

A proposal for a coherent mammalian histone H1 nomenclature correlated with amino acid sequences

MISSAG H. PARSEGHIAN, AGNES H. HENSCHEN, KERSTIN G. KRIEGLSTEIN,¹
AND BARBARA A. HAMKALO

Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92717

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Abstract

Bio-Rex 70 chromatography was combined with reverse-phase (RP) HPLC to fractionate histone H1^o and 4 histone H1 subtypes from human placental nuclei as previously described (Parseghian MH et al., 1993, *Chromosome Res* 1:127–139). After proteolytic digestion of the subtypes with *Staphylococcus aureus* V8 protease, peptides were fractionated by RP-HPLC and partially sequenced by Edman degradation in order to correlate them with human spleen subtypes (Ohe Y, Hayashi H, Iwai K, 1986, *J Biochem (Tokyo)* 100:359–368; 1989, *J Biochem (Tokyo)* 106:844–857). Based on comparisons with the sequence data available from other mammalian species, subtypes were grouped. These groupings were used to construct a coherent nomenclature for mammalian somatic H1s. Homologous subtypes possess characteristic patterns of growth-related and cAMP-dependent phosphorylation sites. The groupings defined by amino acid sequence also were used to correlate the elution profiles and electrophoretic mobilities of subtypes derived from different species. Previous attempts at establishing an H1 nomenclature by chromatographic or electrophoretic fractionations has resulted in several misidentifications. We present here, for the first time, a nomenclature for somatic H1s based on amino acid sequences that are analogous to those for H1^o and H1t. The groupings defined should be useful in correlating the many observations regarding H1 subtypes in the literature.

Keywords: H1 sequences; histone H1 subtypes; nomenclature; protein phosphorylation sites

The presence in the somatic cells of higher eukaryotes of multiple primary amino acid variants of the linker histone H1 is well documented (Cole, 1977, 1984, 1987; Risley & Eckhardt, 1981; Cole et al., 1984; Lennox, 1984; Von Holt et al., 1984; Wells, 1986; Wells & McBride, 1989). Differences have been reported for the timing of synthesis and turnover rates of these subtypes in humans (Sizemore & Cole, 1981), mice (Pehrson & Cole, 1982; Higurashi et al., 1987; Dominguez et al., 1992; Talasz et al., 1993), and chickens (Winter et al., 1985a, 1985b). In addition, there are differences in the amount and pattern of phosphorylation among subtypes in humans (Ajiro et al., 1981a, 1981b), rats (Ohba et al., 1984), and mice (Ajiro et al., 1990). These types of data led to the hypothesis that subtypes possess distinct abilities to condense chromatin in vivo (Pehrson & Cole, 1982; Lennox & Cohen, 1983; Lennox, 1984; Ohba et al., 1984; Winter et al., 1985a; D'Incalci et al., 1986; Ajiro et al., 1990), presumably as a consequence of differing abilities to bind to

DNA and/or other chromosomal proteins. Although in vitro evidence supports this hypothesis (Liao & Cole, 1981a, 1981b, 1981c), in vivo evidence is lacking. Therefore, one cannot dismiss the possibility that subtypes are interchangeable, simply representing genetic drift within a multigene family.

In order to begin to evaluate potential functional differences among subtypes, it is essential to correlate changes observed in the amount and/or modification of a particular subtype under different conditions in different systems. Although an enormous literature describing such changes exists, there is no standard nomenclature to permit direct comparisons. In fact, a survey of the literature reveals at least 12 different H1 nomenclatures that employ Roman (Lennox, 1984; Winter et al., 1985a, 1985b; Ohe et al., 1989) or Greek (Allis et al., 1984; Pehrson & Cohen, 1985) letters, Arabic (D'Incalci et al., 1986; Albig et al., 1991; Parseghian et al., 1993) or Roman (Sizemore & Cole, 1981; Higurashi et al., 1987) numerals, a combination of Arabic and Roman numerals (Hoyer-Fender & Grossbach, 1988), or Arabic numerals and Roman letters (Cole, 1987). Even the low resolution obtained when total H1 is separated into 2 bands (excluding H1^o) on an SDS-polyacrylamide gel is accompanied by 3 different labeling systems, all utilizing the letters A and B (Pehrson & Cole, 1982; Tan et al., 1982; Gabrielli & Tsugita, 1986). Additional confu-

Reprint requests to: Barbara A. Hamkalo, Department of Molecular Biology and Biochemistry, University of California, Irvine, California 92717; e-mail: bhamkalo@uci.edu.

¹ Present address: Institute for Anatomy and Cell Biology, Ruprecht Karls University, 6900 Heidelberg, Germany.

sion in nomenclature arises when one compares other types of separations that have been utilized, such as 1- and 2-dimensional acid-urea gels, ion-exchange chromatography, and reverse-phase (RP) HPLC.

We previously described the preparative fractionation and purification of human placental H1 subtypes using a combination of Bio-Rex 70 chromatography and RP-HPLC (Parseghian et al., 1993). Other than H1^o, 4 somatic H1 subtypes were resolved and designated H1-1, H1-2, H1-3, and H1-4, in the order of their elution from the Bio-Rex ion-exchange column. We have used the human H1 subtypes as a basis for the development of a coherent nomenclature because the complete sequences of the 4 somatic isoforms from human spleen were previously published (Ohe et al., 1986, 1989). In fact, this is the only mammalian system in which complete sequence information is available for all of the subtypes, including H1^o and the testis-specific variant H1t (Doenecke & Toenjes, 1986; Eick et al., 1989; Albig et al., 1991; Drabent et al., 1991). Based on partial peptide sequences obtained from placental subtypes after *Staphylococcus aureus* V8 protease digestion, it was possible to correlate placental isoforms with those from spleen. The human subtypes were subsequently correlated with other mammalian H1s at the primary sequence level as well as by chromatographic and electrophoretic fractionations. A coherent system of nomenclature for mammalian H1 subtypes, which is based on amino acid sequence data, is presented for the first time.

Results and discussion

Correspondence of placental subtypes with spleen sequences

Total H1 is resolved into 4 somatic subtypes and H1^o after Bio-Rex 70 and RP-HPLC; the subtypes are designated H1-1, H1-2, H1-3, and H1-4 based on their elution from the ion-exchange column (Parseghian et al., 1993). Direct comparisons of these subtypes with those sequenced from human spleen (Ohe et al., 1986, 1989) were not possible because the subtypes sequenced were fractionated by an uncommonly used HPLC scheme involving an isopropanol gradient in 1.0 M pyridine-formic acid (pH 5.0) with a C₈ silica column. Thus, we could not assume that the order of elution of subtypes in this system was the same as that obtained by either conventional ion-exchange chromatography (Kinkade & Cole, 1966) or a standard HPLC fractionation (Parseghian et al., 1993). Therefore, in order to determine the identity of placental subtypes, they were digested with V8 protease as described by Ohe et al. (1986, 1989) and the fragments separated by RP-HPLC.

Figures 1, 2, 3, and 4 show RP-HPLC elution profiles of the fragments from V8 digestions of purified subtypes. Retention times are recorded above the peaks, and the sequenced fragments are numbered at the bases of their respective peaks. Several observations, illustrated in the chromatographic profile of protease-digested H1-1 (Fig. 1), were characteristic of all runs. All detectable proteolytic fragments were eluted within the first 70 min of the run and a few peaks consisted of 2 or more fragments that elute simultaneously. Based on the sequences of several fragments, the observation made by Ohe et al. (1989) that V8 protease cleaves at sites other than aspartic and glutamic acid residues was confirmed. They reported cleavage on the carbox-

yl side of serine at lysine-serine-X sites, where X represents one of the apolar residues alanine, leucine, or isoleucine. Cleavage was observed at sites with an alanine residue in subtypes H1-1 (peaks 4 and 5), H1-2 (peak 4), and H1-4 (peak 2). Cleavage also occurred at sites with a leucine residue in H1-3 (peak 6) and an isoleucine residue in H1-2 (peak 2).

