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Purification of the centromere-specific protein CENP-A and demonstration that it is a distinctive histone

(histone H3/scleroderma/autoantibodies/kinetochore/chromatin)

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ABSTRACT **CENP-A, a centromere-specific 17-kDa protein, has histone-like properties. However, in contrast to the common somatic histones, CENP-A is quantitatively retained in bull spermatozoa, and we have exploited this fact to purify CENP-A to apparent homogeneity. Partial sequence analysis of the purified protein indicates that CENP-A is a distinctive gene product. Some CENP-A sequences are highly similar to regions of histone H3. Other segments of CENP-A are not related to H3 or any other histone. These unrelated segments are presumably involved in localizing CENP-A to centromeric DNA or in centromere-specific functions of CENP-A.**

The centromeric region of the chromosome is responsible for its integration into the mitotic spindle and for its proper segregation poleward during anaphase (1–4). Several proteins have been identified that are associated with the centromere (5–10). For the most part, their functions are presently unknown. Recently, however, Bischoff *et al.* (11) reported that a 47-kDa centromere-specific autoantigen is highly homologous to the translation product of *RCC1*, a gene involved in regulating mammalian chromosome condensation. This antigen may be identical to CENP-D, a 50- to 60-kDa centromeric protein reported to be present in a variety of species (7, 9, 10).

CENP-A, a centromere-specific protein of 17 kDa, appears to be associated with kinetochore chromatin or with chromatin closely apposed to the outermost domain of the kinetochore, as judged by indirect immunofluorescence (9) and immunoelectron microscopy (12). This localization suggests it may play a direct role in kinetochore function during mitosis.

We have extensively analyzed CENP-A and have shown that it appears to be a core histone by the following criteria: (*i*) it is extracted with histones from chromatin by dilute mineral acids, (*ii*) it elutes with histones H3 and H4 in apparent tetrameric complexes during ion exchange (13) or sizing-column chromatography (D.K.P., K.O., and R.L.M., unpublished observations), and, most importantly, (*iii*) it is a component of highly purified nucleosome core particles (13). CENP-A is exceptional among somatic histones not only because it is centromere specific but also because it is quantitatively retained in chromatin during spermatogenesis in mammals, even in species where other histones are quantitatively replaced by protamines (14).

The selective retention of this protein in bull sperm, where other histones are absent, has now allowed us to extract and purify CENP-A to homogeneity. We have also subjected the purified CENP-A from bull sperm to partial sequence analysis and report here that it is a distinctive histone, with sequences similar to those of H3, as well as segments that are not related to histones or homologous to any other known mammalian centromeric protein sequences (11, 15).

MATERIALS AND METHODS

Purification and Acid Extraction of Nuclei and Reverse-Phase Chromatography of Acid-Extracted Proteins. Calf thymus nuclei and bull sperm nuclei were purified and extracted with 1 M NaCl/0.25 M HCl as described (14). Acid-extracted proteins dissolved in 36% (vol/vol) acetonitrile/0.1% trifluoroacetic acid were fractionated at 25°C on a 250 mm × 4.6 mm Bio-Rad RP-318 (C_{18}) column by using a gradient of acetonitrile in 0.3% trifluoroacetic acid to elute proteins (16, 17). The concentration of acetonitrile was raised linearly from an initial value of 36% to 60% over 55 min, was held constant at 60% for 5 min, and was then increased to 80% over 25 min, at a flow rate of 1 ml/min. Elution of protein was monitored by absorbance at 230 nm, and 0.5-ml fractions were collected. Elution of CENP-A was monitored by dot-blotting immunoassay of the column fractions (see below).

For preparative purposes, CENP-A-containing fractions from multiple-reverse phase runs, each loaded with acid-extracted protein from up to 5000 260-nm absorbance units of bull sperm nuclei, were pooled, 15 μg of Polybrene was added to aid in subsequent resolubilization of the CENP-A, and the combined sample was concentrated by using a SpeedVac (Savant), and rechromatographed. CENP-A-containing fractions were again pooled, supplemented with Polybrene, and concentrated; the protein was then chromatographed a third time. Purified CENP-A was collected by using a SpeedVac or by precipitation with trichloroacetic acid [20% (wt/vol) final concentration] in the presence of added Polybrene. In the latter case, pellets were washed with acetone/0.2% HCl and were dried by using a SpeedVac.

