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Organization and Expression of H1 Histone and H1 Replacement Histone Genes

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Abstract The H1 family is the most divergent subgroup of the highly conserved class of histone proteins [Cole: Int J Pept Protein Res 30:433–449, 1987]. In several vertebrate species, the H1 complement comprises five or more subtypes, and tissue specific patterns of H1 histones have been described. The diversity of the H1 histone family raises questions about the functions of different H1 subtypes and about the differential control of expression of their genes. The expression of main type H1 genes is coordinated with DNA replication, whereas the regulation of synthesis of replacement H1 subtypes, such as H1° and H5, and the testis specific H1t appears to be more complex. The differential control of H1 gene expression is reflected in the chromosomal organization of the genes and in different promoter structures. This review concentrates on a comparison of the chromosomal organization of main type and replacement H1 histone genes and on the differential regulation of their expression. General structural and functional data, which apply to both H1 and core histone genes and which are covered by recent reviews, will not be discussed in detail. © 1994 Wiley-Liss, Inc.

Key words: histone genes, gene clusters, promoter structures, gene regulation

In contrast to our detailed knowledge of the nucleosomal core structure and its constituent histones, the role of H1 histones is just partly understood. H1 histones participate in the basal repression of the genome. Upon activation of specific gene regions, this H1-mediated repression is counteracted by sequence specific transcription factors [Schlissel and Brown, 1984; Wolffe, 1989; Croston et al., 1991].

Activated portions of the genome must, however, not be devoid of H1 histones. Partial depletion of H1 histones on active vs. inactive genes was observed at globin and ovalbumin gene loci [Kamakaka and Thomas, 1990]. Moreover, Ericsson et al. [1990] have shown in an insect system that the chromatin of highly active Balbiani ring genes contains histone H1. Similarly, binding of antibodies against H1 was observed over puffs in polytene chromosomes of *Drosophila melanogaster* [Hill et al., 1989], and chemical cross-linking of H1 was quantitatively similar for active and inactive *Drosophila* chromatin [Nacheva et al., 1989].

Differences in the intranuclear distribution of individual H1 subtypes were observed in mammalian cells [Breneman et al., 1993]. Thus, the diversity of H1 histones, their differential state of phosphorylation [reviewed in Wolffe, 1991], and their varied distribution may suggest that different H1 subtypes (and their phosphorylation products) differ in their potential to confer repression to the genome and to interact with components of the gene activating system.

H1 histones interact with the chain of nucleosomes at the site of entry and exit of the nucleosomal core particle DNA [Allan et al., 1986]. Furthermore, H1 is a prerequisite for the formation of higher order chromatin structures [Thoma et al., 1979]. The exact location of H1 within the solenoidal structure of the 30 nm fiber is, however, still unknown.

In recent years, several H1 histone genes and neighboring core histone gene clusters have been isolated. The sequence information now allows a detailed characterization of H1 subtypes and the analysis of their tissue specific expression.

HISTONE GENE CLUSTERS

The first data on the molecular organization of histone genes were obtained from several sea urchin species and from *Drosophila melanogas-*

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ter [for an early review, see Hentschel and Birnstiel, 1981]. In these cases, H1 genes formed part of tandemly repeated histone gene clusters, each comprising the five histone classes H1, H2A, H2B, H3, and H4.

In vertebrates, the organization of histone genes differs from the arrangement found in lower eukaryotes. A thorough analysis of histone genes in chicken revealed that about forty histone genes, including five different H1 subtypes, form three major clusters [D'Andrea et al., 1985]. In contrast to lower eukaryotes, groups of histone genes from chicken [and from duck: Tönjes et al., 1989] are not arranged in tandem, and no obvious patterns of association of histone gene classes or spacing between genes was observed. In both the chicken and duck genomes, the H1 subtype gene H5 (which is specifically expressed in erythroid cells) is a solitary gene without neighboring H1 or core histone genes [Ruiz-Carrillo et al., 1983; Krieg et al., 1983; Doenecke and Tönjes, 1984].