Partial sequencing of the fragments from 6 fractions of the H1-1 chromatogram (Fig. 1) reveals sequence identity to the subtype Ohe et al. (1989) refer to as H1d. The polymorphic site at residue 17 (valine/alanine) observed in spleen (Ohe et al., 1989) was not detected in the placental sample (peak 2), which possesses only alanine at this position. Extraction of the H1s from several placentas rules out the lack of a valine variant being peculiar to only 1 particular individual. Based on chromatographic peak areas of subtypes resolved on Bio-Rex 70, H1-1 represents about 31.5% of the non-H1^o subtypes in placenta (data not shown).

Sequences derived from H1-2 fragments (Fig. 2) identified this subtype as spleen H1c. In addition, 3 peaks (1, 3, and 5) contain minor amounts of peptides derived from contaminating H1-1. H1-2, a subtype that represents a relatively small amount of the total in both placenta (<10%; Parseghian et al., 1993) and spleen (13%; Ohe et al., 1989), was the only subtype that was contaminated with another isoform. The absence of contaminating fragments in the other 3 placental isoforms and the correspondence of peptide sequences with those from spleen in all cases confirms the purity of subtypes and strengthens the identification of H1-2 as the placental homolog of H1c.

The sequences obtained from H1-3 digests (Fig. 3) were identical to those published for spleen H1a, except for residues 57 and 59 in peaks 9 and 10. The amino acids at these positions are asparagine and leucine, respectively, in spleen. Peak 9 contained the expected asparagine and leucine residues, whereas peak 10 contained a serine at position 57 and a valine at position 59, amino acids in common at these positions among the other subtypes (compare with sequences from Figs. 1, 2, 4). In order to eliminate the possibility that peak 10 resulted from the comigration during purification of contaminating phosphorylated forms of the other subtypes, a sample of H1-3, which was dephosphorylated and rerun on the RP-HPLC subtype purification gradient (Parseghian et al., 1993), revealed no evidence of contamination by the other subtypes (data not shown). In addition, all peptide sequences obtained corresponded only to the amino- and carboxy-terminal variant regions of H1-3, reaffirming the purity of the sample. These data support the existence of 2 variants of H1-3 in placenta, which may or may not have biological significance. Interestingly, although the authors reported only asparagine and leucine at positions 57 and 59, twin peaks similar to 9 and 10, which may correspond to the 2 placental isoforms, also were present in the chromatograms of H1a published by Ohe et al. (1989). H1-3 represents about 20–25% of the non-H1^o subtypes in placenta (data not shown).

Finally, partial sequences of fragments from 4 fractions of the H1-4 chromatogram (Fig. 4) are identical to those predicted by the sequence of spleen H1b. Although this is the major variant in spleen (Ohe et al., 1986), accounting for 60% of the non-H1^o H1 in that organ, it represents only about 40% in placenta.

In addition to the 4 H1 subtypes characterized in human placenta, a fifth subtype, termed "H1a" by Seyedin and Kistler (1979a) (not to be confused with the H1a of Ohe et al., 1986,

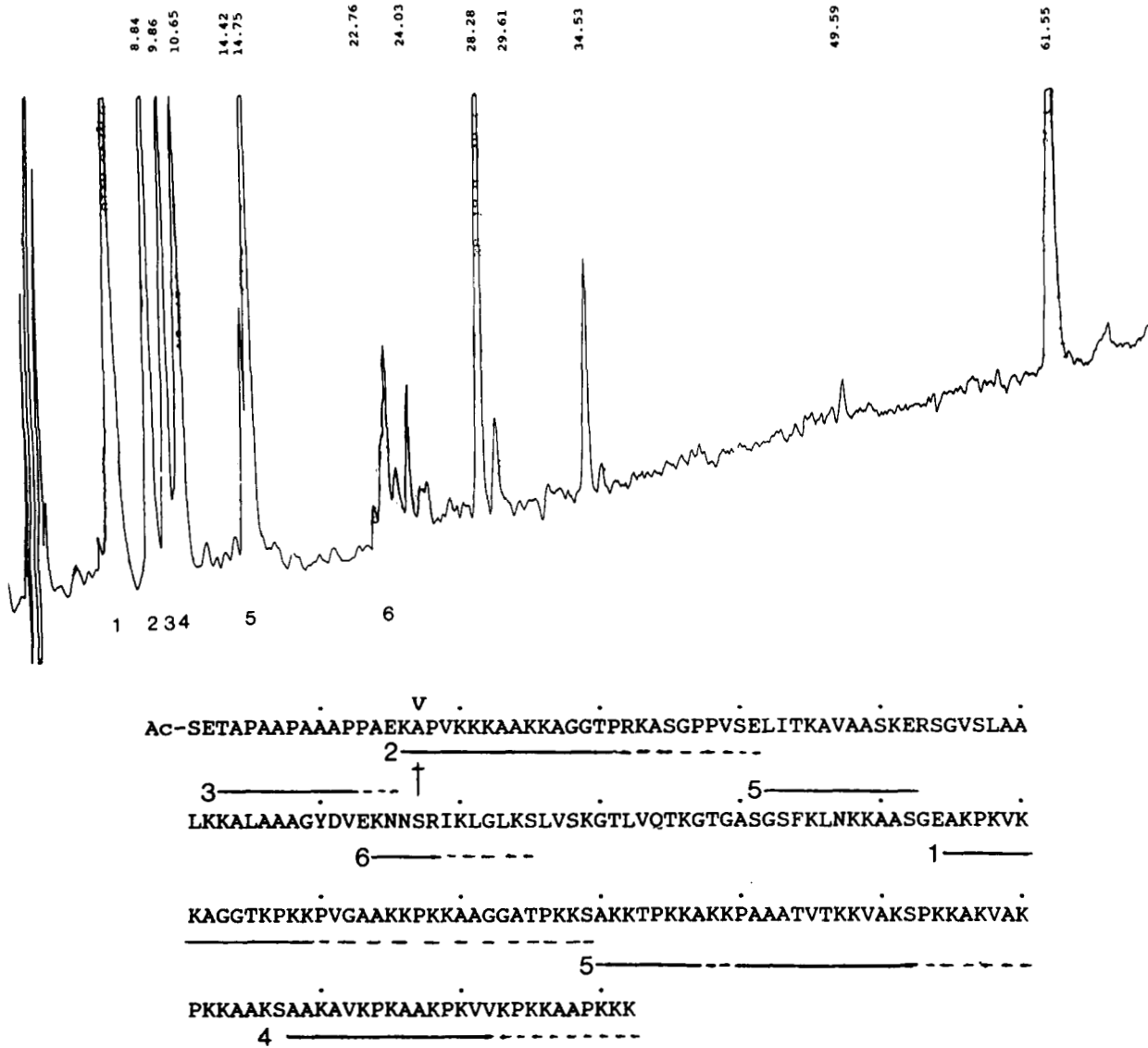


Fig. 1. RP-HPLC chromatogram of V8 protease-digested H1-1 from human placenta. Numbers above peaks are corresponding retention times. Numbers at base of peaks correspond to fragments containing the underlined sequences. Dashed lines represent regions of generated fragment not sequenced or identified. The dagger represents the location of the valine/alanine variant, where only alanine was detected. Complete sequence of H1d by Ohe et al. (1989).

1989), has been reported to occur in thymus, testis, neuronal, and hematopoietic tissue (Seyedin & Kistler, 1979a, 1979b; Lennox et al., 1982; Pina et al., 1987; Rasheed et al., 1989; Dominguez et al., 1992). This isoform appears to be absent in placenta based on the analysis of multiple independent fractionations and the absence of peptide sequences derived from this subtype predicted from the partial sequence published by Baubichon-Cortay et al. (1992) and the sequence of the H1.1 gene that probably codes for this isoform (Eick et al., 1989). Although "H1a" has been reported in other tissues such as, for example, liver, lung, and spleen (Lennox & Cohen, 1983; Lennox, 1984), the absence of transcripts of the H1.1 gene in these tissues (Burfeind et al., 1992) argues that its presence is the result of contaminating proliferating cells such as lymphocytes and macrophages, which do contain "H1a" (Rasheed et al., 1989). Because "H1a" does not

appear to be present in spleen either (Ohe et al., 1986, 1989), it is clearly not a universally expressed subtype.