Dot-Blotting Immunoassay of CENP-A. Small aliquots of column fractions were diluted into 400 μl of water in a dot-blotting apparatus, and protein was drawn onto nitrocellulose (BA 83, Schleicher & Schuell). Blots were then processed as described for an immunoblot transfer (13) by using anticentromere serum from patient GD as the primary antibody and ^{125}I-labeled protein A (NEX-146L, New England Nuclear) as the secondary reagent.

PAGE and Immunoblotting. Procedures for (*i*) electrophoresis of acid-extracted sperm proteins in polyacrylamide gels containing 12% acrylamide, 6.5 M urea, and 0.38% (wt/vol) Triton X-100 and (*ii*) electrophoretic transfer of proteins to Immobilon membrane (Millipore) in 0.7% acetic acid have been described (14). Electrophoresis of proteins in SDS/18% polyacrylamide gels and electrophoretic transfer of proteins to nitrocellulose in Tris glycine/methanol/SDS transfer

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buffer have also been described (13). Procedures for Coomassie brilliant blue staining of detergent/acid/urea or SDS/polyacrylamide gels were identical, as were procedures for immunologic detection of CENP-A on immunotransfers to Immobilon or nitrocellulose; both procedures have been reported (13).

Enzymatic Cleavage of CENP-A and Separation and Analysis of Peptides. Chymotrypsin was obtained from Worthington. *Staphylococcus aureus* V8 protease and *Pseudomonas fragi* endoproteinase Asp-N were purchased from Miles and Boehringer Mannheim, respectively. CENP-A (1 nmol) was digested with chymotrypsin by using a 1:50 (wt/wt) protease/substrate ratio. Digestion was at room temperature in 0.2 M ammonium bicarbonate/0.1 mM $CaCl_2$ for 6 hr and was terminated by adding 20% trifluoroacetic acid. Cleavage of CENP-A (500 pmol) with *S. aureus* V8 protease, 1:25 (wt/wt) was performed in 2 M urea/0.25 M sodium phosphate, pH 8.0, at room temperature for 24 hr. CENP-A (1 nmol) was digested with Asp-N protease using a protease/substrate ratio of 1:50 (wt/wt). Digestion was in 0.1 M guanidine hydrochloride/0.1 M Tris·HCl, pH 7.5, for 24 hr at 37°C. The buffer was then adjusted to contain 6 M guanidine hydrochloride, 0.1 mM EDTA, and 0.25 M Tris·HCl, pH 8.5, and the digest was reduced and pyridylethylated (18).

Peptides were purified by reversed-phase HPLC on RP-300 (2.1 × 100 mm) or RP-18 (2.1 × 30 mm) columns (Pierce) using a Hewlett–Packard 1090 liquid chromatograph equipped with a diode-array detector. Edman degradations were performed on an Applied Biosystems model 470A gas-phase sequencer equipped with an on-line model 120A phenylthiohydantoin–amino acid analyzer.

The SEARCH program (19) was used to identify homologous mammalian histone sequences within the Protein Identification Resource (PIR) protein data base (release 21.0, Natl. Biomed. Res. Found.). Sequence alignments were performed on a VAX/VMS computer by using the ALIGN program (19) with the mutation data matrix and a gap penalty of 10.

RESULTS

Relative to DNA, CENP-A is present in acid extracts of calf thymus nuclei or bull sperm nuclei in comparable amounts (14), and it is the only protein in acid extracts of human, calf thymus, or bull sperm nuclei recognized by antibodies in human CREST (calcinosis, Raynaud's phenomenon, esophageal hypomotility, sclerodactyly, telangiectasia) scleroderma anticentromere serum from patient GD (13, 14, 17) or by antibodies in any of several human anticentromere sera we have tested (data not shown). Comparison of acid-soluble proteins from calf thymus and bull sperm nuclei by reverse-phase chromatography on a C_{18} column reveals that the abundance of histones in sperm extracts is <1% that in thymus nuclei (Fig. 1). Protamines, the quantitatively major acid-soluble proteins from sperm, elute in the C_{18} column flow-through fraction (data not shown). Analysis of column fractions by dot blotting using serum GD as a probe reveals that CENP-A from sperm elutes at ≈60% acetonitrile, as does CENP-A from thymus (Fig. 1) or from human tissue-culture cells (17).

