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Primary Structure of Histone H2B from Trout (*Salmo trutta*) Testes†

Arend Kootstra and George S. Bailey*

ABSTRACT: Large scale purification of histone H2B from trout (*Salmo trutta*) testes was achieved by a simple procedure consisting of extraction enrichment and chromatography on Bio-Gel P10 and Sephadex G-100, without the use of ion exchange chromatography. The complete amino acid sequence of histone H2B was deduced from automated sequence analysis and compositional analysis of intact H2B, cyanogen bromide, and *N*-bromosuccinimide fragments, thermolysin peptide TH 1, and maleylated and demaleylated tryptic fragments. Comparison of the primary structure of histone H2B from calf and trout revealed eight changes out of 125 residues. The calculated rate of change for H2B among vertebrates is 0.16 residue/100 residues per 10^7 years. All changes except one have occurred in the amino-terminal 37 residues of H2B, which is in striking contrast to the highly conserved sequence of this region in histones H2A, H3, and H4. An even more striking series of insertions, deletions, and substitutions is observed on comparison of the vertebrate sequences with the amino-terminal regions of H2B from two genera of sea urchins. In addition, H2B appears to have evolved among echinoderms at a rate approximately tenfold higher than among vertebrates. These results suggest that it is the core regions of histones which have the most critically conserved sequences, in line with the proposed role of these regions in forming the histone–histone contacts necessary for nucleosome formation. By contrast, the variability of the amino-terminal region of H2B suggests a more species-specific interaction with chromatin components, possibly in addition to, or other than, nucleosomal DNA.

Histones are well known to be intimately associated on an approximately equal weight basis with the DNA of all eukaryotes (for a recent review, see Felsenfeld, 1978). The structural basis for this interaction appears to be a stoichiometric association of two each of histones H2A, H2B, H3, and H4 per unit core length of DNA, the core particle containing approximately 140 base pairs of DNA (Kornberg, 1974; Olins & Olins, 1974; Van Holde et al., 1974; Shaw et al., 1976; Compton et al., 1976; Lohr et al., 1977). These core particles, or nucleosomes, are separated by a variable and less well-defined length of nuclease-accessible (Hewish & Burgoyne, 1973) DNA of 20–60 base pairs (Noel, 1976; Morris, 1976; Lohr et al., 1977; Thomas & Furber, 1976) which also appears to be the sight of DNA interaction with histone H1 (Kornberg & Thomas, 1974; Varshavsky et al., 1976). However, the precise details of histone:histone:DNA interaction, as well as the possible role(s) and relationships of the nucleosomal structure to such events as DNA replication (Weintraub, 1976; Seale, 1976), gene activation, and transcription (Piper et al., 1976; Reeves & Jones, 1976; Garel & Axel, 1976) and histone modification are at present not clearly established.

One useful approach toward an understanding of some of the above problems is the delineation through comparative sequence studies of crucial structural features among each class of histones. Indeed the range of evolutionary variability established among histones (Delange & Smith, 1971; Bailey & Dixon, 1973), and in particular the pair-wise conservatism of H3 and H4 formed part of the basis for the original nucleosome model (Kornberg, 1974). We wish to present here the complete primary sequence of histone H2B from trout. The results, when compared with calf H2B, show that H2B is less conservative than H3 or H4, and evolves among vertebrates at a rate identical with H2A. This would further strengthen the suggestion that H2A and H2B are complementary in their interaction

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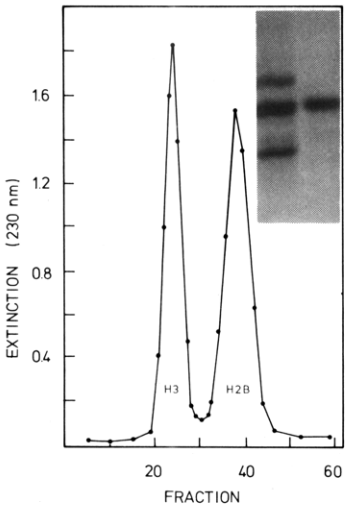

FIGURE 1: Chromatography of histone H2B and H3 on a Sephadex G-100 column (3.5 × 105 cm). The lyophilized histones were dissolved in 0.025 M sodium acetate, 0.25 M sodium bisulfite, pH 5.5, and eluted from the column with the above buffer, at a flow rate 75 mL per h. The effluent fractions (15 mL) were monitored at 230 nm. The first peak contained histone H3 and the second peak contained histone H2B. The photograph shows an electropherogram of the initial testis histone extract (left channel) and the final pool of purified H2B (right channel). Order of migration, top to bottom, is H3, H2B, H2A, and H4 (left gel; H1 migrates slowest toward the top of the gel and is not shown on this photo). The right channel shows, in addition to H2B, a trace of peptide appearing above histone H4 which may be a degradation product.

within the nucleosomal structure. In contrast to H2A, H3, and H4, however, H2B shows considerable variability of sequence in the amino-terminal region.