Correlation of subtypes based on electrophoretic mobilities

Although ion-exchange chromatography is the standard method for H1 subtype fractionation, 1- and 2-dimensional gels have also been employed extensively to separate isoforms. SDS-PAGE of total human or rat H1 results in 3 bands (Fig. 5, lanes H and R). The mobility of isolated human subtypes in such gels permits definitive identification of the composition of each of these bands: the upper band consists of a mixture of H1-2, H1-3, and H1-4, the middle band is pure H1-1, and the fastest migrating band is pure H1^o, as previously reported (compare lane O

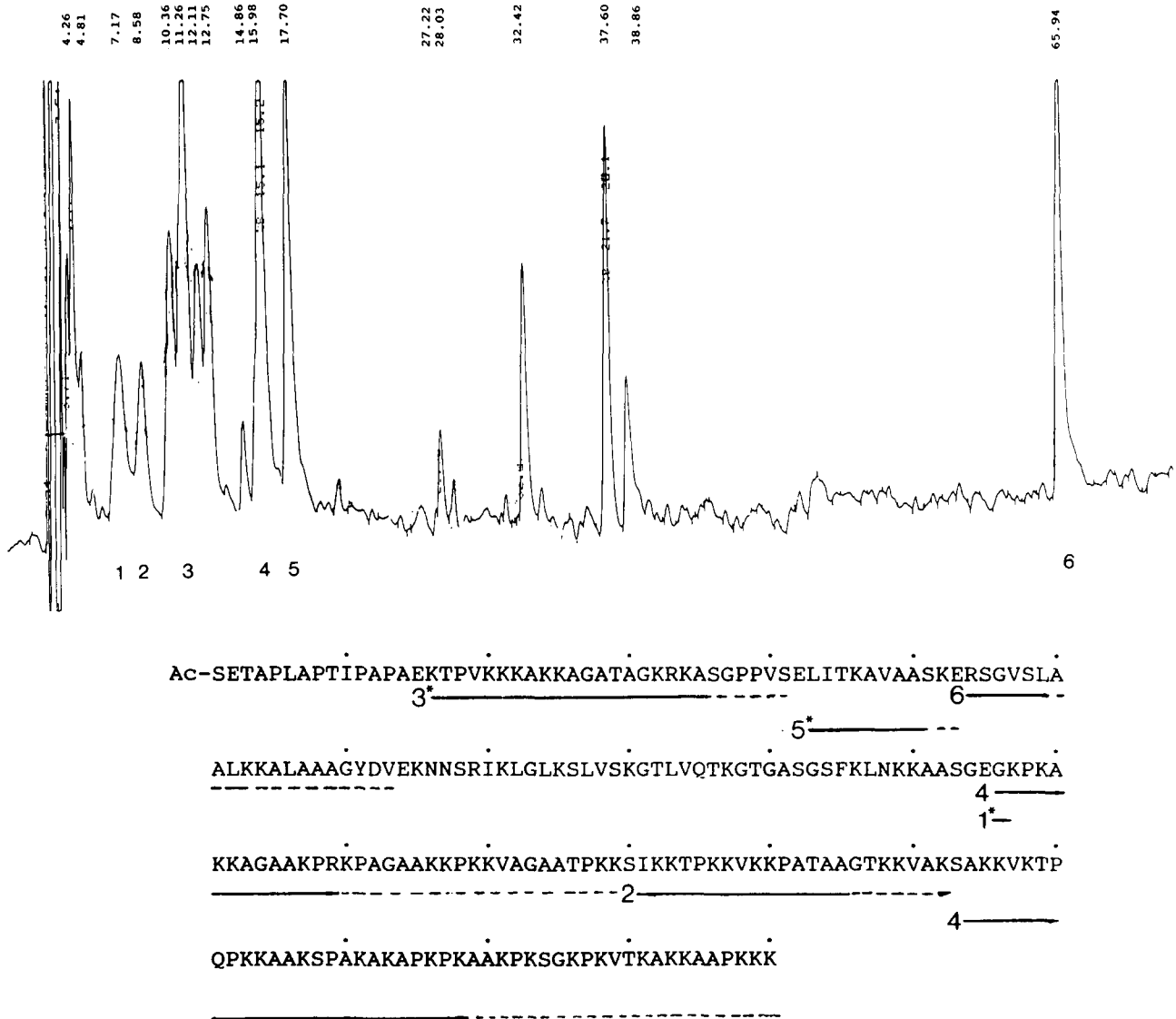


Fig. 2. RP-HPLC chromatogram of V8 protease-digested H1-2 from human placenta. See Figure 1 for details. Asterisks represent fractions contaminated with H1-1 fragments. Complete sequence of H1c by Ohe et al. (1989).

with lanes H and R) (Pehrson & Cole, 1982). Variable amounts of degradation products of H1-1, H1-2, and H1-3 also are detected under the major bands containing these subtypes (Bartley & Chalkley, 1970; Chae et al., 1975; Cole, 1989). Subtype resolution is improved by 2-dimensional separation. For example, when the first dimension is an SDS-PAGE gel and the second is an acid-urea-cetyltrimethylammonium bromide (AUC) gel (Bonner et al., 1980; D'Incalci et al., 1986), human fibroblast H1 is resolved into 5 somatic H1s and 2 H1^o variants (D'Incalci et al., 1986). Based on 2-dimensional separations of total placental H1 supplemented with each purified subtype, H1-1 is variant 2 of D'Incalci et al. (1986), and H1-2, H1-3, and H1-4 are variants 4, 3, and 1, respectively (data not shown). As predicted from the sequence analysis, a fifth H1 could not be detected in 2-dimensional gels of placental H1. However, D'Incalci's variant 5 may not be "H1a," because variant 5 in the first dimension migrates more slowly than the migration reported for "H1a" (Seyedin & Kistler, 1979a, 1979b).

Two-dimensional separation of H1 from quiescent cells revealed an increase in the synthesis of H1^o and variants 1 and 2 (placental H1-4 and H1-1) along with a concomitant decrease in variants 3, 4 (placental H1-3 and H1-2), and 5 (D'Incalci et al., 1986). The decrease in H1-2 and H1-3 in quiescent cells, along with the observation that H1-2 and H1-3 are synthesized early in S-phase (Higurashi et al., 1987) when many potentially active genes are replicated (Goldman et al., 1984), suggest that these isoforms may be preferentially associated with active chromatin. One observation that supports this hypothesis is the immunolocalization of H1-3 to the nuclear periphery in HeLa cells (Parseghian et al., 1993), a region that contains both preferentially nick-translatable chromatin (Hutchison & Weintraub, 1985; Krystosek & Puck, 1990; Puck et al., 1991) and a higher concentration of active RNA polymerase II molecules (Clark et al., 1991). Similarly, the increase in H1-1 and H1-4 in quiescent cells and their synthesis in late S-phase, when constitutive and facultative heterochromatin are replicated, suggest a pos-

