NOTES

Histone-Like Proteins of the Dinoflagellate *Crypthecodinium cohnii* Have Homologies to Bacterial DNA-Binding Proteins

J. T. Y. Wong,* D. C. New,† J. C. W. Wong, and V. K. L. Hung

*Department of Biology, Hong Kong University of Science and Technology,
Kowloon, Hong Kong, People's Republic of China*

The dinoflagellates have very large genomes encoded in permanently condensed and histoneless chromosomes. Sequence alignment identified significant similarity between the dinoflagellate chromosomal histone-like proteins of *Crypthecodinium cohnii* (HCCs) and the bacterial DNA-binding and the eukaryotic histone H1 proteins. Phylogenetic analysis also supports the origin of the HCCs from histone-like proteins of bacteria.

The evolutionary development of histones and nucleosomes led to more efficient packaging of DNA into structured chromosomes, which was probably a key step in the evolution of eukaryotes from prokaryotes (13). The dinoflagellates represent an exception to this histone-based increase of genome size among eukaryotes (2). The dinoflagellate genome ranges from 2 to 3 pg (similar to the haploid human genome) to 200 pg (18), but ultrastructural and biochemical studies of the dinoflagellate chromatin identified an absence of nucleosome structures (3, 9) and histone proteins (17). Even more surprisingly, despite the lack of histones the dinoflagellate chromosomes are permanently condensed throughout interphase and have been proposed to exist in a liquid crystal state (4). Proteins associated with DNA have been isolated and characterized as predominantly low-molecular-mass proteins (14 to 17 kDa) rich in basic amino acids (17). Six chromosomal proteins were identified that represented only 10% of the DNA mass (25), a ratio even smaller than that of the prokaryotes. Immunoscreening of a cDNA expression library constructed from mRNA isolated from *Crypthecodinium cohnii* identified two clones (HCC1 and HCC2) with high similarity to the previously purified proteins. These clones confirmed the small size and basic nature of the proteins and allowed localization of the encoded proteins at the periphery of the permanently condensed chromosomes (19). Database searches at the time found no convincing homology to the histones and other chromosomal proteins. The ciliates and the apicomplexans, which together with the dinoflagellates form the alveolates, possess chromosomal proteins similar to histones of other eukaryotes (16), which generates an intriguing question as to the origin of the dinoflagellate histone-like proteins of *C. cohnii* (HCCs). A thorough investigation of the relationship between dinoflagellate HCCs

and other prokaryotic and eukaryotic chromosomal binding proteins is necessary to facilitate a more complete understanding of the evolution of histones.

Accordingly, we designed two PCR primers against the 5′ and 3′ ends of the previously identified clones HCC1 and HCC2: pHCC1 sense, 5′-TATGGATCCATGGCCCCCAAG ATG-3′, and antisense, 5′-TATAAGCTTGAAGTCTGTCTT GAGGGC-3′. Reverse transcription-PCR with mRNA isolated from *C. cohnii* amplified a number of DNA fragments of the expected size. Subsequent sequencing of these fragments allowed identification of two clones, HCC3 and HCC4, with homology to HCC1-HCC2 (Fig. 1A). The alignment of all four HCC variants enabled the proteins to be separated into two regions (Fig. 1A). The first 40 to 45 amino acids of each protein are highly variable apart from a region of 10 amino acids that is 100% conserved among HCC1, -2, and -4. Conversely, the final 70 to 75 amino acids of all four variants are highly homologous, with 64% absolute identity and 77% similarity when conservative substitutions are considered. HLP, an HCC-like gene product isolated by PCR from the dinoflagellate *Lingulodinium polyedrum* (6), has a high degree of homology to the HCCs (Fig. 1B).