Experimental Procedure

Materials

Trypsin (Tos-PheCH_2Cl,[1] treated) and thermolysin were obtained from Sigma Chemical Co. Sephadex resins were obtained from Pharmacia Ltd. Bio-Gel P10 was obtained from Bio-Rad Laboratories. Quadrol and DMAA sequencer grade buffers were obtained from Beckman Instruments Inc. All other chemicals used in sequencing procedures were purified essentially according to Edman & Begg (1967). Polyamide sheets were purchased from Cheng Chin Trading Co. Ltd. S-PITC was obtained from Pierce. Testes were taken in February from trout caught by angling in Otago streams, transported on ice to the laboratory, and stored at −80 °C until needed.

Methods

Isolation and Purification of Histone H2B. Histone H2B was extracted from whole trout testes as described by method I of Johns (1964), except that the ethanol extraction step contained 0.1% β-mercaptoethanol. The impure histone H2B fraction (contaminated with H2A, H3, H4, and protamine) was separated from H2A, H4, and protamine by chromatography on a Bio-Gel P10 column (100–200 mesh, 4 × 240 cm) previously equilibrated with 0.01 M HCl (Sung & Dixon, 1970). The fractions containing histone H3 and H2B were pooled, concentrated on a PM10 membrane, and lyophilized.

TABLE I: Amino Acid Composition of Histone H2B from Brown Trout Testes.

Residue	Residues per mol[a]	Nearest integer	No. of residues in total sequence: Trout	No. of residues in total sequence: Calf
Lys	19.51 ± 0.99	20	19	20
His	2.96 ± 0.75	3	3	3
Arg	8.13 ± 0.31	8	8	8
Asp	5.28 ± 0.59	5	5	6
Thr	8.89 ± 0.71[b]	9	9	8
Ser	13.37 ± 0.63[b]	13	14	14
Glu	9.43 ± 0.56	9	9	10
Pro	4.74 ± 0.54	5	5	6
Gly	9.11 ± 1.57	9	9	7
Ala	12.06 ± 1.16	12	12	13
Val	7.73 ± 0.89	8	8	9
Met	2.04 ± 0.64	2	2	2
Ile	6.76 ± 0.45	7	7	6
Leu	6.05 ± 0.51	6	6	6
Tyr	4.71 ± 0.38	5	5	5
Phe	2.05 ± 0.18	2	2	2
			Σ123	Σ125

[a] Mean ± standard deviation from seven analyses. [b] Extrapolated to zero hydrolysis time.

Final purification of histone H2B to adequate homogeneity was achieved by chromatography on a Sephadex G-100 column (Figure 1) as described by Van der Westhuyzen & Von Holt (1971). The fractions containing histone H2B were pooled, dialyzed against 0.01 M HCl and lyophilized. Throughout the purification procedure the column effluents were subjected to starch gel electrophoresis (Sung & Smithies, 1969) before individual fractions were pooled. Examination of the preparation of H2B by polyacrylamide gel electrophoresis (Panyim & Chalkley, 1969), amino acid analysis, and quantitative N-terminal analysis by Edman degradation (Edman & Begg, 1967) failed to reveal the presence of significant contaminating proteins.

During the subsequent sequencing procedures no unaccountable peptides were isolated or observed, and the amino acid compositions of the intact preparation and of the final proposed sequence agree within experimental error. The amino acid composition of histone H2B is shown in Table I.

Maleylation and Tryptic Digestion. Histone H2B (100 mg) was dissolved in 10 mL of deionized water and cooled to 0 °C in an ice bath. The solution was adjusted to pH 9.0 with 0.1 M NaOH, and maleic anhydride (30-fold excess to amino groups) was added in small aliquots over a period of 1 h. The solution was maintained at pH 9.0 ± 0.5 with the aid of a pH stat. The reaction was considered complete after 4 h when no further uptake of base took place. The pH was then lowered to 8.5 and the maleylated histone H2B digested directly with trypsin (Tos-PheCH_2Cl treated) at 38 °C for 4 h at an enzyme–substrate ratio of 1:100 (w/w).