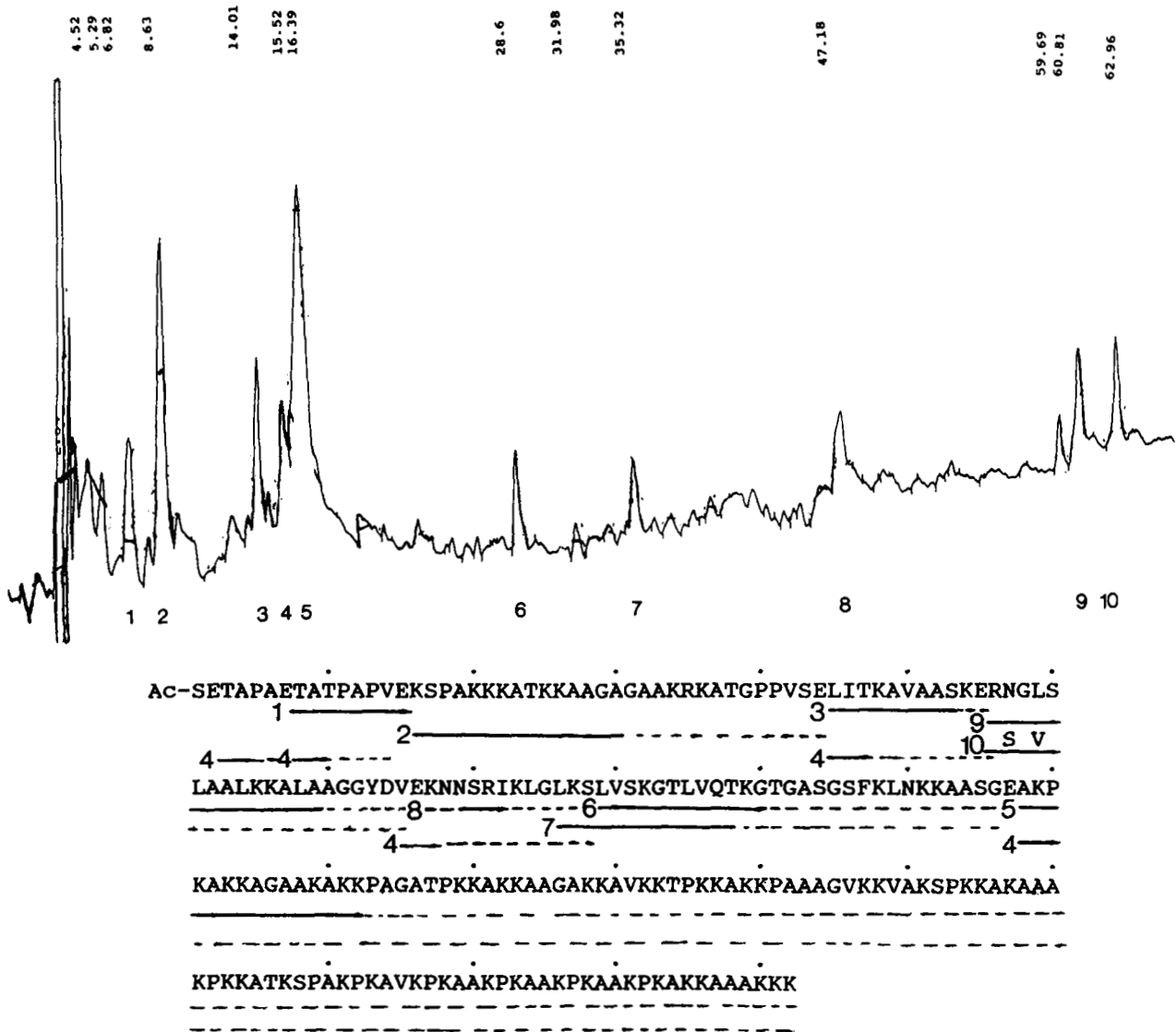


Fig. 3. RP-HPLC chromatogram of V8 protease-digested H1-3 from human placenta. See Figure 1 for details. Complete sequence of H1a by Ohe et al. (1989).

sible association of these subtypes with inactive or more highly condensed chromatin.

A coherent mammalian H1 nomenclature

The sequences of human H1 subtypes have been compared to published sequence data from other mammals, as well as other comparative data, to classify the isoforms into 5 families (Fig. 6; Table 1). In all cases, subtypes are grouped based on the highest percentage of amino acid identity in pairwise comparisons. The first 4 families correspond to closely related subtypes present in all normal somatic cells. The nomenclature is based on the order of their elution from a Bio-Rex column with the superscript "S" ("somatic") to distinguish them from the highly variant subtype H1^o and the testis-specific variant H1t. The fifth class contains members of Seyedin and Kistler's "H1a" family and retains their nomenclature in order to avoid further confu-

sion but to distinguish this tissue-specific variant from the closely related "somatic" subtypes.

Comparisons of the published DNA sequences coding for human H1 subtypes with amino acid sequences permit the identification of the subtype corresponding to each gene. Carozzi et al. (1984) reported the first cloned human H1 gene, which, based on translation of the entire sequence (G. Stein, pers. comm.), would code for a protein that is 95.5% identical to H1-3. More recently, 4 additional human H1 genes, including the H1.1 mentioned above, were cloned and sequenced (Eick et al., 1989; Albig et al., 1991); the others are designated H1.2, H1.3, and H1.4. Based on the published DNA sequences, the latter 3 genes code for H1-1 (H1d; Ohe et al., 1989), H1-2 (H1c; Ohe et al., 1989), and H1-4 (H1b; Ohe et al., 1986), respectively, although Albig et al. (1991) incorrectly identified the H1.4 gene as that coding for H1-1 (H1d; Ohe et al., 1989). Nevertheless, cloned genes are now available for all of the human H1 sub-

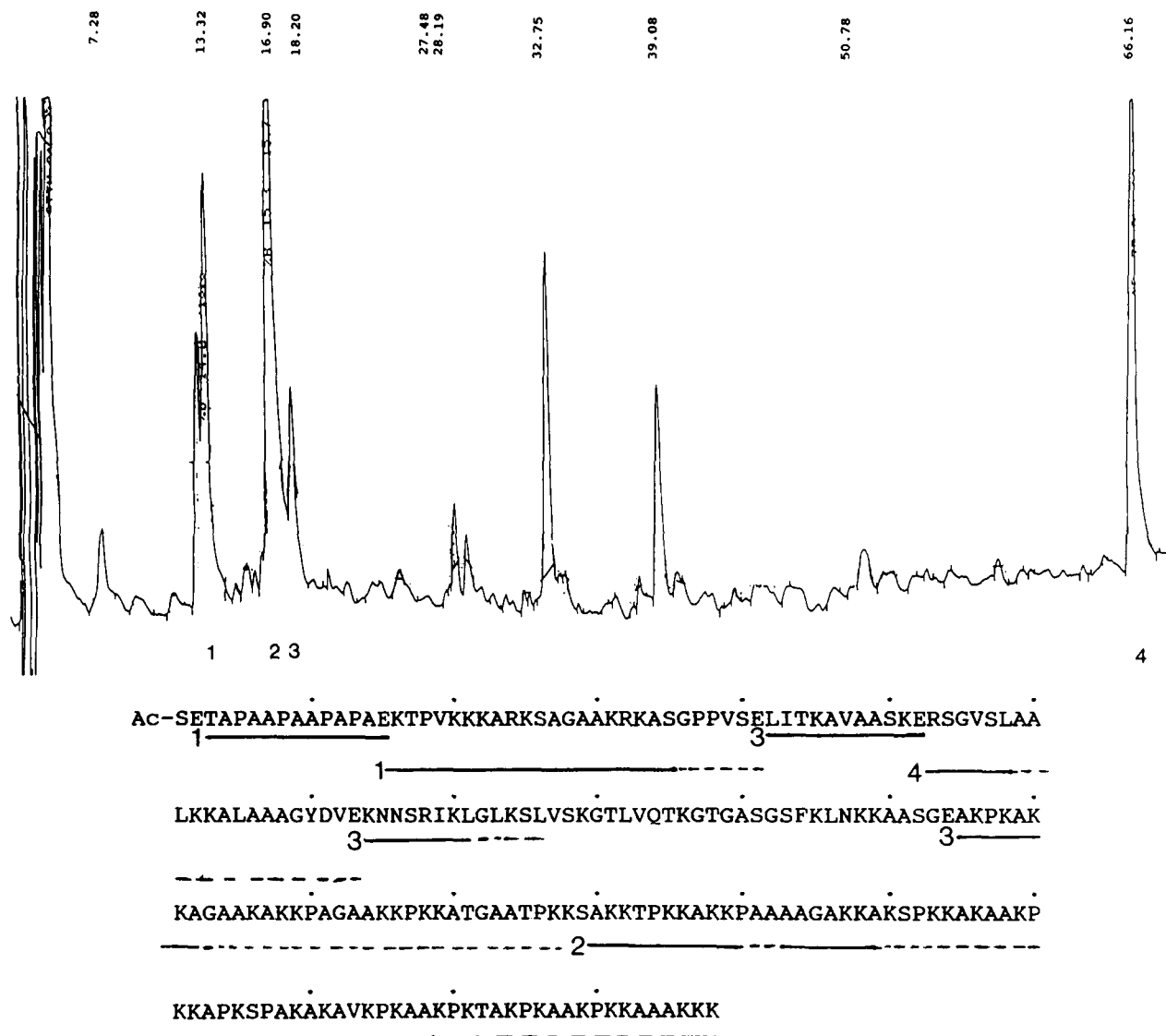


Fig. 4. RP-HPLC chromatogram of V8 protease-digested H1-4 from human placenta. See Figure 1 for details. Complete sequence of H1b by Ohe et al. (1986).

types, including H1^o and H1t (Doenecke & Toenjes, 1986; Drabent et al., 1991).