Our database searches with BLASTX revealed similarity to a number of bacterial DNA-binding proteins, especially the bacterial HUs (Fig. 1A). HUs are the most abundant small molecular multifunctional proteins in the *Escherichia coli* nucleoid (10). With the help of the software program ClustalX, we aligned the putative amino acid sequences of the HCCs with five randomly selected HUs (Fig. 1A). HUs are polypeptides of about 100 amino acids, while the HCCs are about 110 amino acids in length. However, since our PCR strategy was based on the amino and carboxyl termini of HCC1 and HCC2, we cannot completely rule out the possibility of extra sequences at the C terminus. Similarly, since the amino-terminal region of the protein contains a number of methionine residues, further library construction and screening as well as mRNA extension experiments will be required to determine whether the isolated genes encode full-length proteins. Given this, compared with the HUs, the HCCs have an extra amino-

* Corresponding author. Mailing address: Department of Biology, Hong Kong University of Science and Technology, Kowloon, Hong Kong, People's Republic of China. Phone: 852-2358-7343. Fax: 852-2358-1559. E-mail: Botin@ust.hk.
† Present address: Department of Biochemistry and the Biotechnology Research Institute, Hong Kong University of Science and Technology, Kowloon, Hong Kong, People's Republic of China.

A

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HCC1  ----------MAPKMKAAMKAMKAPAM-KGKAMTKTGLAEAWRRRPSFLRRTAGQSWRAWPRSCAAEVKKTGKLTIPGL  68
HCC2  ------------------MKAMKA----TKKAMTKTGLAEALAPKPSSARRIAPPSSRAWPPS-AQEVKKTGKLIIPGL  56
HCC3  ---------MAPKMKAAMKAKAMKAR--SVAMSKGALCQAIADATENKKSAIVKFMDALAEVVTAEVKKTGKMTIPGV  67
HCC4  ---MAPKMKAAMKAAMKAPMKAMKATAMKGGKAMTKTGLAEALANATEVSKKDCASIIESLATICATEVKKTGKLTIPGL  77
HU-Sp ---------------------------MANKQDLIAKVAEATELTKKDSAAAVEAVFAAVADYLAAGEKVQLIGF  48
HU-Hi ----------------------------MNKTDLIDAIANAAELNKKQAKAALEATLDAITASLKEGEPVQLIGF  47
HU-St ----------------------------MNKSQLIEKIAAGADISKAAAGRALDAIIASVTESLKEGDDVALVGF  47
HU-Dr MTKKSTKAPAKKAAPAAKAAPAAKRGAAADSGKVAKTQLVEMVADQTGLTKKQSEEAISSMLECVVSALQSGKSVGLPGL  80
HU-Pa -----------------------MALTKDQLIQDIAEAIDAQKTTVRSALDQLAEIVKDALENDGEITLPGI  49
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HCC1  VMVKTRKKPATKAGKREMFGKVVLVKAS-CQDVVKAYPVKALKTDF  113
HCC2  VMVKTRKKPATKAGKREMFGKVVLVKAQPAKTVVKAYPVKALKDNF  102
HCC3  TMIKTRKKPATKAGKREMFGKVVLVKAQPAKTVVKAFPVKALKTDF  113
HCC4  VMVKTRKKPATKAGKREMFGKVVLVKAQPAKTVVKAYPVKALKTDF  123
HU-Sp GNFEVRERAE-RKGRNPQTGKEMTIAASKVPAFKAGKALKDAVK--  91
HU-Hi GTFKVNERAA-RTGRNPQTGAEIQIAASKVPAFVSGKALKDAIK--  90
HU-St GTFAVKERAA-RTGRNPQTGKEITIAAAKVPSFRAGKALKDAVN--  90
HU-Dr GTLSVKETAA-RTGVRPGTSEKIQIPAGKKVAFKVASTLKGNL---  122
HU-Pa GKLKVSERPA-RTGRNPQTGKAIEIAAAKRVAKFVPAKALTDAING-  93
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B