Purification of Maleylated Tryptic Fragments. Nine maleylated tryptic peptides (MT 1–9) from H2B were purified by the scheme summarized in Figure 2. After the first Sephadex step, all fractions were demaleylated[2] by incubation in 0.1 M pyridine acetate (pH 3.5) for 60 h at 40 °C, then 8 h

[1] Abbreviations used: Tos-PheCH_2Cl, L-1-tosylamido-2-phenylethyl chloromethyl ketone; DMAA, dimethylallylamine; S-PITC, 4-sulfophenyl isothiocyanate, sodium salt; NBS, *N*-bromosuccinimide; CNBr, cyanogen bromide; Pth, phenylthiohydantoin; CM, carboxymethyl.

[2] Rather harsh conditions appeared necessary for complete demaleylation. Although possible deamidation of asparaginyl and glutaminyl residues under these conditions was not quantitated, no indication of excessive deamidation was noted during subsequent isolation and sequencing of demaleylated peptides.

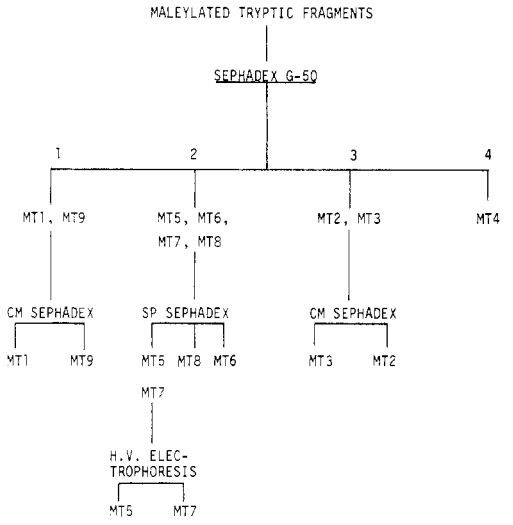

FIGURE 2: Summary of the purification scheme for tryptic peptides from maleylated H2B.

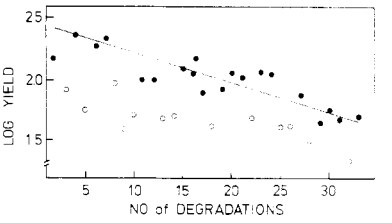

FIGURE 3: Automated degradation of intact H2B. The intact protein (7.8 mg) was treated with 4-sulfophenyl isothiocyanate and degraded as in Methods, using the single cleavage 1 M Quadrol program. Each residue was identified by amino acid analysis after hydrolysis of the phenylthiohydantoin in HCl or HI. All yields were corrected for mechanical loss, overlap, and hydrolysis efficiency (except proline and lysine) as described elsewhere (Bailey et al., 1977b). The regression line showing a 95% repetitive yield was determined excluding the values for Pth-proline and S-Pth-lysine (shown by open circles) which are known to recover poorly in this system. The run was terminated when nonoverlap background (not shown) approached the regression line.

at 60 °C. A 27-residue fragment, dMT9, was further digested by trypsin and four peptides (dMT9a-d) were isolated by high voltage electrophoresis at pH 5.0. The dMT9 peptide was also cleaved at its single tyrosine with *N*-bromosuccinimide (Ramachandran & Witkop, 1962) and two fragments (NBS 1, 2) were isolated by high voltage at pH 5.0.

Cyanogen Bromide Cleavage. Intact H2B (100 mg) was cleaved with CNBr as described by Gross (1962). Separation of three fragments (CNBr 1–3) was achieved by gel filtration (Sephadex G-50 medium, 2.5 × 110 cm in 0.1 M acetic acid) followed by ion exchange on CM-Sephadex using a linear gradient (0 to 1.0 M NaCl in 50 mM sodium acetate, pH 5.0, 250-mL reservoirs).