We have purified CENP-A from bull sperm nuclei, after acid extraction, by three cycles of reverse-phase chromatography on C_{18} reverse-phase columns, as described. Fig. 2*A* shows the elution profile and dot analysis of the fractions obtained in a third cycle of reverse-phase chromatography of acid-extracted sperm protein, and a Coomassie blue-stained Triton/acid/urea gel of protein in the CENP-A-containing peak. A single major band of CENP-A is present in the gel. Most gels of purified CENP-A contain three quantitatively minor protein bands that migrate more slowly than the bulk of the CENP-A and one band that migrates more rapidly. All

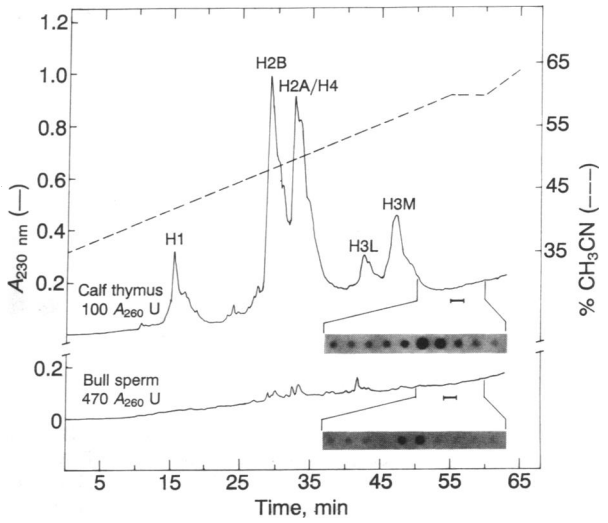

FIG. 1. Reverse-phase chromatography of acid-extracted calf thymus and bull sperm nuclear proteins and dot blotting analysis of the elution of CENP-A. Samples of acid-soluble calf thymus protein (representing 470 260-nm absorbance units of nuclei) and bull sperm nuclear protein (representing 100 260-nm absorbance units of nuclei) were fractionated and assayed for total protein and for CENP-A. Peaks containing calf thymus histone are labeled according to Gurley *et al.* (16); H3L and H3M represent peaks containing less and more hydrophobic species of H3, respectively. Histones are essentially absent from the bull sperm protein, but CENP-A is present, eluting as a single peak at ≈60% acetonitrile. Immuno-dot blots do not reflect the relative amounts of CENP-A in sperm and thymus, as they represent the results of separate experiments; only the CENP-A-containing regions of the dot blots are shown.

four of these quantitatively minor protein bands represent forms of CENP-A (see below).

We first addressed the question of the purity of the protein in the main CENP-A band. The major band present in the Triton/acid/urea gel shown in Fig. 2*A* was excised and equilibrated with SDS sample buffer; the protein resolved in an SDS/polyacrylamide gel as a second dimension. Only two protein species are visible in the Coomassie blue-stained second-dimension gel (Fig. 2*B*, lane 1): a quantitatively major species, migrating at the position of CENP-A (i.e., as a 17-kDa protein), and a minor species (arrow), migrating as a 34-kDa protein. A small fragment of the CENP-A band was similarly electrophoresed in an SDS/polyacrylamide second-dimension gel but was subjected to immunoblotting rather than staining. The immunoblot (Fig. 2*B*, lane 2) reveals that both species bind antibodies in the CREST serum, suggesting that the 34-kDa species is a dimer of CENP-A. As a control, histone H3, similarly processed through the first- and second-dimension gels, did not bind CREST antibodies (data not shown).

To confirm that the 34-kDa protein and CENP-A are related, the spots corresponding to CENP-A and to the putative dimer were excised from the stained SDS/polyacrylamide gel, were equilibrated with SDS application buffer (containing 5% 2-mercaptoethanol), and were subjected a second time to electrophoresis in an SDS/polyacrylamide gel. An immunoblot probed with anticentromere serum (Fig. 3) shows that the dimer was regenerated in the lane containing electrophoretically pure CENP-A, and CENP-A was regenerated in the lane containing dimer. Proteins that represent apparent higher multimers are also visible on the immunoblot. This behavior is similar to that of histone H3, which forms multimers after reverse-phase chromatography or two-dimensional gel electrophoresis (ref. 16, and data not shown).