Bovine and rabbit subtypes fractionated on Bio-Rex 70 and sequenced in Cole's laboratory (1987; pers. comm.) are readily correlated with the human subtypes eluted from the same column in the same order. Sequence identity is particularly striking within the amino- and carboxy-terminal tail regions, which contain the greatest variation in sequence even among subtypes from the same species (Cole, 1987). Further sequence comparisons permit additional correlations. Two mouse gene sequences in the literature (Yang et al., 1987; Cheng et al., 1989) code for an identical H1 subtype that possesses 86.7% predicted protein sequence identity to human H1-1 (Fig. 6A); the partially sequenced rabbit H1.3 and H1.4 proteins are 88.2% and 82.9% homologous to their human counterparts with the same designation (Fig. 6C,D).

One rat H1 gene has also been cloned and sequenced (Cole et al., 1990). Although the gene product was designated "H1d"

based on its electrophoretic mobility, the translated DNA sequence exhibited 94% identity with human H1-4 (Fig. 6D). Analysis of the H1-4 homolog in mouse (H1e in Brown & Sittman, 1993) also reveals a 94% identity, despite the conspicuous lack of a characteristic phosphorylation site in the carboxy-terminal tail (see below and Fig. 6D). Because histones are coded for by multiple gene copies, amino acid sequence differences among members of a given family may or may not have functional significance. Likewise, differences in the amino acid sequence of a given family in different species could result from expression of multiple genes all of which are functionally equivalent. Further isolation of H1 genes may reveal variants with even greater identity to the human subtypes.

Assignment of two of Cole's bovine sequences (H1.3a and H1.3b) (Cole, 1987) into categories was difficult due both to the availability of only partial sequences and to the evolutionary distance between cows and the more closely related orders of primates, rodents, and lagomorphs. Although each sequence

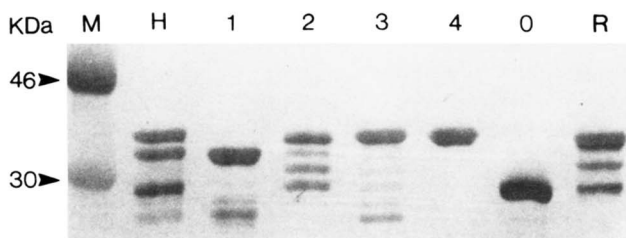


Fig. 5. Fractionation of total H1 and purified subtypes on a 15% SDS-PAGE. kDa, kilodaltons; M, molecular weight markers (Rainbow Markers, Amersham); H, total human H1 from placenta; 1, human H1-1; 2, human H1-2; 3, human H1-3; 4, human H1-4; 0, human H1^o; R, total rat H1 from liver.

has characteristics of the H1^{S-3} class with asparagine/leucine at positions 57/59 for H1.3a and the absence of a cAMP-phosphorylation site for H1.3b (see below), the best overall alignments exist with the H1^{S-4} class. Obviously this classification could change as additional sequence data become available.

An analysis of the grouped sequences shows that a number of amino acid positions are conserved among subtypes in the same group. Examples of these are the location of leucine or valine at residue 6 of H1^{S-2}, a position occupied by alanine in the other subtypes, and the asparagine/leucine residues found in H1^{S-3} positions 57 and 59 discussed above. Another example is the occurrence of glutamic acid-threonine residues at positions 7 and 8 in the H1^{S-3} subtypes of humans and rabbits (also found in 2 H1 subtypes from *Xenopus* [Wells & McBride, 1989]; data not shown). The glutamic acid residue represents an additional potential site for poly(ADP-ribosylation), a modification common to H1 molecules and known to occur *in vitro* at the 3 glutamic acid residues and at the carboxy-terminus highlighted in Figure 6 (Riquelme et al., 1979; Ogata et al., 1980). *In vitro* studies showed that chromatin structure is relaxed upon poly(ADP-ribose) synthesis and the native condensed structure is restored with its degradation (de Murcia et al., 1986). Thus, poly(ADP-ribose)-associated relaxation of chromatin structure could be accompanied by decreased stability of nucleosomes, thought to be a feature of transcriptionally active chromatin. Additional characteristic sites include the tripeptide lysine-proline-lysine (KPK), predicted to be a DNA binding motif with preferential affinity for AT-rich sequences (Churchill & Travers, 1991). The distribution and number of these sites, along with a previously undescribed glycine-alanine-alanine (GAA) tripeptide, vary among the subtypes possibly resulting in differential DNA binding abilities. Each of these diagnostic positions will facilitate classification of a newly sequenced H1 subtype relative to the others.

Characteristic phosphorylation sites

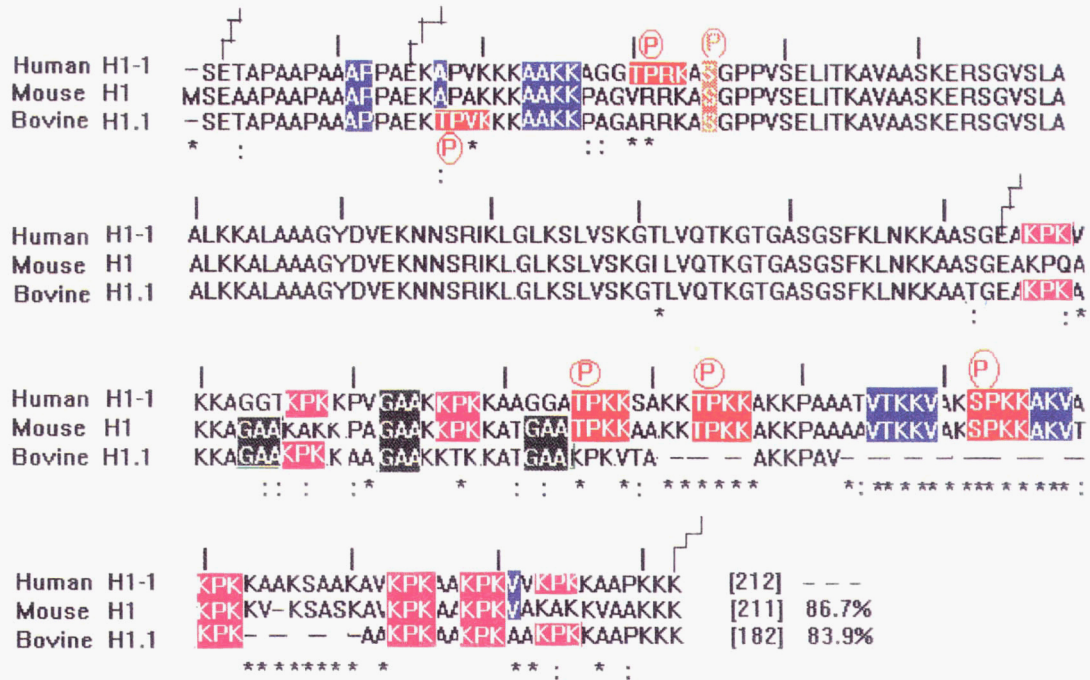
Each H1 class possesses a characteristic pattern of so-called growth-related phosphorylation sites, which vary in number among subtypes. These sites are located in both amino- and carboxy-terminal H1 tails and are predominantly at the quadruple serine/threonine-proline-any amino acid-lysine/arginine (S/T-P-X-K/R) (Langan et al., 1980; Hill et al., 1991; Shetty et al., 1993), a second region implicated in preferential binding to AT-rich DNA (Suzuki, 1989; Churchill & Travers,

1991). Figure 6 shows that subtypes in both H1^{S-3} and H1^{S-4} classes possess 5 sites, 4 in the carboxy-terminal tail and 1 in the amino-terminal tail (2 in the case of the rabbit for H1^{S-4}). In the course of making these comparisons, we noted that one of the conserved phosphorylation sites is shifted 11 amino acid residues toward the amino-terminal tail in all members of the H1^{S-3} class analyzed (Fig. 6C). The existence of this shift in all members of the family suggests that modification of this site may be biologically important.

