V., Karpov, V. L., Ebralidse, K. K. and Mirzabekov, A. D. (1989). Change in the pattern of histone binding to DNA upon transcriptional activation. *Cell* **58**, 27-36.

Pauli, U., Chiu, J. F., Ditullio, P., Kroeger, P., Shalhoub, V., Rowe, T., Stein, G. and Stein, J. (1989). Specific interactions of histone H1 and a 45 kilodalton nuclear protein with a putative matrix attachment site in the distal promoter region of a cell cycle-regulated human histone gene. *J. Cell. Physiol.* **139**, 320-328.

Postnikov, Y. V., Shick, V. V., Belyavsky, A. V., Khrapko, K. R., Brodolin, K. L., Nikolskaya, T. A. and Mirzabekov, A. D. (1991). Distribution of high mobility group proteins 1/2,E and 14/17 and linker histones H1 and H5 on transcribed and non-transcribed regions of chicken erythrocyte chromatin. *Nucl. Acids Res.* **19**, 717-725.

Rocha, E., Davie, J. R., van Holde, K. E. and Weintraub, H. (1984). Differential salt fractionation of active and inactive genomic domains in chicken erythrocytes. *J. Biol. Chem.* **259**, 8558-8563.

Rose, S. M. and Garrard, W. T. (1984). Differentiation-dependent chromatin alterations precede and accompany transcription of immunoglobulin light chain genes. *J. Biol. Chem.* **259**, 8534-8544.

Schlissel, M. S. and Brown, D. D. (1984). The transcriptional regulation of Xenopus 5 S RNA genes in chromatin: the roles of active stable transcription complexes and histone H1. *Cell* **37**, 903-913.

Segers, A., Muyldermans, S. and Wyns, L. (1991). The interaction of histone H5 and its globular domain with core particles, depleted chromatosomes, polynucleosomes, and a DNA decamer. *J. Biol. Chem*. **266**, 1502-1508.

Sevall, J.S. (1988). High resolution analysis of a histone H1 binding site in a rat albumin gene. *Biochemistry* **27**, 5038-5044.

Shimamura, A., Sapp, M., Rodriguez-Campos, A. and Worcel, A. (1989). Histone H1 represses transcription from minichromosomes assembled in vitro. *Mol. Cell. Biol.* **9**, 5573-5584.

Shimamura, A., Tremethick, D. and Worcel, A. (1988). Characterization of the repressed 5 S DNA minichromosomes assembled in vitro with a high speed supernatant of Xenopus laevis oocytes. *Mol. Cell. Biol.* **8**, 4257-4269.

Srebreva, L. N. and Zlatanova, J. S. (1992). Antibodies specific to histone H1 inhibit in vitro transcription in isolated mammalian nuclei. *Mol. Cell. Biochem.* **110**, 91-100.

Srebreva, L., Zlatanova, J., Miloshev, G. and Tsanev, R. (1987). Immunological evidence for the existence of H1-like histone in yeast. *Eur. J. Biochem.* **165**, 449-454.

van Holde, K. E. (1988). *Chromatin*, Springer-Verlag, New York, chapter 8.

van Holde, K. E., Lohr, D. E. and Robert, C. (1992). What happens to nucleosomes during transcription? *J. Biol. Chem.* **267**, 2837-2840.

Weintraub, H. (1984). Histone H1-dependent chromatin superstructures and the supression of gene activity. *Cell* **38**, 17-27.

Wolffe, A. P. (1989). Dominant and specific repression of Xenopus oocyte 5 S RNA genes and satellite I DNA by histone H1. *EMBO J.* **8**, 527-537.

Xu, M., Barnard, M. B., Rose, S. M., Cockerill, P. N., Huang, S.-Y. and Garrard, W. T. (1986). Transcription termination and chromatin structure of the active immunoglobulin κ gene locus. *J. Biol. Chem.* **261**, 3838-3845.

Yaneva, J. and Zlatanova, J. (1992). Histone H1 interacts specifically with certain regions of the mouse α-globin gene. *DNA Cell Biol.* **11**, 91-99.

Zlatanova, J. (1990). Histone H1 and the regulation of transciption of eukaryotic genes. *Trends Biochem. Sci.* **15**, 273-276.

Zlatanova, J., Srebreva, L., Banchev, T., Tasheva, B. and Tsanev, R. (1990). Cytoplasmic pool of histone H1 in mammalian cells. *J. Cell Sci.* **96**, 461-468.

Zlatanova, J. and Yaneva, J. (1991a). Histone H1-DNA interactions and their relation to chromatin structure and function. *DNA Cell Biol.* **10**, 239-248.

Zlatanova, J. and Yaneva, J. (1991b). DNA sequence specific interactions of histone H1. *Mol. Biol. Rep.* **15**, 53-56.