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HCC1  MAPKMKAA--------MKAMKAPAM------------------KGKAMTKTGLAEAWRRRPSFLRRTAGQSWRAWPRSCA  54
HCC2  ---------------MKAMKA----------------------TKKAMTKTGLAEALAPKPSSARRIAPPSSRAWPPS-A  42
HCC3  MAPKMKAAMK------AKAMKA----------------------RSVAMSKGALCQAIADATENKKSAIVKFMDALAEVVT  53
HCC4  MAPKMKAAMKAAMKAPMKAMKATAMK-----------------GGKAMTKTGLAEALANATEVSKKDCASIIESLATICA  63
HLp   --------------MAPMKAA----------------------AKKAMTKGAIADALATQFELKKTVCGKLINSLAEIAT  44
HAf   -----------------------------------MSPSATRSRTRTAGASRRTRLRTSSARG-SARAPVAT  36
BpH2  MATKAKAPAKKVTKTAAKTPAKAPAKKAPAKPAVKKTAAAVRPIKEALNKSQLIAYLVENTGVEAKSVKAVLAGLESSVL  80
XHLP  MAKIAKN---------AKAAPKKAVKKQTAKPG------TIKPIKETLSKSALVAHLAESSALAAKDVRTLLGALENTIH  65
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HCC1  AEVKKT--GKLTIPGLVMVKTRKKPAT--KAGKREMFGKVVLVKAS-CQDVVKAYPVKALKTDF-  113
HCC2  QEVKKT--GKLIIPGLVMVKTRKKPAT--KAGKREMFGKVVLVKAQPAKTVVKAYPVKALKDNF-  102
HCC3  AEVKKT--GKMTIPGVTMIKTRKKPAT--KAGKREMFGKVVLVKAQPAKTVVKAFPVKALKTDF-  113
HCC4  TEVKKT--GKLTIPGLVMVKTRKKPAT--KAGKREMFGKVVLVKAQPAKTVVKAYPVKALKTDF-  123
HLp   KEVKSS--GVFTIPGVCKIKTRTKPAT--KAGKREIFGKTVVKAKPARKVVKAFPVAALKKSI-  104
HAf   AEVKKL--GVFTVPGPYNIKLREKPAT--QASKWVCLRVAW----------HVFPLKACKLC-  85
BpH2  ASVDKKGAGEFTLPGLFKVAVQKVPAKAKRFGKDPFTGEERWFPAKPASVKVKVRPLKKLKDAAQ  145
XHLP  ASLHKKGVGSFTLPGLLKISTVNVPAKPKRKGINPFTKEEQWFAKPASVKIKTRPLKKLKDATL  130
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C