With the exception of the mouse, H1^{S-1} class subtypes possess 1 amino-terminal site and 3 carboxy-terminal phosphorylation sites. H1^{S-2} class subtypes also possess a site in the amino-tail and, possibly, only 3 carboxy-terminal sites, although complete sequences of rabbit and bovine members are required to make a definitive statement. These comparisons agree with previous work by Langan (1982), who reported the differential distribution of phosphorylation sites among 5 rat H1 subcomponents fractionated by Bio-Rex 70 chromatography. He noted the absence of an amino-tail growth-related phosphorylation site in rat subcomponent 3, which is also observed in mouse H1^{S-1} (Fig. 6A), and the presence of 3 sites in that subtype, as well as 5 sites each in subcomponents 2 and 5 (H1^{S-3} and H1^{S-4}, respectively). Because phosphorylation of H1 reduces binding to DNA (Hill et al., 1991), the variable number and location of sites among subtypes could result in differential abilities to stabilize or destabilize higher order chromatin structure. Hyperphosphorylation of these sites prior to mitosis, resulting in a reduction in H1-DNA interactions could facilitate chromosome condensation by increasing H1-H1 interactions as proposed by Bradbury et al. (1974). Alternatively, Roth and Allis (1992) have suggested that hyperphosphorylation and reduction in H1-DNA interactions could lead to transient chromatin decondensation providing access to non-histone chromosomal proteins, which, by binding to H1 and/or other chromosomal proteins, condense more effectively than H1 alone. For example, recent observations by Shibata and Ajiro (1993) demonstrate that H1 hyperphosphorylation in mitosis is required for H3 phosphorylation, possibly as a consequence of increased accessibility of kinase to H3.

In addition to the growth-related phosphorylation sites, a characteristic cAMP-dependent site at serine 37 was identified in total rat liver H1 (Langan, 1969). This site was phosphorylated *in vivo* after administration of glucagon or insulin to rats. Based on these data, Langan suggested that histone phosphorylation could be the mechanism by which hormones induce transcription. Because these studies did not distinguish among subtypes, with respect to modifications at this site, Ajiro et al. (1990) studied the cAMP-phosphorylation of mouse neuroblastoma cells *in vitro* and rat liver tissue *in vivo* (Table 1). They found that of 5 H1 subtypes isolated and partially sequenced, those corresponding to H1^{S-1}, H1^{S-2}, and H1^{S-4} possess the serine 37 phosphorylation site, however, only H1^{S-4} is preferentially phosphorylated *in vivo*. These results, in conjunction with Langan's (1969), suggest a possible functional role for H1^{S-4} in the hormonal regulation of transcription. The possibility also exists that the other 2 subtypes possessing serine 37 could be phosphorylated *in vivo* under different circumstances. The absence of such sites in "H1a" and H1^{S-3} (Ajiro et al., 1990, and Fig. 6C) implies that they are not involved in hormone-regulated transcription. In fact, analysis of the observations of Ohba et al. (1984) also shows preferential phosphoryla-

A



C

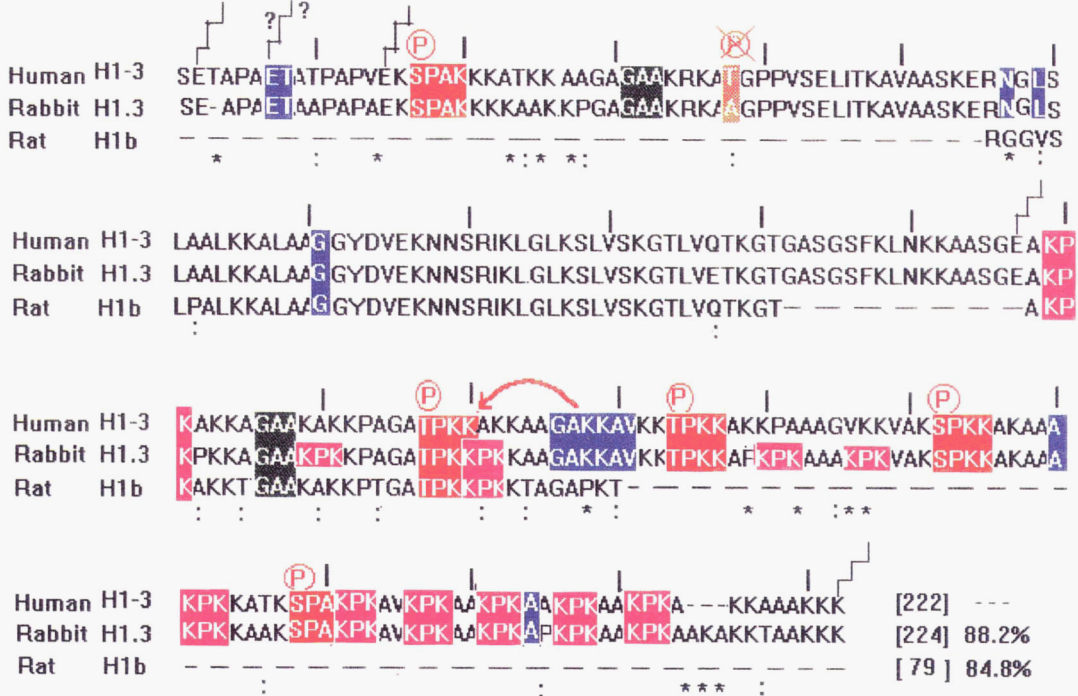


Fig. 6. Classification of human and mammalian H1 sequences into 4 subtype families. **A:** H1^{S-1}. **B:** H1^{S-2}. **C:** H1^{S-3}. **D:** H1^{S-4}. Vertical lines mark every tenth amino acid of the human sequence. Dashed lines represent gaps in the sequence for alignment or unsequenced portions of the protein. Colons represent a conservative amino acid substitution, and asterisks represent a nonconservative substitution or mismatch (Dayhoff et al., 1978). Identical amino acid sequences are unmarked. Stepladder structures designate known sites of poly(ADP-ribosylation) (Riquelme et al., 1979; Ogata et al., 1980), whereas a question mark designates a possible such site. Boxed sequences with a P designate known sites of growth-dependent phosphorylation (in red) (Suzuki, 1989; Churchill & Travers, 1991; Hill et al., 1991; Shetty et al., 1993) or cAMP-dependent phosphorylation (in orange) (Langan, 1969). Boxed sequences in green designate GAA sequences, those in purple designate KPK sequences, and those in blue are other sequences unique to the class. An arrow designates an 11-amino acid shift in the growth-dependent phosphorylation site of H1^{S-3} class subtypes. Bovine and rabbit sequences reported by Cole and colleagues (1987; pers. comm.); mouse sequences reported by Yang et al. (1987), Cheng et al. (1989), and Brown and Sittman (1993); and rat sequences reported by Cole et al. (1990) and Baubichon-Cortay et al. (1992). (Continues on facing page.)