The histone gene distribution in amphibia (e.g., *Xenopus laevis*) varies from the organization in birds and mammals. In contrast to the apparently irregular avian or mammalian histone gene clusters, histone gene groups of the *Xenopus* genome are not restricted to unique fragments, but also occur in repeated clusters [van Dongen et al., 1984].

The situation in mammals is even more complicated due to higher numbers of H1 histone subtype and core histone genes. Several partial mouse histone gene clusters have been described; again, these did not show regular arrangements [Yang et al., 1987; Gruber et al., 1990]. Similarly, screening the human genome for H1 and core histone genes yielded a great number of clones containing clustered histone genes [Heintz et al., 1981; Carozzi et al., 1984; Zwollo et al., 1984; Eick et al., 1989; Albig et al., 1991].

Recently, we could show that six human H1 genes, including the testis specific subtype H1t, map to a small region on the short arm of chromosome 6 [Albig et al., 1993]. In contrast, the subtype H1°, which is structurally related to the avian subtype H5, maps to chromosome 22 and is not associated with core histone genes. This result implies that the expression of the H1 genes, which form part of this major histone gene cluster, must be coordinated with the expression of core histone genes within that cluster.

H1 HISTONE GENES IN VERTEBRATES

The first complete set of H1 histone genes of a vertebrate species was isolated from the three chicken histone gene clusters [D'Andrea et al., 1985]. In amphibia (*Xenopus laevis*), several H1 genes have been described [Turner et al., 1983; Perry et al., 1985], but the exact number of the complete set of H1 genes is still unknown. In addition to the main type H1 genes, the *Xenopus laevis* genome contains a homologue to the avian H5 (and mammalian H1°) gene [Rutledge et al., 1988; Khochbin and Wolffe, 1993] and an H1-like protein (B4), which is the embryonic H1 homologue of the early development until preneurula stages of embryogenesis [Smith et al., 1988]. The replacement of B4 by somatic H1 histones correlates with a more compact and repressed chromatin structure of the later stages of embryogenesis [Wolffe, 1991].

Until now, five main type H1 histones have been identified in mammalian chromatin [Lennox, 1984]. Genes coding for rat H1 [Cole et al., 1990; Drabent et al., 1993], mouse H1 [Yang et al., 1987], and five human H1 histone isoforms [Carrozzi et al., 1984; Eick et al., 1989; Albig et al., 1991] have been described. In addition, a testis specific H1 subtype gene (H1t) has been isolated from rat and man [Cole et al., 1986; Grimes et al., 1987; Drabent et al., 1991]. In the human genome, it is part of the histone gene cluster on chromosome 6 [Albig et al., 1993].

The chromatin in several highly differentiated mammalian cells contains the H1 subtype H1°, which is the structural homologue of the amphibian H1° and avian H5 subtypes [for review, see Smith et al., 1984]. Its accumulation can be induced by inhibition of cell proliferation or by induction of differentiation (see next section).

DIFFERENTIAL EXPRESSION OF H1 HISTONE GENES

Variations of H1 histone subtype patterns have been observed in several vertebrate tissues. During development and in terminally differentiated cells, changes in H1 subtype and phosphorylation patterns might control the accessibility of DNA to trans-acting factors [Wolffe, 1991]. One example for a developmental change of an H1 subtype and its functional implications has been mentioned above: the replacement of the H1-like protein B4 of early *Xenopus* by a somatic H1, which correlates with repression of 5S RNA genes [Wolffe, 1989].

In mammals, varied patterns of H1 subtypes have been documented, for example in different mouse tissues, depending on the state of development or differentiation [Lennox, 1984]. Similarly, patterns of human H1 histones vary between different tissues or between normal cells and tumor cell lines [D'Incalci et al., 1986].

The mammalian H1t histone is an example for a cell specific H1, which is synthesized at a specific stage of differentiation. The H1t gene is exclusively transcribed during the pachytene stage of spermatogenesis [Grimes et al., 1990], while the H1t protein persists until the late spermatid stage [Bucci et al., 1982]. The increasing rate of H1t synthesis is paralleled by a decrease of the H1a subtype, which is the predominant H1 species at the stage of spermatogonia.