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HCC1   18 KGKAMTKTGLAEAWRRRPSFLRRTAGQSWRAWPRSCAAEVKKTGKLTIPGLVMVKTRK--------------------  75
HCC2    7 TKKAMTKTGLAEALAPKPSSARRIAPPSSRAWPPS-AQEVKKTGKLIIPGLVMVKTRK--------------------  63
HCC3   17 RSVAMSKGALCQAIADATENKKSAIVKFMDALAEVVTAEVKKTGKMTIPGVTMIKTRK--------------------  74
HCC4   27 GGKAMTKTGLAEALANATEVSKKDCASIIESLATICATEVKKTGKLTIPGLVMVKTRK--------------------  84
H1-Ce  79 QINAHLRQALKRGVTSKALVQAAGSGANGRFRVPEKAAAAKKPAAAKKPAAAKKPAAA------KKATGE--------  142
H1-Gg  76 KNNSRIKLGLKSLVSKGTLVQTGTGASGSFKLNKKPGETKAKATKKKP-AAKPKKPA--AKKPAAAAKKPKKAAAVKKS  152
H1-Mm  75 KNNSRIKLGLKSLVSKGTLVQTKGTGASGSFKLNKKAASGEAKPKAKRAGAAKAKKPAGAAKKPKKAAGTATAKKSTKKT  154
H1'-Hs 63 NADSQIKLSIKRLVTTGVLKQTKGVGASGSFRLAK--SDEPKKSVAFKKTKKEIKKVA----TPKKASKPKKAAS-----  131
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HCC1   -----KPATKAGKREMFGKVVLVKAS-CQDVVK--AYPVKALKTDF--------------------  113
HCC2   -----KPATKAGKREMFGKVVLVKAQPAKTVVK---AYPVKALKDNF--------------------  102
HCC3   -----KPATKAGKREMFGKVVLVKAQPAKTVVK---AFPVKALKTDF--------------------  113
HCC4   -----KPATKAGKREMFGKVVLVKAQPAKTVVK---AYPVKALKTDF--------------------  123
H1-Ce  -KKAKKPAAAKPKKAATGDKKVKKAKSPKKAKSPAKKAAPKKIAKPAAKKAAKPAAKA---------  208
H1-Gg  PKKAKKPAAAATKKAAKSPKKATKAGRPKKTAK--SPAKAKAVKPKAAKSKAAKPKAAKAKKAATKKK  218
H1-Mm  PKKAKKPAAAGAKKAKSPKK-AKATKAKKAPK--SPAKATVKPKAAKPKTSKPKAAKPKKTAAKKK  219
H1'-Hs KAPTKKPKATPVKKAKKKLAATPKKAKKPKTVK--AKP-VKASKPKKAKP--VKPKAKSSAKRAGKKK  194
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FIG. 1. Alignment of HCCs with HUs, histone-like proteins, and histone H1. (A) Multiple alignments of HCCs and selective bacterial HUs: HCC1 (A56581), HCC2 (B56581), HCC3 (AY128510), and HCC4 (AY128511); HU-Pa (*Pseudomonas aeruginosa*, JC4061); HU-Dr (*Deinococcus radiodurans*, Q9RZ89); HU-St (*Salmonella enterica* serovar Typhimurium, S01732); HU-Hi (*Haemophilus influenzae*, P43722); and HU-Sp (*Streptococcus pneumoniae*, AAK75224). (B) Multiple alignments of dinoflagellate and bacterial histone-like proteins: HLP (*L. polyedrum*, AF482694), HAF (*A. fundyense*, AAD20317), BpH2 (*B. pertussis*, AAB40156), and XHLP (*X. fastidiosa* 9a5c, AAF84745). (C) Multiple alignments of HCCs and selective eukaryotic histone H1 proteins: H1-Ce (*Caenorhabditis elegans*, NP_510410), H1-Gg (*Gallus gallus*, P09987), H1'-Hs (*Homo sapiens*, XP_009973), and H1-Mm (*Mus musculus*, AAA37814). Asterisks indicate positions which have a single, fully conserved residue; colons indicate that one of the "strong" groups in parentheses is fully conserved (STA/NEQK/NHQK/NDEQ/QHRK/MILV/MILF/HY/FYW); periods indicate that one of the "weaker" groups in parentheses is fully conserved (CSA/ATV/SAG/STNK/STPA/SGND/SNDEQK/NDEQHK/NEQHRK/FVLIM/HFY).
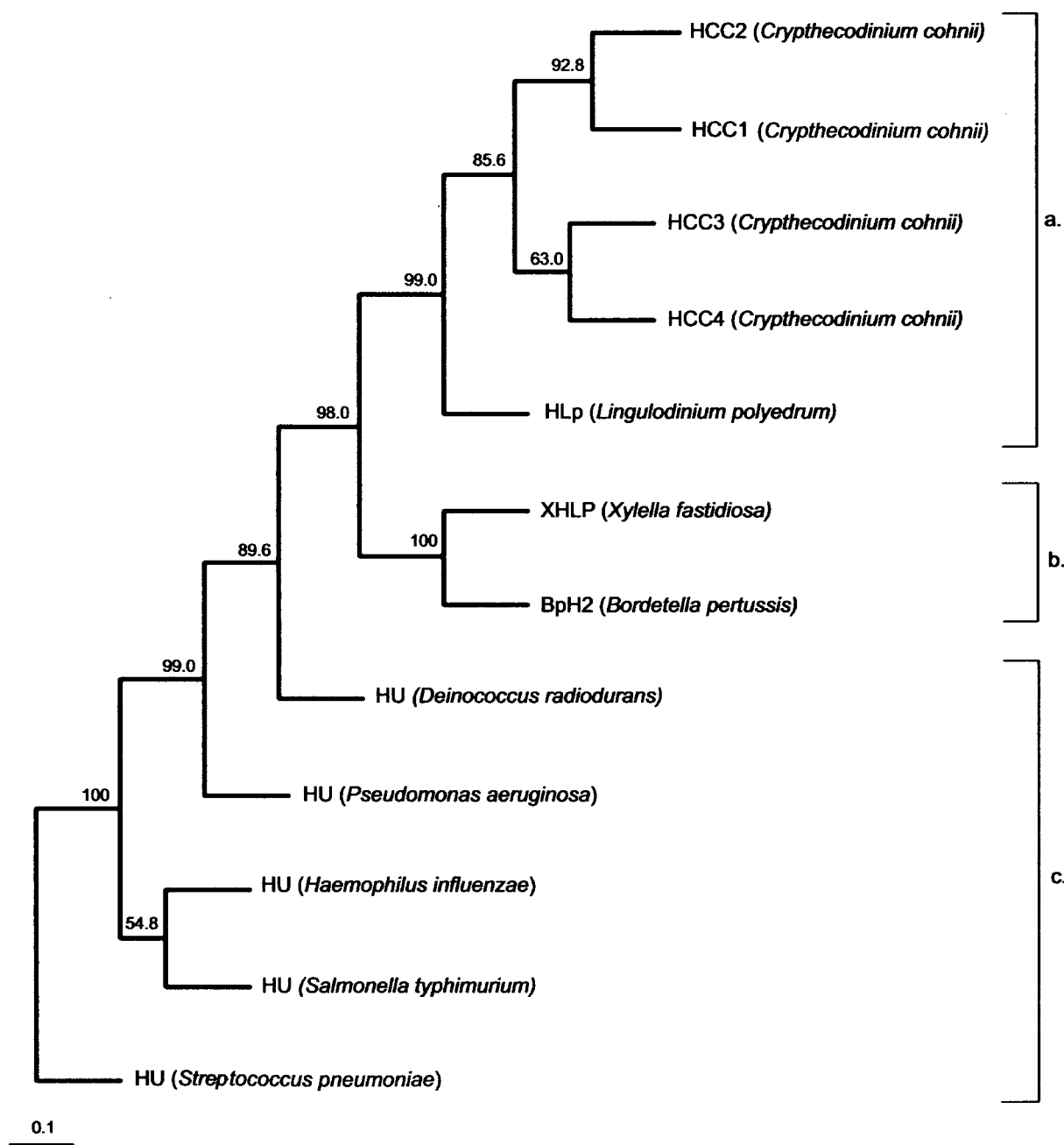
FIG. 2. A phylogenetic tree (UPGMA) inferred from the multiple alignments of amino acid sequences of histone-like proteins from dinoflagellate HCCs (a), bacterial histone-like protein family (b), and bacterial DNA-binding HU proteins (c) was constructed with PHYLIP version 3.5. The numbers at the nodes represent the bootstrap percentages by creation of 500 replicated data sets with SEQBOOT. The accession number for each protein sequence is as follows: HCC1 (*C. cohnii*), A56581; HCC2 (*C. cohnii*), B56581; BpH2 (*B. pertussis*), AAB40156; XHLP (*X. fastidiosa*), AAF84745; HU (*Salmonella enterica* serovar Typhimurium), S01732; HU (*Streptococcus pneumoniae*), AAK75224; HU (*Haemophilus influenzae*), P43722; HU (*Pseudomonas aeruginosa*), JC4061; HU (*Deinococcus radiodurans*), Q9RZ89.