We addressed the compositions of the quantitatively minor bands detected after Triton/acid/urea-gel electrophoresis of column-purified CENP-A with similar techniques. Again, we

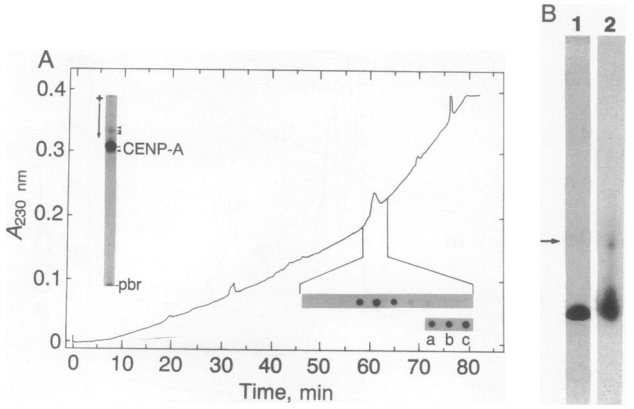

FIG. 2. (*A*) Reverse-phase chromatography of CENP-A and Triton/acid/urea-gel electrophoresis of purified CENP-A. Acid-extracted protein from 14,000 260-nm absorbance units of bull sperm nuclei was fractionated by reverse-phase HPLC. Elution of protein, monitored by absorbance at 230 mm, is shown for the third cycle of chromatography. (*Horizontal inset*) Elution of CENP-A as monitored by immuno-dot blotting analysis of column fractions. The three dot-blotting fractions, designated a, b, and c, correspond to sample applied for the column run; they represent protein from 3.5, 7, and 14 260-nm absorbance units of nuclei, respectively. Summing the dot-blotting fractions containing eluted protein, 100% recovery of CENP-A would generate a signal corresponding to 28 260-nm absorbance units of nuclei; our results, while not rigorously quantitative, indicate that CENP-A is recovered from the reverse-phase column in high yield. (*Vertical inset*) Coomassie blue-stained Triton/acid/urea gel loaded with the purified CENP-A (≈7 μg) obtained by pooling and trichloroacetic acid-precipitating protein in the three major CENP-A-containing column fractions. Control experiments showed that staining material that migrates at the dye front represents Polybrene (data not shown). (*B*) Analysis of purified CENP-A by electrophoresis in an SDS/polyacrylamide gel as a second dimension and by immunoblotting. The major band in the Triton/acid/urea gel shown in *A* was excised, equilibrated at 22°C with SDS gel sample buffer containing 5% 2-mercaptoethanol, and divided into two unequal portions before applying the fragments to SDS/polyacrylamide gels. The gel containing protein from the larger fragment was stained with Coomassie brilliant blue R (lane 1), whereas the gel containing protein from the smaller fragment was immunoblotted to reveal CENP-A (lane 2). Protein in the CENP-A band migrates predominantly as a single band in the second dimension and has a mobility corresponding to a 17-kDa protein. A faint upper band is also visible in the stained gel (arrow), and has a mobility corresponding to an ≈34-kDa protein. The immunoblot shows that both the 17- and 34-kDa proteins bind antibody. A histone H3 control (data not shown) did not bind antibody.

found that all quantitatively minor Triton/acid/urea-gel bands resolve in a second dimension into multiple protein species that can be identified as monomeric CENP-A and multimers of CENP-A, as judged by both antibody binding and mobilities of the proteins in a second dimension (data not shown). The proteins that migrate more slowly than CENP-A in Triton/acid/urea gels probably represent CENP-A multimers. Triton/acid/urea gels are exquisitely sensitive to protein conformation and can resolve histone variants that differ by a single neutral amino acid or in which a single methionine has been oxidized (20). Although other explanations are possible, we consider it likely that the fastest migrating CENP-A species is an artifact generated during our column-purification procedures. In summary, we conclude that all bands detectable on Triton/acid/urea gels represent forms of CENP-A and that we have successfully purified CENP-A to apparent homogeneity.