The regulation of H1° histone genes differs substantially from other human H1 genes. As stated above, the H1° gene is a solitary gene with no neighboring core histone genes [Doenecke and Tönjes, 1986; Albig et al., 1993]. It was initially described as a gene which was expressed solely in highly differentiated, nondividing cells [Panyim and Chalkley, 1969]. Its expression even in the presence of inhibitors of DNA replication [Zlatanova, 1980] showed the unique mode of regulation of this histone gene: the H1° gene can be expressed in the absence of DNA replication. Moreover, inhibitors of DNA replication [e.g., Pehrson and Cole, 1980] and inducers of differentiation can induce an accumulation of H1° mRNA and protein [Keppel et al., 1977; Pieler et al., 1981; Alonso et al., 1988; Cheng and Skoultchi, 1989; Hochhuth and Doenecke, 1992].

This inducibility of H1° synthesis in parallel with a decreased rate of cell proliferation is in marked contrast to the regulation of most other histone genes (including H1 genes), which are transcribed in coordination with DNA replication [Heintz et al., 1983; Plumb et al., 1983]. In the meantime, several reports have proven that the H1° gene expression does not depend on the absence of DNA replication. Cell lines (like the hepatoma line HepG2), which have a high content of H1° in their chromatin, synthesize H1° constitutively even at a high rate of proliferation [Hochhuth and Doenecke, 1990]. Moreover, an increased rate of transcription of the H1° gene has been observed during the S phase in murine erythroleukemia cells [Grunwald et al., 1991] and in proliferating rat hepatocytes [Khochbin et al., 1991].

THE H1°/H5 HISTONE FAMILY

As summarized in the preceding paragraph, the chromatin of several terminally differentiated cell types shows a partial replacement of main type H1 genes by H1° (in mammals) or its amphibian (H1°) and avian (H5) homologues [Smith et al., 1984]. These replacement linker histone genes can be expressed in the absence of DNA replication (e.g., H5 in avian erythroblasts or H1° in terminally differentiated mammalian cells). As stated above, the initial concept of a non–S-phase dependent expression of the H1° gene must, however, be modified, since it was shown recently in several systems that the transcription of the H1° gene is increased during the S phase of the cell cycle [Grunwald et al., 1991] and that proliferative growth of a tumor cell line like HepG2 is compatible with a high level of H1° synthesis [Hochhuth and Doenecke, 1990].

The H1°/H5 histone structures differ from main type H1 in many respects. First, these replacement linker histones are generally shorter (about 190 amino acids) than main type H1 proteins (about 215). H5 is enriched in arginine residues, and most of these are replaced by lysine in the otherwise closely related H1° subtype [Doenecke and Tönjes, 1986]. Even the C-terminal domain, which is highly conserved in most H1 species, shows only partial structural homologies between H5 and H1° on one hand and main type H1 species on the other hand.

The C-terminal domains vary among all H1 species described thus far. However, most H1 species, including H1° and H5, show a highly conserved octapeptide (TPKKAKKP in mammals, SPKKAKKP in others) in their C-terminal domain [Tönjes and Doenecke, 1987; Albig et al., 1991]. This may be a specific, cell cycle dependent phosphorylation site [for review, see Wolffe, 1991] and DNA binding motif [Suzuki, 1989]. It is not fully conserved in H1t, but is found in main type as well as replacement type H1 histone C-terminal tails.

The function of H1° is as yet unknown. Hybridization analysis showed its association with inactivated portions of the genome [Roche et al., 1985], but immunocytochemical studies suggest its location in the vicinity of active portions of the genome [Gorka et al., 1993]. In any case, the presence of H1° in differentiated, transcriptionally active cells suggests a specific role in local modulations of higher order chromatin structures.