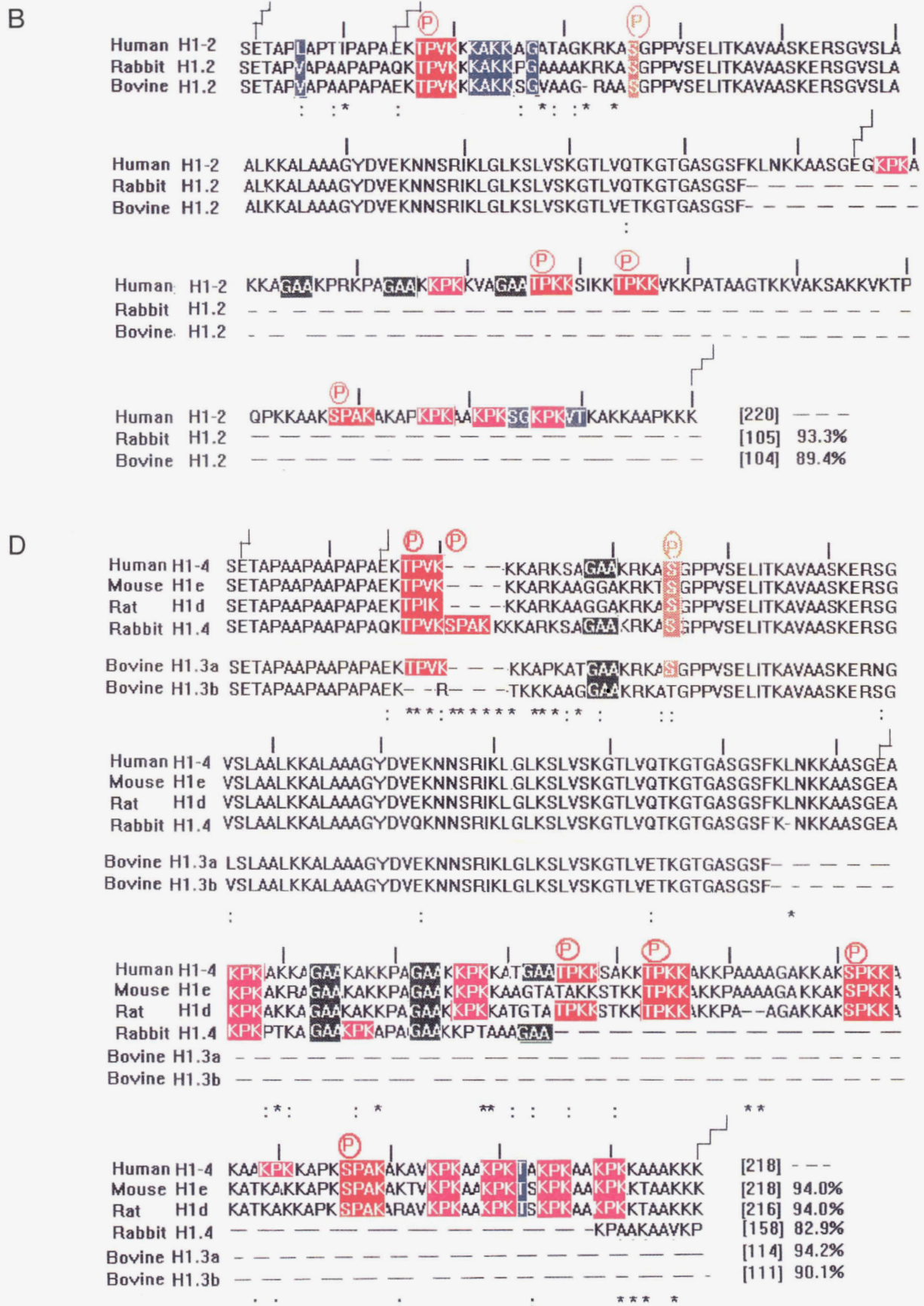


Fig. 6. Continued.

Table 1. Classification of the published sequences and numerous nomenclatures of fractionated H1 somatic subtypes into 5 distinct families^a

	H1 ^S -1	H1 ^S -2	H1 ^S -3	H1 ^S -4	H1a	References
Primates	H1-1	H1-2	H1-3	H1-4	Not detected	Parseghian, Clark, Hauser, Dvorkin, Harris, & Hamkalo, 1993, <i>Chromosome Res</i> 1:127-139 (human placenta)
	H1d	H1c	H1a	H1b	Not detected	Ohe, Hayashi, & Iwai, 1989, <i>J Biochem (Tokyo)</i> 106:844-857; also: 1986, <i>J Biochem (Tokyo)</i> 100:359-368 (human spleen)
	Variant 2	Variant 4	Variant 3	Variant 1	Not detected	D'Incalci, Paola, Wu, & Bonner, 1986, <i>Eur J Biochem</i> 154:273-279 (human fibroblast)
	H1e	H1d	H1a	H1c	H1b	Ajiro, Shibata, & Nishikawa, 1990, <i>J Biol Chem</i> 265:6494-6500 (mouse neuroblastoma)
Rodentia	p6	p4	p2	p5	p3	Lindner, Helliger, & Puschendorf, 1990, <i>Biochem J</i> 269:359-363 (rat liver and testis)
	H1d ^b	H1C	H1A	H1D ^b	H1B	Karhu, Mahonen, & Paivimo, 1988, <i>J Chromatogr</i> 426:65-73 (rat liver, spleen, testis, and thymus)
	III	IV	II	V	I	Higurashi, Adachi, & Ohba, 1987, <i>J Biol Chem</i> 262:13075-13080 (mouse lymphoma)
	H1-2	Probably too minute to detect	H1-1	H1-3	Not detected	Ohba, Higurashi, & Hayashi, 1984, <i>J Biol Chem</i> 259:2942-2948 (rat liver)
	H1c	H1d	H1b	H1c	H1a	Lennox, Oshima, & Cohen, 1982, <i>J Biol Chem</i> 257:5183-5189; Lennox & Cohen, 1983, <i>J Biol Chem</i> 258:262-268 (mouse teratocarcinoma, lung, kidney, liver, spleen, fibroblast, and thymus)
	3	4	2	5	1	Langan, 1982, <i>J Biol Chem</i> 257:14835-14846 (rat thymus)
	H1c	H1d	H1b	H1e	H1a	Seyedin & Kistler, 1979a, <i>Biochemistry</i> 18:1371-1375 (rat thymus and testis)
	H1b	H1c	H1d	H1e	H1a	Seyedin & Kistler, 1979b, <i>Biochemistry</i> 18:1376-1379 (rabbit thymus and testis)
	RTL-1	RTL-2	RTL-3	RTL-4	Not reported	Cole, 1987, <i>Int J Pept Protein Res</i> 30:433-449; also pers. comm. (rabbit thymus)
	CTL-1	CTL-2	?	CTL-3a + CTL-3b	Not reported	Cole, 1987, <i>Int J Pept Protein Res</i> 30:433-449; also pers. comm. (calf thymus)
Artiodactyla	1b	2	3a	3b	1a	Snerdon & Isenberg, 1976, <i>Biochemistry</i> 15:4233-4242 (calf thymus)
	H1.2	H1.3	Not detected	H1.4	H1.1	Eick, Nicolai, Mumberg, & Doenecke, 1989, <i>Eur J Cell Biol</i> 49:110-115; Albig, Kardalhou, Drabent, Zimmer, & Doenecke, 1991, <i>Genomics</i> 10:940-948 (gene sequence from human granulocyte DNA)
Primates			Human H1 gene isolated corresponds to H1 ^S -3			Carozzi, Marashi, Plumb, Zimmerman, Zimmerman, Coles, Wells, Stein, & Stein, 1984, <i>Science</i> 224:1115-1117; also G. Stein, pers. comm.
			Human gene Hh8c			La Bella, Zhong, & Heintz, 1988, <i>J Biol Chem</i> 263:2115-2118; also F. La Bella, pers. comm.
Rodentia	Mouse H1 var. 1			Rat H1d		Cole, Kandala, Kremer, & Kistler, 1990, <i>Gene</i> 89:265-269
	H1c			H1e		Cheng, Nandi, Clerk, & Skoutchi, 1989, <i>Proc Natl Acad Sci USA</i> 86:7002-7006
						Yang, Brown, Wellman, & Sittman, 1987, <i>J Biol Chem</i> 262:17118-17125; Brown & Sittman, 1993, <i>J Biol Chem</i> 268:713-718 (mouse DNA)

^a Mammalian H1 protein and gene sequences published before September 1993 have been included. The families are organized according to the nomenclature we propose. A complete amino acid sequence of Seyedin and Kistler's "H1a" has yet to be published. The assignments in this table were made by evaluating the published data on Bio-Rex 70, RP-HPLC, SDS-PAGE, acid-urea PAGE, and 2-dimensional gel separations, as well as data on subtype phosphorylation patterns, and then assigning members to a group based on consistent correlations among the various published observations.