Aliquots of CENP-A, purified by the procedures described above, were digested with chymotrypsin, *S. aureus* V8 protease, or *P. fragi* Asp-N endoprotease. Digests were separated by reverse-phase HPLC, and individual peptides were analyzed for

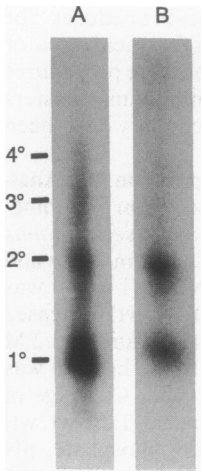

FIG. 3. Demonstration that CENP-A forms multimers *in vitro*. The 17-kDa CENP-A band and the 34-kDa band in the Coomassie blue-stained SDS/polyacrylamide gel of Fig. 2*B* were excised, reequilibrated in gel application buffer, electrophoresed a second time in an SDS/polyacrylamide gel, and immunoblotted to reveal CENP-A. Lanes: A, protein from the 17-kDa CENP-A band excised from the second-dimension SDS/polyacrylamide gel; B, protein from the 34-kDa band excised from the second-dimension SDS/polyacrylamide gel. The immunoblot shows that both 17- and 34-kDa bands regenerate a ladder of CENP-A-containing bands, with major species at 17 kDa (monomer) and 34 kDa (dimer).

sequence on a gas-phase sequenator. Analysis of these digests provided eight nonoverlapping sequences for a total of 107 residues (≈70% of expected residues). Most of these sequences were obtained from analysis of the 12 major peptide fractions that were separated from the chymotryptic digest (Fig. 4). Eight of these fractions yielded single sequences; of which, six (C1, C2, C3, C5, C8, C10) are represented in Table 1. The sequence from one of the *S. aureus* V8 protease products, E1, overlapped sequences from chymotrypsin-cleaved C2 and C3 to provide a single proven sequence of 14 residues that is indicated as C2/E1/C3 in Table 1. The sequence of one of the *P. fragi* Asp-N products, D5, contained the sequence of C5 and overlapped sequence in C10 to provide a single proven sequence of 27 residues, which is indicated as C10/D5/C5 in Table 1. The two additional single-sequence chymotryptic fractions, C7 and C4, yielded short sequences that were not distinctive because they are contained within the sequences of the incomplete cleavage products, C10 and C8, respectively. No sequence was detected in fractions C11 and C12, suggesting that both fractions contain peptides with blocked N termini. Both the presence of these peptides and failure to obtain sequence from the whole protein (data not shown) suggest that the native protein is N-terminally blocked.

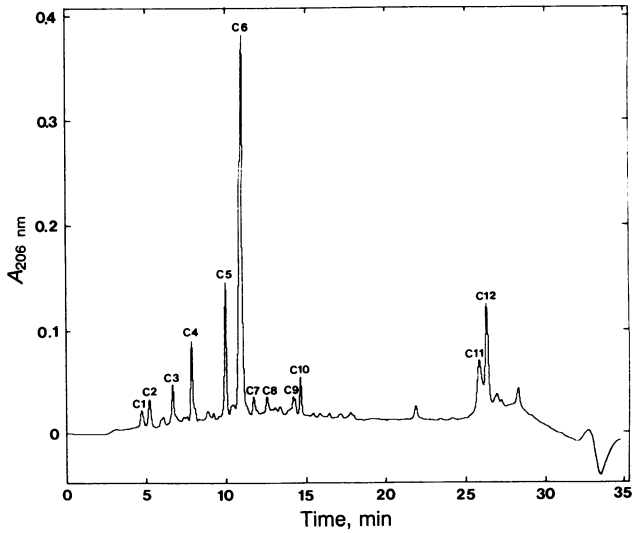

FIG. 4. Separation of peptides after cleavage of CENP-A with chymotrypsin. CENP-A was digested with chymotrypsin, and the resulting peptides were resolved by reverse-phase chromatography on an Aquapore RP-300 C_8 column (2.1 mm × 100 mm, 7-μm particle size). Peptides were eluted with a linear gradient of 0–75% acetonitrile (vol/vol) over 30 min at a flow rate of 0.3 ml/min, and were detected by absorbance at 206 nm.