An association of H1° with inactivated portions of the genome, on the other hand, agrees with the putative function of its avian homo-

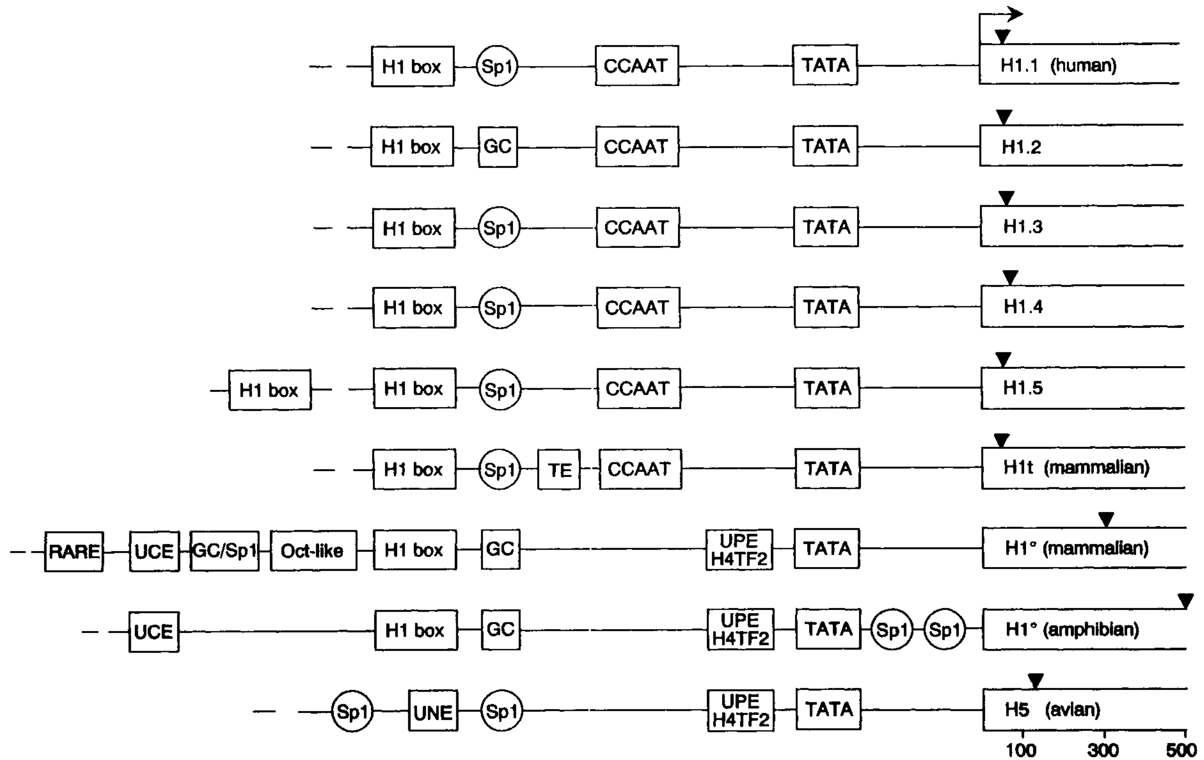

Fig. 1. Organization of vertebrate H1 gene promoters. TATA, CCAAT, Sp1, and H4TF2 sites are consensus sequence elements. The term *Sp1 binding site* was only used when a GC-rich element agreed with the consensus sequence defined by Briggs et al. [1986]. TE is a testicular element involved in the spermatocyte specific expression [Grimes et al., 1992]. The sequence ATTTGCT (Oct-like) is used to emphasize the similarity of this element with the OTF1-binding site ATTTGCAT [Fletcher et al., 1987]. UCE (upstream conserved element [Khochbin and Wolffe, 1993]) is found in both amphibian and mammalian promoters. RARE: retinoic acid receptor binding element [Evans, 1988]. A negatively acting upstream element (UNE) has been described in the chicken H5 promoter [Rousseau et al., 1989]. The arrowheads and numbers at the transcribed portions of genes (on the right) indicate beginning of coding sequences in relation to transcription start sites. Main type H1 genes are labeled tentatively H1.1 to H1.5 (H1.1, H1.2: Eick et al., 1989; H1.3, H1.4: Albig et al., 1991; H1.5: Carozzi et al., 1984). H1t data are from rat [Cole et al., 1986; Grimes et al., 1987] and man [Drabent et al., 1991]. Mammalian H1° data refer to human H1° [Doenecke and Tönjes, 1986; Bouterfa et al., in press] and murine H1° [Breuer et al., 1989] and amphibian H1° data are taken from Khochbin and Wolffe [1993] and Rutledge et al. [1988]. Avian H5 data are taken from Ruiz-Carrillo et al. [1983], Krieg et al. [1983], and Doenecke and Tönjes [1984].

logue H5. This arginine-rich avian histone protein plays a major role in the condensation of the transcriptionally silent erythrocyte chromatin and in the control of DNA synthesis and cell proliferation [Sun et al., 1989].