Cleavage by Thermolysin. Histone H2B (80 mg) was dissolved in 18 mL of 2 mM $CaCl_2$ (pH 7.0) at 37 °C. Thermolysin (1:250 w/v) was added and the solution was maintained at 37 °C and pH 7.0 for 3 h (Iwai et al., 1972). The fractionation of thermolysin peptides was achieved on a CM-Sephadex column (2.5 × 20 cm) using a linear gradient of NaCl (0 to 2.0 M, in 0.03 M sodium acetate, pH 5.0). The most basic peptides, which eluted last from the column, were pooled, desalted on a Sephadex G-10 column (2 × 30 cm), and lyophilized. Thermolysin peptide Th 1 was purified from this fraction by high voltage electrophoresis at pH 5.0. (No attempt was made to examine other thermolysin peptides as they were not needed for establishing the sequence of H2B.)

Amino Acid Analysis. Histone H2B was hydrolyzed in 6 M HCl at 110 °C under nitrogen and reduced pressure for 24, 48, and 72 h. Peptides were hydrolyzed for 24 h under the same conditions, except that the 6 M HCl contained 0.1% phenol. Amino acids were identified on a JOEL 3LC-6AH, or a modified Beckman 120 C (Bailey et al., 1977a) amino acid analyzer.

Sequencing Procedures. Proteins and peptides were subjected to automated Edman degradation using a Beckman 890C sequencer. The Fast protein Quadrol program (072172C) was used to degrade the intact protein. The 0.1 M Quadrol program (Brauer et al., 1975) and the peptide DMAA program (102974) were used to sequence peptides. In all programs the restricted vacuum times were doubled to facilitate initial removal of solvent and a single cleavage and extraction procedure was used (Bailey et al., 1977b).

Polypeptides containing lysyl residues were first coupled with S-PITC (Braunitzer et al., 1970) as described elsewhere (Bailey et al., 1977b).

Identification of Pth-Amino Acids. Pth-amino acids were analyzed by gas chromatography as described by Pisano & Bonzert (1969), by thin-layer chromatography (Summers et al., 1973) and by amino acid analysis after hydrolysis of the Pth-amino acids in 6 M HCl or 57% HI, as described by Smithies et al. (1970). All Glu and Asp residues were examined on thin layer for the presence of amides.

The acquisition of data from all sequencing runs was terminated either when the carboxyl residue was reached, or before the yield of nonoverlap background residues became sufficiently high as to preclude positive identification of the major residue. For simplicity of presentation, overlap background yields are given, but nonoverlap residues (always less than overlap) are omitted.

Results

Sequence Analysis of Intact H2B. Native histone H2B was dissolved in deionized water and dried in the sequencer cup. The protein film was subjected to one complete degradation cycle, using the fast protein Quadrol program without PITC, to precondition the protein film. The protein was then coupled to S-PITC, as described in Materials and Methods. During the first cycle only, a double cleavage and extraction procedure was used. Table II shows the yields obtained under various conditions, including a 34-cycle degradation of S-PITC treated H2B. Linear regression analysis of this degradation indicated a repetitive yield of 95% (Figure 3). Further attempts to achieve more extended degradations using varied buffer programs under standard sequencer configurations were unsuccessful. Characteristically we observed low recovery of lysyl residues and low extrapolated initial yield values when the S-PITC was used as initial coupling reagent. A similar observation was made by Bailey et al. (1977b).

Sequence Analyses of H2B Fragments. Procedures similar to those above were used to examine the sequences of an overlapping set of H2B fragments including the nine tryptic fragments of maleylated H2B, three cyanogen bromide fragments, and one thermolysin fragment. (The compositions of these peptides, the quantitative results from multiple sequencer degradations, and the arguments on which the proposed sequence is based are available as supplementary material; see paragraph concerning supplementary material at the end of this paper.)

The complete primary structure which we propose for histone H2B from brown trout (*Salmo trutta*) testes is shown in

TABLE II: Sequence Analysis of N-Terminal Region of Histone H2B.