Table 1. Peptide sequences from CENP-A

CENP-A peptides*	Peptide source†	Residues, no.	Comparison to bovine H3: Identical, no.	Comparison to bovine H3: Sequence identity, %
Aligned with H3				
QKTTHL	C1	6	4	67
SLHAGRVTLFPKDVQLARRIRGIQEGL	C10/D5/C5‡	27	16	60
XVLKEIRTL	C8	8	4	50
lrlareipvqf	C9a	11	6	55
AFLVvyF	E2	6	4	67
Unaligned				
DVSGGTEGEGTEGGEET	D1	17	—	—
DRTPSEKKTPSVGsIPpA	D4	18	—	—
KAEKAFKVSETFky	C2/E1/C3‡	14	—	—
$K^{kKE}_{rPP}E^{PP}_{TR}RR^{PPAS}_{RAsP}PA^{P}_{A}$§	C6	16	—	—

*Sequences are given in single-letter code with lowercase letters designating residues that are tentative assignments.
†Prefixes C, D, and E designate fragments generated from cleavage with chymotrypsin, *P. fragi* endoproteinase Asp-N, and *S. aureus* V8 protease, respectively.
‡Sequence was deduced from the overlap of the three peptides given.
§Several cycles from the Edman degradation of C6 yielded nearly equal quantities of two phenylthiohydantoin–amino acids; both sequences are given—one above and one below the line. The 16 residues of C6 are not counted among the 107 (total) residues assigned.

Fraction C9 was a 1:1 mixture of two peptides; one of which was identical to a 13-residue segment present at the C terminus of C10, allowing the sequence C9a (Table 1) to be tentatively assigned by subtraction. Fraction C6 was comprised of at least two different peptides. As shown in Table 1, 6 of the 16 cycles of C6 peptide analysis gave a single phenylthiohydantoin–amino acid, whereas the remaining cycles gave two different derivatives, each of which was present at about one-half the amount detected in the other cycles. These observations suggest, but do not prove, that fraction C6 is comprised of a 1:1 mixture of two structurally related, but different, proline-rich peptides.

Asp-N cleavage of CENP-A provided fractions with additional distinctive sequences. Two Asp-N fractions, D1 and D2, yielded single sequences that were identical (D1 shown in Table 1). The peptide common to D1 and D2 may contain a site of posttranslational modification, possibly phosphorylation, that may explain the difference in retention time. Fraction D4 yielded a single 18-residue sequence (Table 1).

A computer-assisted search demonstrated that the CENP-A sequences were *not* identical to any of the 30 mammalian histone sequences examined; at least one representative of each of the five major histone classes was included. Hence, CENP-A is clearly not a posttranslationally modified derivative of the major histones of the nucleosome core but is a distinctive gene product. However, as shown in Table 1 and Fig. 5, six of the sequences obtained from chymotryptic fragments of CENP-A can be aligned with bovine histone H3 (21) with ≥50% sequence identity. The similarity is most striking at the C terminus of histone H3, where 27 residues from peptides C10 and C5 can be aligned with residues 111–135 of histone H3 to give a Dayhoff alignment score (19) of 11 SD. The probability of such an alignment score occurring randomly is $<10^{-23}$. This degree of sequence similarity is sufficient to conclude that at least a portion of CENP-A is homologous to histone H3.

Despite such strong sequence homology in these fragments, the homologous relationship between CENP-A and histone H3 must be restricted to limited segments of each protein. Clearly nonhomologous regions in CENP-A include the 17-residue sequence of peptides D1 and D2, the 18-residue sequence of D4, and the 17-residue sequence deduced from peptides C2, C3, and E1. None of these sequences can be unambiguously aligned to H3 by either visual inspection or computer-assisted searches.

The mixed sequences of C6, a possible partial internal repeat, are also distinctive to CENP-A. Although interpretation of these sequences is difficult, histone H3 obviously does not contain a similar proline-rich region. Also of interest is the amino acid sequence EGEGTEGGEE, which comprises a portion of the D1/D2 sequence of CENP-A (Table 1). This decameric sequence contains an 8 of 10 match with