The amphibian H1° appears to be a functional intermediate between the avian H5 and mammalian H1° histones, since it is expressed in varied tissues at different developmental stages and is finally confined to several differentiated tissues, including erythrocytes [Moorman et al., 1987].

H1 HISTONE GENE PROMOTER SEQUENCES

Figure 1 shows a compilation of promoter regions of human main type H1 histones, the mammalian H1t and H1°, the amphibian H1°, and the avian H5 histones. All vertebrate H1 promoter sequences, notably including the amphibian H1°/H5 [Khochbin and Wolffe, 1993] but not the avian H5 promoters [Krieg et al., 1983; Ruiz-Carrillo et al., 1983; Doenecke and Tönjes, 1984], show the sequence AAACACA (H1 box) about 80 nucleotides upstream of a TATA box. A CCAAT sequence is found invariantly in the promoter region of main type H1 genes and the H1t gene, but it is missing in the H1° and H5 5′ flanking regions. GC-rich elements, which had been initially described as a conserved element upstream of H1 genes [Coles and Wells, 1985], are found in all histone H1 promoter sequences. In most cases, they contain Sp1 binding site consensus sequence motifs [Briggs et al., 1986]; their numbers and positions vary.

The sequence AAACACA [Coles and Wells, 1985], which has been found thus far in all

vertebrate H1 promoters, was initially considered as the main binding site for trans-activating factors mediating S-phase control [Dalton and Wells, 1988]. Van Wijnen et al. [1988a, b] and Gallinari et al. [1989] have identified additional H1 specific transcription factors (termed HiNF-B or H1TF2, they may be identical), which both interact with the CCAAT-sequence. DNA binding studies indicate that the CCAAT box binding factor plays a major role in the S-phase regulation, since the H1TF2 activity is increased in S-phase nuclear extracts [La Bella et al., 1989; Heintz, 1991].

As shown in Figure 1, the CCAAT box is missing in H1°/H5-type gene promoters but is present in the upstream regions of H1t and main type H1 genes. Thus, differences in the S-phase regulation of main type and replacement H1 histone genes may in part be due to their differential dependence on the H1TF2/HiNF-B system, but additional factors must contribute to this gene control (see e.g., the testicular element in the H1t promoter [Grimes et al., 1992]).

The initial view that the H1 box binding protein was the main factor for an S-phase expression of H1 genes did not agree with the presence of an H1 box in the H1° promoter, since H1° synthesis was considered as strictly independent of the S-phase of the cell cycle. As mentioned above, recent evidence indicates that in some systems the transcription of the H1° gene can increase during S-phase. Thus, replication of chromatin in cells, where H1° gene transcription is inducible or constitutively high, may depend on H1-box binding factor (H1TF1, but not H1TF2) and further trans-acting proteins, whereas H1° replacement in nondividing cells may also occur in the absence of DNA replication. Thus, the absence of the H1-box motif in the H5 promoter suggests that expression of this gene is in fact independent from S-phase regulatory steps.

The association of H1° gene expression and terminal cell differentiation [Panyim and Chalkley, 1969; Gjerset et al., 1982; Alonso et al., 1988; Cheng and Skovetchi, 1989] is reflected in a very complex promoter structure. In addition to the TATA box, the H1 box, and an Sp1 site (see above), the H1° promoter of mouse and man contains a binding site for the retinoic acid receptor [Breuer et al., 1989, 1993; Bouterfa et al., in press] and other conserved sequence motifs within otherwise divergent promoter sequences. The presence of a retinoic acid receptor binding site agrees with the induction of differentiation of murine F9 cells, which is induced by retinoic acid and includes the synthesis of H1° mRNA and protein [Alonso et al., 1988].