Cycle	Residue identified	A Unmodified load = 510 nmol 1.0 M Quadrol Single coupling Yield[f]	A Overlap	B Unmodified load = 320 nmol 1.0 M Quadrol Double coupling Yield[f]	B Overlap	C Unmodified load = 700 nmol 1.0 M Quadrol Double coupling Yield[f]	C Overlap	D S-PITC treated[e] Load = 520 nmol 1.0 M Quadrol Double coupling Yield[f]	D Overlap
1	Pro	50.3		43.8					
2	Glu[a]	140.1	Pro 11.5		Pro 12.1	210.3		133.51	
3	Pro	50.5	Glu 13.0	45.1		109.1	Ala 21.3	71.96	Glu 29.2
4	Ala	165.0	Pro 20.3	149.6		241.8	Pro 19.0	201.07	Pro NQ
5	Lys	150.3	Ala 17.6		Ala 18.3	10.4	Ala 20.8	34.13	Ala 33.7
6	Ser[b]							151.85	Lys 25.8
7	Ala	136.3		110.1		207.3		166.37	Ser 21.9
8	Pro	12.6	Ala 23.8	8.9	Ala 12.3	71.9	Ala 34.1	73.79	Ala 44.9
9	Lys	130.1	Pro 19.8			72.2		28.75	Pro NQ
10	Lys	145.0				83.5		36.79	
11	Gly	53.4		41.3		101.0		73.88	Lys 30.4
12	Ser[b]							68.65	Gly 16.3
13	Lys	34.2				43.0		32.28	Ser 26.1
14	Lys	39.9				49.8		34.46	
15	Ala	16.2		34.8		81.3		82.52	Lys 28.2
16	Val			23.2		71.3	Ala 12.9	72.03	Ala 38.6
17	Thr[c]						Val 11.0	48.46	Val 43.8
18	Lys					29.9		25.11	Thr 37.9
19	Thr[c]							50.36	Lys 16.9
20	Ala			9.3		35.5		68.34	Thr 28.5
21	Gly					10.9		59.66	Ala 44.7
22	Lys					6.4		28.21	Gly 41.4
23	Gly							66.19	Lys 23.6
24	Gly							69.81	
25	Lys							22.56	Gly 37.9
26	Lys							24.49	
27	Arg[d]							39.49	Lys 23.3
28	Lys							16.63	Arg 24.9
29	Arg[d]							22.32	Lys 18.2
30	Ser[b]							28.39	Arg 22.6
31	Arg[d]							23.72	
32	Lys							10.68	Arg 16.2
33	Glx							24.60	Lys 15.9
34	Ser[b]							10.52	Glu 28.4

[a] Confirmed by thin-layer chromatography. [b] Isolated as alanine after HI hydrolysis. [c] Isolated as α-aminobutyric acid after HI hydrolysis. [d] Isolated from the aqueous phase. [e] Coupled for 40 min at 56 °C. [f] Yield in nanomoles.

Figure 4. A partial sequence of the first 22 residues of H2B from rainbow trout (*Salmo gairdneri*) has been previously published (Candido et al., 1972). Those residues which were positively identified are identical with the brown trout sequence, though threonine 19 might be substituted by serine in rainbow trout.

A preliminary communication of this sequence has been presented elsewhere (Kootstra & Bailey, 1976).

Discussion

Histone Purification and Sequencing. The simple procedure for histone H2B purification reported here involves selective extraction enrichment (Johns, 1964), removal of contaminants except H3 on Bio-Gel P-10 (Sung & Dixon, 1970), and separation of H2B and H3 on Sephadex G-100. This procedure permits the purification of large quantities of H2B (and H3) in a short time, without the use of ion-exchange chromatography.

The primary sequence of H2B was determined entirely using the protein sequenator, by degradation of the intact protein and a combination of overlapping fragments. Quantitation at each cycle, including background and overlap yields (Smithies et al., 1970; Bailey et al., 1977b), provides a set of confidence limits on which the proposed sequence is based. For example, the availability of quantitative overlap information (Bailey et al., 1977b) is sometimes necessary in order for repeated sequences (e.g., Lys-Lys, Leu-Leu-Leu) to be confidently established. Similarly, greater confidence can be placed on a region of sequence which has been determined more than one time. In establishing the sequence reported here, intact H2B was degraded in five separate experiments. This information, together with the fragments CNBrI (degraded three times), MT2, MT3, and MT4 (degraded twice), means that each of the first 50 residues in H2B was identified and placed in sequence on the basis of 4 to 15 independent quantitative observations. The only peptides from H2B which were degraded but once were three small fragments of 3–7 residues (CNBr 2, MT6 and MT7). Because of their unique compositions, the low background observed during sequencing runs of such short duration, and the secondary confirmation of certain residues through overlap, we consider the probability of error in these regions to be very low.