terminal sequence of about 20 to 30 amino acids in which the peptide sequence MAPK appears frequently. The highly variable nature of the amino termini suggests that these proteins are not redundant in their functions. A recent study (11) identified regions of similarity between the basic regions of the sea urchin H1b protein and the HCC2 protein. We have also been able to identify similar regions of homology between histone H1 proteins and HCCs (Fig. 1C). There is an approximately

10% identity between the two groups of proteins, predominantly in C-terminal positions occupied by basic residues. Some of these residues are also conserved between HCCs and HUs (Fig. 1A). The repeated occurrence of these residues has been postulated previously to result in a proline-kinked AK α-helix organization (7). Prototypical examples of this structure in histones of animals confer the ability to bind to DNA and to facilitate chromatin folding and condensation (22). One

of the basic residues, K-107 of HCC1, is conserved in all eukaryotic H1 proteins and bacterial HUs analyzed.

The extent of similarity (23%) between HCCs and HUs is not restricted to the basic residues at the C terminus, as is found in the histones. Three glycine residues are identical in all HUs and HCCs but not in the H1 histones. One of these glycine residues (Gly-66 in HUs) lies within the DNA-binding β-ribbon arms of the dimeric HUs (23) and is located at the arm's flexible tip, which has been postulated to encircle the DNA (26).