The avian H5 gene promoter differs from other H1 (including H1°) promoters in several respects. It is the only H1 gene devoid of an H1 box. A sequence element involved in downregulation of H5 gene expression (UNE) was identified upstream of an activating promoter element (UPE) and an Sp1 site [Rousseau et al., 1989].

The upstream positive element (UPE) is a common feature of the three H1°/H5 promoters. It has the same sequence as the binding site of the transcription factor H4TF2, which is normally involved in the control of the H4 histone gene expression [Dailey et al., 1988]. In *Xenopus laevis* A6 cells, this H4TF2 system cooperates with a second cis-acting control site (UCE) in maintaining the basal transcription rate [Khochbin and Wolffe, 1993]. The UCE (upstream conserved element) is highly conserved in amphibian, murine, and human H1° promoters. Its function in the regulation of mammalian H1° gene expression remains to be shown.

The mammalian H1t promoter structure contains the same array of H1 box, Sp1-binding site, and CCAAT and TATA box as most main type H1 genes, although the expression of the H1t gene occurs in pachytene spermatocytes in the absence of DNA replication. Thus, testis specific expression of the H1t gene requires additional regulatory factors. Grimes et al. [1992] have identified an 18 base pair element including the palindromic sequence (CCTAGG) between the Sp1 and CCAAT promoter elements as the binding site for testicular proteins obtained from primary spermatocytes. The palindromic sequence and most of the 18 base pair element is conserved in the human H1t promoter [Drabent et al., 1991].

Until now, the work on replacement histone gene expression has concentrated on the function of proximal promoter sites. Tissue specificity and developmental control of H1° expression may depend on distant enhancer or silencer binding sites, which remain to be identified. Recently, Rousseau et al. [1993] have shown that the transcriptional activation of the chicken H5 gene depends on two upstream enhancer binding sites (around 2,000 and 1,200 nucleotides upstream of the start site of transcription) and on a downstream enhancer, which was identified before by Trainor et al. [1987]. A stabilization of TFIID binding to the TATA box in the

duck H5 promoter is mediated by an additional factor (erythroid upstream stimulating factor [eUSF]), which interacts with a sequence element downstream of the transcription start site [Bungert et al., 1992].

H1 HISTONE 3′-FLANKING SEQUENCES

Most histone mRNA 3′ ends show a conserved sequence element with a potential stem-loop structure, just upstream of the 3′ mRNA end [Birnstiel et al., 1985]. Processing of the initial transcript at this site involves the formation of a U7 snRNP [Gick et al., 1986; Schümperli, 1988] and results in nonpolyadenylated histone mRNA. A detailed analysis of this complex processing mechanism is beyond the scope of this review, since it is not an H1 specific but a general histone mRNA maturation system [Birnstiel et al., 1985; Schümperli, 1988; Liu et al., 1989; Heintz, 1991].

This system of generation of histone mRNA 3′ ends by processing in a snRNP-dependent reaction is found in all main type H1 genes but is missing in H5 and H1° genes (or mRNAs, respectively). Both H5 and H1° mRNAs are polyadenylated [Molgaard et al., 1980; Kress et al., 1986] and have long 3′ noncoding segments.

Two conserved palindromic sequence elements are found at similar sites in the chicken and duck H5 mRNA 3′ flanking mRNA sequences [Krieg et al., 1983; Doenecke and Tönjes, 1984]. H1° mRNA has a 1.3 kb non-coding 3′ end, which carries a poly(A) tail [Kress et al., 1986]. In contrast to the avian H5 mRNA, which shows no consensus polyadenylation signal, poly(A) addition to the H1° histone mRNA is mediated by a conventional AAUAAA sequence [Kress et al., 1986].

TRANSLATIONAL REGULATION OF H1 HISTONE GENE EXPRESSION

The synthesis of histones is controlled at the transcriptional level as well as at the level of mRNA stability [for reviews, see Marzluff and Pandey, 1988; Schümperli, 1988; Heintz, 1991]. The highly conserved sequence elements at the ends of replication-dependent histone mRNAs are involved in both processing and cell cycle regulated degradation of both core and H1 histone mRNAs.