^b Subtypes not resolved sufficiently.

tion of H1^S-4 in vivo with the administration of glucagon, as well as the lack of cAMP-dependent phosphorylation of H1^S-3 in vitro.

Confusion in the literature

Identification of homologous mammalian H1s based on amino acid sequence data rather than an array of chromatographic or electrophoretic techniques allow, with minimal confusion, direct correlations of observations made in different species. The complications that can arise when only biochemical properties are used to designate isoforms is illustrated in the extensive literature that employs the nomenclature of Seyedin and Kistler (1979a). In their nomenclature, each subtype from rat thymus was designated by a lowercase letter based on the order of their elution from a Bio-Rex 70 column. This system should not be confused with the separate lowercase letter nomenclature of Ohe et al. (1986, 1989). Subsequently, Lennox et al. (1982) adopted this nomenclature for subtypes resolved on acid-urea gels in the first dimension and SDS gels in the second. However, "H1a," which elutes ahead of H1^S-1 on a Bio-Rex column, is not present in many cell types resulting in difficulties with data comparison among different experiments. For example, in the course of studying H1 subtype phosphorylation in rat liver (Ohba et al., 1984) and subtype turnover in mouse lymphoma cells (Higurashi et al., 1987), Ohba and Higurashi's group separated both samples on Bio-Rex 70 and designated peaks in the order of elution 1, 2, etc. However, the corresponding peaks do not contain the same subtypes because "H1a," which elutes first, is present only in the lymphoma cells.

Additional difficulties arise when subtypes from different species possess dramatically different orders of elution from an ion-exchange column. For example, rat and mouse H1^S-3 elute ahead of the remaining subtypes (with the exception of "H1a"), a property that is not shared with H1^S-3 from humans, rabbits, or cows. The designation of this rat subtype as a member of the H1^S-3 group is supported by its coincident migration with the upper band of total H1 on an SDS-polyacrylamide gel (see Fig. 5, lane 3), an RP-HPLC retention time characteristic of human H1-3 (Lindner et al., 1990; Parseghian et al., 1993), lack of phosphorylation with cAMP-dependent protein kinase (Ohba et al., 1984), and the partial amino acid sequence for rat "H1b" (Baubichon-Cortay et al., 1992, and Fig. 6C). The shift in ion-exchange elution suggests that there is a significant difference in the polar amino acid composition of rat and mouse H1^S-3, which does not affect its retention on an RP-HPLC column. Thus, reliance on only chromatograph elution can, and has, resulted in the incorrect identification of both rat H1^S-3 and rabbit H1^S-1 as "H1b" in the Seyedin and Kistler (1979a, 1979b) nomenclature.

Another example of an incorrect designation was published recently by Giancotti et al. (1993), who used ion-spray mass spectrometry to determine molecular weights of mouse subtypes after RP-HPLC elution in an attempt to characterize the subtypes completely. Their reliance on chromatographic elutions to designate isoforms also resulted in the misidentification of H1^S-3 as H1^S-1.

Strict reliance on electrophoretic mobility can also be misleading, as evidenced by a report from the Kistler lab (Cole et al., 1990) in which a rat H1 gene was cloned, expressed, and tentatively identified as H1d on a polyacrylamide gel. However, as

noted above, sequencing of the gene revealed a homology to mouse H1e and the H1^S-4 family rather than that of H1^S-2 as expected from correlations with the Seyedin and Kistler (1979a) and Lennox et al. (1982) nomenclatures (Table 1).

Beyond the development of a consistent subtype classification, this nomenclature will permit direct comparisons of changes in subtype ratios under various conditions made in the same system by different investigators as well as correlations between systems. For example, it was possible to identify H1^S-1 as the subtype with a rapid turnover rate in the absence of DNA synthesis in various mouse tissues (Pehrson & Cole, 1982; Lennox & Cohen, 1983; Higurashi et al., 1987; Dominguez et al., 1992), and H1^S-4 as the subtype preferentially phosphorylated by cAMP-dependent protein kinase in rats and mice (Ohba et al., 1984; Higurashi et al., 1987; Ajiro et al., 1990).

At present, data to support putative subtype-specific functions of H1 in vivo are lacking, and the available data are scattered in the literature. We have presented a correlation of the various nomenclatures existing for histone H1 and propose a standard nomenclature based on amino acid sequences (Table 1). This nomenclature is analogous to that used for H1^o (Doenecke & Toenjes, 1986) and the testis-specific H1t (MacLeod et al., 1977; Seyedin & Kistler, 1980; Cole et al., 1984; Drabent et al., 1991). Rat and mouse sequences are included based on available data. However, the wealth of biological information concerning, for example, changes in mouse and rat subtype ratios, subtype turnover rates, and patterns of phosphorylation (Pehrson & Cole, 1982; Pina et al., 1987; Dominguez et al., 1992; Helliger et al., 1992; Brown & Sittman, 1993) could be more profitably exploited if the rodent subtypes were fully sequenced. Such data could be then used for analysis of homologous regions in the subtypes of other vertebrates and subsequently extended to other eukaryotes in order to gain insight into possible structure/function relationships of subtypes in organisms that can be readily manipulated genetically.

Materials and methods

Histone H1 isolation and subtype fractionation

Total histone H1 was purified from human placenta and subtypes were fractionated by RP-HPLC followed by Bio-Rex 70 chromatography as described by Parseghian et al. (1993). Total H1 was extracted from male Wistar Munich rat livers using the method of Goodwin et al. (1978).

Protease cleavage and peptide fractionation

Fifty grams of each subtype was subjected to *S. aureus* V8 protease (Sigma) cleavage (1:30, w/w) according to Ohe et al. (1986). Digestion was carried out in 50 mM NH₄HCO₃ (pH 7.8) buffer for 22 h at 37 °C with 1 μL of 10% toluene in absolute ethanol to prevent bacterial contamination; the solution was frozen at -80 °C and lyophilized. V8 protease cleaves preferentially on the carboxylic side of glutamic and aspartic acid residues. Peptide fragments were suspended in double-distilled water and fractionated by RP-HPLC on a Vydac C₁₈ column. The system included Hewlett Packard 1084A pumps and injector, with both column and solvents heated to 40 °C. The column was eluted with a 10–50% acetonitrile/0.1% trifluoroacetic acid gradient at a flow rate of 1.0 mL/min for 125 min, although a flow rate

of 1.5 mL/min was required for improved resolution of H1-1 peptide fragments. Peaks were identified by absorbance readings at 214 nm, manually collected, numbered in the order of their elution, and lyophilized.

Peptide sequencing and sequence alignments

Peptides were sequenced by Edman degradation in an Applied Biosystems model 477A pulsed-liquid protein sequencer equipped with an on-line model 120A HPLC analyzer. Typically, 20–600 pmol of a peptide sample were run for 10–25 cycles as required for an unambiguous identification.

Amino acid and nucleotide sequences determined by other researchers were obtained either from the literature (Wells, 1986; Cole, 1987; Wells & McBride, 1989) or from the PIR, Swiss-Prot, GenBank, and EMBL databases. Sequence alignments were created using a Clustal V protein alignment program.

PAGE

Total histone H1 and purified subtypes were electrophoresed on SDS-PAGE mini-gels (Bio-Rad) at 200 V for 1 h (Laemmli, 1970). Gels were silver stained according to the method of Wray et al. (1981).

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