Misidentification of a residue is most likely to occur toward the end of long degradations, where yields are low and nonoverlap background high, or through regions of repeating residues. Hence, the particularly difficult sequence of residues 20–29 was confirmed by isolation and sequencing of the thermolysin peptide Th 1.

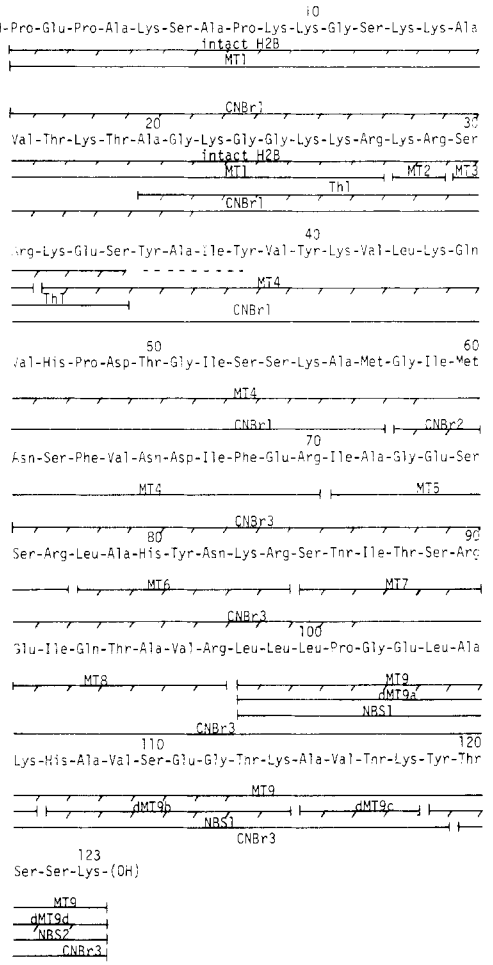

FIGURE 4: Summary of the sequence of trout histone H2B, as deduced from the sequencing of overlapping peptides.

Histone/DNA Interactions. The sequences of histone H2B from trout and calf are compared in Figure 5, together with DNA-derived and direct protein sequences of H2B from two genera of sea urchins. The most striking feature is that H2B, in contrast to histones H2A, H3, and H4, shows remarkable variability in the amino-terminal region. These variations are particularly surprising in view of the accumulating evidence that the amino-terminal regions of the core histones provide sites for interaction with nucleosomal DNA (Li & Bonner, 1971; Adler et al., 1974; Weintraub, 1975). The variations in sequence observed within the first 50 alignment positions (Figure 5) of H2B do not appear to fit into any readily apparent pattern of histone/DNA interaction which would be common to all species. For example, molecular models (not shown) demonstrate that, despite the sequence differences, the regularly spaced basic residues in region 35–50 (Figure 5) of calf and trout H2B can both readily fit into the major groove of a DNA double helix with maximal phosphate interaction, even in as conformationally restrictive a model as an α-helical arrangement. However, a similar arrangement of this region of the sea urchin sequences shows only a partial fit. By contrast, it is the first 25 residues of the sea urchin sequences which show the greatest regularity of spacing of basic residues (e.g., Lys-4, Lys-8, Lys-13, Lys-19, Arg-23) but much of this region is deleted in the vertebrate sequences. It therefore appears that the amino-terminal region of this histone has much more conformational freedom or species specificity in its mode of interaction in chromatin than is apparent for histones H2A, H3, and H4 (Delange & Smith, 1971; Sung & Dixon, 1970; Bailey & Dixon, 1973).

Comparison of the H2B sequences in Figure 5 also reveals that, beyond the variable amino terminal region, all sequences possess a short intensely basic region of 7–8 residues (alignment positions 50–57), followed by central and carboxyl regions which are generally highly conserved. These results might therefore suggest that, for histone H2B, the exact mode of histone/histone interaction within the nucleosome core may be a more critically conserved feature than is the mode of interactions with DNA. Considerable support for this suggestion derives from recent studies on histone reassociations in vitro, which show a remarkable conservation of interaction parameters between H2A and H2B, even between combinations of plant and vertebrate histones (Spiker & Isenberg, 1977, 1978; Isenberg, 1978). By contrast, any interaction between H2B and DNA which involves the first 30–50 residues must be less tightly specified. An attractive alternative is that this region of H2B might be involved in higher order, extranucleosomal interactions with chromatin components other than, or in addition to, DNA. The very highly basic sequence of 7–8 residues