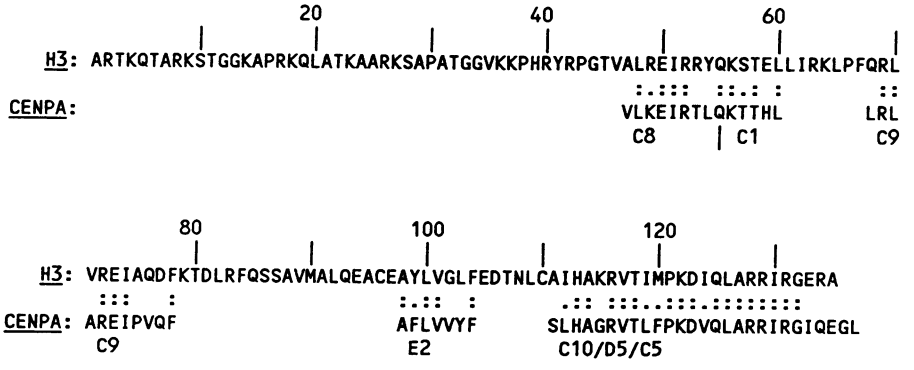

FIG. 5. Summary of the probable homologies between bull sperm CENP-A and bovine H3. Six chymotryptic peptides derived from CENP-A, which have ≥50% sequence identity, are aligned with the sequence of bovine histone H3 (21). Names of the peptides are given below their sequences. Identical residues are indicated by double dots; conservative substitutions are indicated by single dots. Alignment of segment C10/D5/C5 from CENP-A (Table 1) with the corresponding region of histone H3 was performed using the ALIGN program. Other peptides were aligned by visual inspection of the sequences.

residues 442–451 (EGEGEEEGEE) of mammalian α-tubulin (22) and a 7 of 9 match with residues 432–440 (GEGEELGEE) of the centromere-specific protein CENP-B (15). Within these segments, α-tubulin and CENP-B differ from CENP-A at two corresponding positions.

DISCUSSION

Its similar biochemical characteristics (13) and structural relationship with histone H3 demonstrate that CENP-A is a distinctive centromere-specific histone. Selected regions of histone H3 and CENP-A are homologous and must have evolved from a common progenitor. Nonhomologous regions of CENP-A may be presumed to engage in centromere-specific functions—perhaps involving recognition of centromeric DNA sequence, interaction with other centromere-associated proteins, and packing of centromeric chromatin into arrays characteristic of the fully formed centromere.

Interesting sequences specific to CENP-A include the distinctive proline-rich segment(s), representing a possible partial internal repeat (in chymotryptic fragment C6) and an acidic stretch (within the D1/D2 sequence). The latter region has homology to Glu-Gly regions in both α-tubulin (22) and in CENP-B (15). The homologous region in α-tubulin resides at the C terminus of α-tubulin, which is subject to unusual posttranslational covalent modifications, such as addition of a terminal tyrosyl residue (23) and addition of a polyglutamate side chain (24). This region is also apparently the specific recognition site for binding of various microtubule-associated proteins (25, 26). Significance of the similarity of two centromeric proteins to tubulin in this acidic stretch is unknown.

CREST scleroderma antisera routinely exhibit cross-reactive recognition of CENP-A and CENP-B antigens (7), suggesting sequences in common between these two proteins. With 70% of CENP-A (this publication) and >90% of CENP-B (15) reported, the only homologous region found thus far is the acidic stretch within the D1/D2 fragment. It is unlikely that cross-reactivity between CENP-A and CENP-B can be explained by this homology, as the antisera do not recognize α-tubulin that shares sequence homology in this region.

The presence of sequences specific to CENP-A demonstrate that the homologous relationship with histone H3 must be restricted to limited segments of each protein. Position of these H3-like sequences within the molecule is not yet known. The central globular domain of H3 is a highly conserved site of interaction with other histones to form octamers (for review, see ref. 27). We have determined that CENP-A is present in nucleosome-like structures in chromatin (13) and participates in apparent histone H3/H4 tetramers (ref. 13 and D.K.P., K.O., and R.L.M., unpublished data). Therefore, the essential elements from this conserved globular domain of H3 have probably been retained in CENP-A.

The identification of CENP-A as a centromere-specific core histone suggests that CENP-A has a direct role in centromeric chromatin packaging and function. Indirect immunofluorescence and immunoelectron microscopy suggest that one or more of the centromeric antigens are located either within or closely apposed to the kinetochores (9, 12, 28). Recent microinjection experiments (29, 30) have shown that at least one component among the centromeric antigens recognized by scleroderma sera is essential for kinetochore function in mitosis. The role of each of the individual centromeric proteins remains unknown. The information now at hand will make it possible to address whether CENP-A is essential to kinetochore function in mitosis.