The regulation of replacement H1 histone gene expression again appears to differ in several respects from main type H1 histone synthesis. As stated above, H5 as well as H1° synthesis may take place at cell cycle stages other than S-phase, but an increase in H1° gene transcription occurs during S-phase. In the case of H1°, translational control might play a major role in the synthesis of this protein. Rousseau et al. [1991, 1992] have shown in several systems that increases in mRNA levels can exceed the resulting increase in newly synthesized H1° histone protein. This implies that mechanisms exist which prevent the translation of H1° mRNA beyond the actual needs of a given cell type. It was shown that high levels of H1° mRNA were organized in polysomes without being translated. A particularly striking example of this phenomenon is the dissociation of the rat H1° gene transcription and H1° mRNA translation after partial hepatectomy [Khochbin et al., 1991]. In this case, transcription of the H1° gene started immediately after hepatectomy (i.e., in rapidly dividing cells), at the same time when the amount of H1° protein decreased. Thus, control mechanisms must exist which uncouple the transcription of the H1° gene and the translation of the mRNA.

H1 AND CORE HISTONE GENE REGULATION OCCURS AT SEVERAL CONTROL LEVELS

All presently known human H1 genes are clustered, together with core histone genes, on the short arm of chromosome 6 [Albig et al., 1993]. Additional core histone genes map to chromosome 1, and the solitary human H1° histone gene maps to chromosome 22. It remains to be shown whether similar major clusters of histone genes occur in other mammalian genomes, too.

The known portions of the human histone gene cluster on chromosome 6 cover more than 300 kb, and overlapping portions of this cluster remain to be found. This suggests that this major group of histone genes may extend over more than 500 kb. Thus, the coordinated expression of histone genes during S-phase suggests that the chromatin of the cluster region must be kept in an active state. Since the length of the potentially active region exceeds the expected size of a chromatin domain [Bonifer et al., 1991], the cluster may be interrupted by several nuclear scaffold or matrix attachment sites [Gasser and Laemmli, 1987; Bonifer et al., 1991]. In addition to its structural role, the nuclear matrix is involved in the localization of promoter regulatory elements and sequence specific transcription factors [Stein et al., 1991]. If a specific pattern of matrix attachment sites delimiting chromatin domains within the large histone gene cluster

can be identified (such as in the vicinity of a human H4 gene [Pauli et al., 1989]), this may provide a basis for a differential control of histone genes in separate cluster domains. Thus, a coexpression of a specific H1 and neighbouring core histone genes may be regulated at this higher order chromatin level.

A general activation of histone gene clusters at the level of chromatin domains may increase the accessibility of individual H1 and core histone promoter sites for specific trans-acting factors. Thus, promoter elements, which are specific for individual H1 genes and each of the core histone genes, respectively, provide a next level of control of histone gene expression.

At a third level of regulation, mRNA processing, stability, and translational control will influence the *de novo* synthesis of stoichiometrically correct amounts of histones and delimit the period of histone synthesis to the S-phase of the cell cycle and/or to defined developmental stages.

Specific factors must be involved in the expression of the H1 subtypes H1° and H1t and their restriction to certain tissues or cell types. An individual regulation of H1° is easily conceivable, since this histone gene is a solitary gene outside any major histone gene cluster. In addition, its promotor sequence appears to be the target of several regulatory factors, which are not involved in main type H1 gene expression. The testis specific expression of H1t, on the other hand, appears to be particularly interesting, since (at least in the human genome [Albig et al., 1993]) it is part of the major histone gene cluster on chromosome 6 and in the rat genome it is associated with at least an H4 gene [Grimes et al., 1990]. Thus, cell type specific control mechanisms must restrict the expression of the H1t gene (in contrast to its neighbouring genes) to a short period during spermatogenesis.

The regulation of H1t expression is just one example for a tissue specific regulation of H1 gene expression. Similarly, the patterns of H1 histone subtypes in any other tissue may be the result of a complex interplay of control steps at the levels of higher order chromatin structures, transcriptional regulation at individual genes, and translational control based on the functional state of the cell.

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