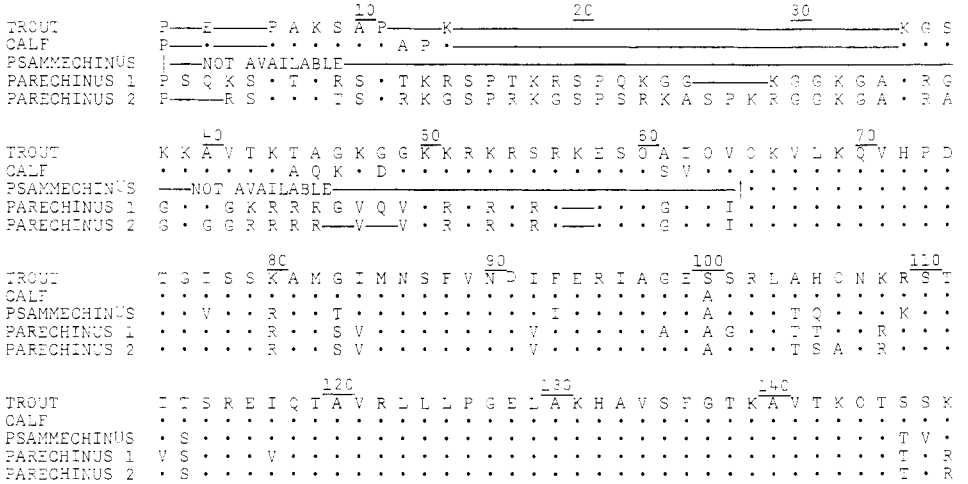

FIGURE 5: Comparison of histone H2B in trout, calf, and the sea urchins *Psammechinus* and *Parechinus*. The calf sequence is taken from Iwai et al. (1972). The sea urchin sequences are taken from Birnstiel et al. (1977), where the *Psammechinus* sequence is deduced by sequencing of cloned histone DNA, and from Strickland et al. (1977a,b), where the *Parechinus* sequence is provided by direct protein sequencing. The alignment of the vertebrate and sea urchin sequences is based on that suggested by Strickland et al. (1977a,b). *Parechinus* 1 and 2 are H2B tissue-specific variants from the same species; the significance of these variants is unclear.

would thus serve as a conserved hinge region which separates the more variable amino-terminal function from the conservative core region of H2B. Recent data on the composition of H2B tryptic peptides from pea (Hayashi et al., 1977), though as yet incomplete, suggest that the pattern of distribution of variable and conserved regions of histone H2B extends to plants as well as animals.

The Variable Rate of Histone H2B Evolution. Considering each of the differences between trout and calf H2B as a single mutational event, the rate of change of H2B among vertebrates is 0.16 residues/100 residues per 10^7 years (Kootstra & Bailey, 1976). This rate is identical with that shown by vertebrate histone H2A (Bailey & Dixon, 1973) and offers further evidence for the mutual interaction of these two histones within the nucleosomal structure (Kornberg, 1974). Further, as noted above, the central and carboxyl regions of trout and calf H2B are essentially identical in sequence, which probably reflects a high degree of conservatism in H2B folding and histone/histone contact within the nucleosomal structure among vertebrates. In contrast, however, the two genera of sea urchin for which DNA and histone H2B sequence information is available show enhanced variation throughout the central and carboxyl regions. Though many of the changes are normally considered conservative, some are not. It is surprising that the changes among the above species at residues 105 (His, Gln, and Thr/Ser), 106 (Tyr, Ala), and 147 (Ser, Val) can function as alternatives (see Figure 5) in an otherwise remarkably conservative core region.

Whether or not all such changes represent truly functionally equivalent states (neutral substitutions) among these species,[3] it is unexpected that the observed rate of evolutionary change for all substitutions in this region of H2B should be so conservative among vertebrates yet so variable within a family of arthropods. For residues 42–125 (Figure 4), trout and calf differ by 1 residue in 4×10^8 years of divergence (Kootstra & Bailey, 1976). By comparison the two sea urchins, both classified as Echinidae (Moore, 1966), differ over the same sequence region by 12 amino acid changes, comparing *Psammechinus* with the *Parechinus* 2 sequence. Yet the earliest trace of Echinidae in the fossil record is Meiocene (2×10^7 years), and the divergence of irregular sea urchins from regular ones which include Echinidae is dated maximally at 1.5×10^8 years. Even taking the maximum possible divergence times for these two sea urchins as beginning with the earliest traces of Echinodermata in the late Ordovician ($4.5\text{–}5.5 \times 10^8$ years), their rate of H2B divergence is still an order of magnitude greater than that shown between H2B in trout testes and calf thymus. The reason for this discrepancy is unclear. Birnstiel et al. (1977) have suggested that sea urchin sperm H2B histones may represent unusual specialized tissue variants. Hence these protein (and DNA) sequences may not correspond to the major nucleosomal form for H2B from somatic tissues. They also differ strikingly from vertebrate H2B in having an extra 17 residues on the amino terminus and this may affect the variability of the rest of the protein.