Interestingly, histone H1-like sequences from *Bordetella pertussis* (BpH2) and *Xylella fastidiosa* (XHLP) were identified during the search for HCC homologues. Sequence comparison reveals that BpH2-XHLP has more than 35% sequence homology with all HCCs. Similar to sequence identities among the HCCs themselves, the C-terminal 65 amino acids show the highest identity with BpH2-XHLP (over 50%), including the three glycines. Based on sequence similarities, BpH2 and XHLP are more closely related to the HCCs, and together they form a group that is intermediate between the histone H1 proteins and the bacterial HUs. HCCs have higher homology to BpH2-XHLP than to HAF, the other histone-like protein from the dinoflagellate *Alexandrium fundyense* (Fig. 1C). The genomes of *B. pertussis* and the intercellular bacterium *Chlamydia trachomatis* contain other histone H1-like gene products, BpH1 and Hc1, respectively (8, 21), which are more closely affiliated with the histone H1 proteins and do not contain the glycine residues (sequences not shown). It is possible that the Hc1-BpH1 group was derived from an ancestral form between the ancestral BpH2-HCC and the eukaryotic histone H1.

Using the HCC sequences and randomly chosen published sequences of HUs and HLPs, we constructed an unrooted UPGMA (unweighted pair group method with averages) tree by use of the PHYLIP program (Fig. 2). As expected, the three families form distinct clades. The HLPs form an intermediate clade between the HCCs and the bacterial HUs, confirming the intermediate nature of their primary sequences. The bootstrap values for the major nodes are highly significant. Assuming the evolution of histone H1 from the HUs, the dinoflagellate HCCs probably evolved from ancestral bacterial BpH2-like genes and represent an intermediate form before the loss of some bacterium-specific residues (e.g., the conserved glycines) and the acquisition of the wing domain. The ancestral alveolates did develop eukaryotic histones, as observed for the ciliates and the apicomplexans. Dinoflagellates may have subsequently lost these proteins, and a set of primitive bacterial BpH2-XHLP-like proteins may have been reintroduced via gene transfer from a prokaryotic source. This was supported by the isolation of the nucleus-encoded dinoflagellate RuBisCo (ribulose-1,5-biphosphate carboxylase-oxygenase) gene in *Gonyaulax polyedra*, which is more homologous to those of the proteobacteria than to other eukaryotes (14). Based on the condensed states and birefringence, three different chromosomal types can be observed in dinoflagellates (5), suggesting that different groups of dinoflagellates may have different basic chromosomal proteins. Interestingly, the homology between the HCCs and HAF, the putative DNA-binding protein from the dinoflagellate *A. fundyense* (24), is actually lower than the homology between the HCCs and BpH2-XHLP. One possible explanation may be the occurrence of a high evolutionary rate

after the initial establishment of the BpH2-like gene in the alveolates.

The conservation of histones and the nucleosomes in the majority of eukaryotes implies that the eukaryotic histone-DNA complex probably existed in their last common ancestor. Both primary sequence data and three-dimensional structural comparisons support the origin of the eukaryotic core histones from the archaeal histones (15, 20). This contrasts with the evolution of the histone H1 as predicted from the sequence homologies with the eubacterial HU family. Both the conserved "histone fold" motif (three α-helices) of the core histones and the N-terminal α-helices of the eubacterial HUs, while having no primary sequence homology, function to enable dimer formation (1, 26). Interestingly, the lack of the histone fold motif in "histone-like proteins" in eubacteria, mitochondria, and dinoflagellates is correlated with the aggregation sensitivity of their DNA during conventional cytological fixation for electron microscopy (12). The HCCs and BpH2, BpH1, and other bacterial "histone-like" sequences probably represent a continuum in the evolution of the eukaryotic histone H1 from the prokaryotic HU families (including integration host factor and HN-S). Further phylogenetic analysis with these additional histone-like sequences will be required to give a more accurate account of the evolution of chromosomal proteins and chromosome structures.

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