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1. Gorbsky, G. J., Sammak, P. J. & Borisy, G. (1987) *J. Cell Biol.* **104**, 9–18.
2. Gorbsky, G. J., Sammak, P. J. & Borisy, G. (1988) *J. Cell Biol.* **106**, 1185–1192.
3. Koshland, D. E., Mitchison, T. J. & Kirschner, M. W. (1988) *Nature (London)* **331**, 499–504.
4. Nicklas, R. B. (1989) *J. Cell Biol.* **109**, 2245–2255.
5. Earnshaw, W. C., Halligan, N., Cooke, C. & Rothfield, N. (1984) *J. Cell Biol.* **98**, 352–357.
6. Guldner, H., Lakomek, H. J. & Bautz, F. A. (1984) *Clin. Exp. Immunol.* **58**, 13–20.
7. Earnshaw, W. C. & Rothfield, N. (1985) *Chromosoma* **91**, 313–321.
8. Valdivia, M. M. & Brinkley, B. R. (1985) *J. Cell Biol.* **101**, 1124–1134.
9. Kingwell, B. & Rattner, J. B. (1987) *Chromosoma* **95**, 403–407.
10. Hadlaczky, G., Praznovsky, T., Rasko, I. & Kereso, J. (1989) *Chromosoma* **97**, 282–288.
11. Bischoff, F. R., Maier, G., Tilz, G. & Ponstingl, H. (1990) *Proc. Natl. Acad. Sci. USA* **87**, 8617–8621.
12. Cooke, C. A., Bernat, R. L. & Earnshaw, W. C. (1990) *J. Cell Biol.* **110**, 1479–1488.
13. Palmer, D. K., O'Day, K., Wener, M. H., Andrews, B. S. & Margolis, R. L. (1987) *J. Cell Biol.* **104**, 805–815.
14. Palmer, D. K., O'Day, K. & Margolis, R. L. (1990) *Chromosoma* **100**, 32–36.
15. Earnshaw, W. C., Sullivan, K. F., Machlin, P. S., Cooke, C. A., Kaiser, D. A., Pollard, T. D., Rothfield, N. F. & Cleveland, D. W. (1987) *J. Cell Biol.* **104**, 817–829.
16. Gurley, L. R., Valdez, J. G., Prentice, D. A. & Spall, W. D. (1983) *Anal. Biochem.* **129**, 132–144.
17. Palmer, D. K., O'Day, K. & Margolis, R. L. (1989) *Prog. Clin. Biol. Res.* **318**, 61–72.
18. Friedman, M., Knull, L. H. & Carvins, J. F. (1970) *J. Biol. Chem.* **245**, 3868–3871.
19. Dayhoff, M. O., Barker, W. C. & Hunt, L. T. (1983) *Methods Enzymol.* **91**, 524–545.
20. Franklin, S. G. & Zweidler, A. (1977) *Nature (London)* **266**, 273–275.
21. DeLange, R. J., Hooper, J. A. & Smith, E. L. (1973) *J. Biol. Chem.* **248**, 3261–3274.
22. Valenzuela, P., Quiroga, M., Zaldivar, J., Rutter, W. J., Kirschner, M. W. & Cleveland, D. W. (1981) *Nature (London)* **289**, 650–655.
23. Thompson, W. C. (1982) *Methods Cell Biol.* **24**, 235–255.
24. Edde, B., Rossier, J., LeCaer, J.-P., Desbruyeres, E., Gros, F. & Denoulet, P. (1990) *Science* **247**, 83–85.
25. Serrano, L., Avila, J. & Maccioni, R. B. (1984) *Biochemistry* **23**, 4675–4681.
26. Serrano, L., Montejo de Garcini, E., Hernandez, M. A. & Avila, J. (1985) *Eur. J. Biochem.* **153**, 595–600.
27. van Holde, K. E. (1989) *Chromatin* (Springer, New York).
28. Brenner, S., Pepper, D., Berns, M. W., Tan, E. & Brinkley, B. R. (1981) *J. Cell Biol.* **91**, 95–102.
29. Simerly, C., Balczon, R., Brinkley, B. R. & Schatten, G. (1990) *J. Cell Biol.* **111**, 1491–1504.
30. Bernat, R. L., Borisy, G. G., Rothfield, N. & Earnshaw, W. C. (1990) *J. Cell Biol.* **111**, 1519–1533.