Acknowledgment

We wish to thank Dr. I. Isenberg for helpful discussion on histone interactions and evolution.

[3] Among these species of H2B, where several substitutions exist, it is of course incorrect to consider any single substitution in isolation as to its possible effect. The total allelic form of the protein is the functioning unit and this in turn must be influenced by the other histone species with which H2B interacts. Ultimately, x-ray crystallographic structures (Finch et al., 1977) may clarify the possible conformational effects of any substitution.

Supplementary Material Available

A complete description of the amino acid compositions and sequence determinations of all peptides, together with the arguments on which the proposed sequence is based (10 pages). Ordering information is given on any current masthead page.

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Phosphate Transport in Yeast Mitochondria: Purification and Characterization of a Mitoribosomal Synthesis Dependent Proteolipid Showing a High Affinity for Phosphate†

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ABSTRACT: It is possible to obtain from yeast mitochondria a proteolipid able to bind phosphate, by two different procedures. One of them, generally used for lipid extraction, leads to the preparation of a more active crude proteolipid. This crude proteolipid has been purified by various chromatographic procedures and the active fraction, in phosphate binding, is always associated with cardiolipin. Its molecular weight seems to be close to 10 000. The phosphate binding shows ligand saturation behavior and is inhibited by arsenate and *N*-ethylmaleimide; succinate is noninhibitory. This protein seems to be dependent on the mitoribosomal synthesis since it is not present in mitochondria of mutant "petite colonie" and its amount largely decreases in mitochondria from yeast grown in the presence of chloramphenicol. It is possible to extract a proteolipid from the oligomycin sensitive ATPase, showing the same activity and properties. The hypothesis that this proteolipid acts as a part of the P_i carrier and constitutes the oligomycin-sensitive ATPase complex is discussed.

Recently the effects of some physiological or genetic manipulations on yeast mitochondria were studied with regard to phosphate transport (Arselin de Chateaubodeau et al., 1976; Rigoulet et al., 1977). It was shown that the phosphate carrier assembly was under the dual dependence of the cytosolic and the mitochondrial protein synthesis. For instance, saturation kinetics for the phosphate transport was detected only in mitochondria isolated from cells in which the mitochondrial protein synthesis was functional.

Products of the mitoribosomal protein synthesis are known to have a high degree of hydrophobicity and to be extracted only with detergent or organic solvents (Tzagoloff et al., 1973; Kadenbach & Hadvary, 1973a; Kuela et al., 1975; Brambl and Handshin, 1976). Several studies have been reported in which these mitochondrial proteins, defined as proteolipid (Folch-Pi & Lees, 1971), were extracted with mixtures of chloroform–methanol. Some of these products are well defined and characterized: the DCCD binding protein (Cattell et al., 1971; Stekhoven et al., 1972); a subunit of the oligomycin-sensitive ATPase (Tzagoloff et al., 1973); a low molecular weight proteolipid in rat liver mitochondria (Dianoux et al., 1976). Particularly, Kadenbach & Hadvary (1973a) have shown that a chloroform extract of rat liver mitochondria was able to bind specifically phosphate.

Studies described in this paper were initiated to gain better understanding of this proteolipid in yeast mitochondria. Purification procedures, some properties, and the origin of the proteolipid able to bind phosphate are investigated. The possibility that this proteolipid could be a part of the phosphate carrier system and/or of the OSATPase is discussed. Preliminary data of these studies have already been reported (Napias & Guérin, 1976; Guérin et al., 1977).

Experimental Procedures

Materials

Saccharomyces cerevisiae, yeast foam diploid wild strain, is grown aerobically on a 2% galactose medium, supplemented

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0006-2960/78/0417-2510$01.00/0