Vol. 184, No. 1, 1992
April 15, 1992

BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
Pages 317-322

EVIDENCE FOR POSSIBLE STRUCTURAL CHANGES OF PARTICULAR H3 AND H2B HISTONE GENES IN DIFFERENT CHICKEN TISSUES (CELLS)

Tatsuo NAKAYAMA* and Yoshiko SETOGUCHI

Department of Biochemistry, Miyazaki Medical College,
Kihara, Kiyotake, Miyazaki 889-16, Japan

Received March 4, 1992

SUMMARY: The two chicken histone gene families H3 and H2B contain nine and eight members, respectively. To clarify whether the structures of these genes differ in different tissues (and cells), we analyzed DNAs from chicken lung, kidney, oviduct, and sperm. An H3-specific probe (probe 1.3SS) hybridized with a 10 kb EcoRI fragment carrying two H3 genes (H3-II and H3-III) from the lung, kidney, and oviduct with intensities of about one quarter of that of the fragment from the sperm. On the other hand, the intensities of hybridization of the H2B-specific probes (probes H2B-Ia, H2B-IIb, and H2B-III) with a 12 kb EcoRI fragment carrying two H2B genes (H2B-IV and H2B-V) from the oviduct, lung, and sperm were about a quarter of the intensity of hybridization with this fragment from the kidney. These findings, together with those reported previously, suggest that these particular histone genes H3 and H2B possess inherent abilities to form either a tight or loose structure, and that they exist in a loose form in the sperm and kidney but in a tight form in the other tissues tested.

Among higher eukaryotes, chickens appear to have one of the lowest histone gene repetitive frequencies, ranging from six for H1 genes to approximately ten for core histone genes (H2A, H2B, H3, and H4) (1-3). With some exceptions, most of these genes fall into two major histone gene clusters (4). Several of these histone genes have been sequenced (4-11). The H2B gene family consists of eight highly homologous members (H2B-I to H2B-VIII), seven of which have been sequenced and shown to encode three different protein variants (5, 7). All the H2B genes sequenced contain an H2B-specific element including the common OTF-1 binding site (12), in addition to common CCAAT and TATA boxes. Six of the nine H3 genes (belonging to the two major gene clusters) have also been sequenced (4, 8, 10, 11). These H3 genes all have several copies of possible AP-1-binding (13) and Sp1-binding (14) elements as well as the common CCAAT and TATA boxes within their 5'-flanking regions.

Based on their 5' consensus elements, we have been investigating the regulation of expression of H2B-IV, H2B-V, H3-II, and H3-III, which we have cloned and sequenced (7,8).

*To whom correspondence should be addressed.

0006-291X/92 $1.50

These 5' sequence blocks have been shown to be potential promoter elements (data to be reported elsewhere), such as an octamer and an adjacent CCAAT elements essential for control of expression of the chicken H2A and H2B (H2B-II in Fig. 1) gene pair (15) and also a bi-directional S phase-specific transcriptional regulatory element for the rat testis-specific TH2A and TH2B gene pair (16).

However, our unpublished results have suggested that, in addition to this regulation based on *cis*-acting elements, there may be another, as yet unknown, but essential mechanism for tissue-specific regulation of the expressions of H2B-IV, H2B-V, H3-II, and H3-III. To obtain information on this unknown mechanism, we examined the DNA structures of the H2B genes in various tissues. In Southern analyses (17), various H2B gene-specific probes hybridized with two particular H2B genes (H2B-IV and H2B-V), which are located in close proximity within a 12 kb EcoRI fragment, but the intensities of hybridization with fragments from the oviduct and lung were about a quarter of that with the fragment from the kidney (18). This structural difference of H2B-V may result in its relatively lower expression in the oviduct and lung.

To determine whether this biological event is common in other tissues (cells) and/or other histone gene subfamilies, we analyzed DNAs from the sperm, kidney, lung, and oviduct with an H3-specific probe and various H2B-specific probes. The results indicated that some undefined structural change occurs to form a tight DNA structure in a narrow region surrounding the H3 genes (H3-II and H3-III) of the kidney, lung, and oviduct, resulting in decreased hybridization intensity.

MATERIALS AND METHODS

DNA preparation and Southern blotting

High-molecular-weight DNA was purified from the oviduct, lung, kidney, and sperm of chickens (white leghorn) essentially as described (19). DNA (200 μg) was digested with EcoRI according to the supplier's recommendations (Takara Shuzo). Analyses with three vitellogenin II-related probes as controls showed that the restriction enzyme digested all the DNA preparations completely (data now shown). The digests were divided into several fractions, and 20 μg of each fraction was separated by electrophoresis on 0.8% agarose gel. The resulting DNA fragments were transferred to nitrocellulose membrane filters and examined by the method of Southern (17). The filters were hybridized with the respective probe DNAs (see later section), washed under stringent conditions as described (19), and autoradiographed at -70°C with an intensifying screen for several days.

Probes

Probe H2B-Ia consists of 5 bp of the 5'-noncoding region and the 215 bp coding region of H2B-I. Probe H2B-IIb consists of 147 bp of the 5'-noncoding region and the 118 bp coding region of H2B-II. A 3.2 kb EcoRI fragment carrying the entire H2B-III coding region was used as probe H2B-III. Because the coding regions of the seven H2B genes sequenced share extensive sequence homology (92% to 99%) (5, 7), these three probes hybridize well with the H2B gene-carrying fragments. A 1.3 kb SalI/SalI fragment (probe 1.3SS) contains almost all the coding region but lacks the 30 bp 5' coding sequences of two H3 genes (H3-IV and H3-V), which are located in close proximity in the same 12 kb EcoRI fragment (10). The

coding regions of all the H3 genes sequenced (4, 8, 10, 11) also show high homology (98% to 99%). The DNA probes were labeled with ^{32}P by random-priming as recommended by the supplier (Amersham). Their specific activities were about 1×10^9 cpm/μg DNA.

RESULTS AND DISCUSSION

We analyzed EcoRI digests of DNAs from the sperm, oviduct, kidney, and lung with an H3-specific probe (probe 1.3SS) and three H2B-specific probes (probes H2B-Ⅰa, H2B-Ⅱb, and H2B-Ⅲ) whose locations are shown in Fig. 1.

In the lung, kidney, and sperm, probes H2B-Ⅰa, H2B-Ⅱb, and H2B-Ⅲ hybridized with seven discrete fragments of 3.2 kb, 3.6 kb, 4.5 kb, 7.0 kb, 12 kb, 14 kb, and 20 kb, and probe H2B-Ⅲ also hybridized with a 4.0 kb fragment (Fig. 2A, 2B, and 2C). Six of these fragments corresponded completely to the H2B gene-carrying EcoRI fragments in the two major gene clusters A and B (see Fig. 1), but the 3.6 kb and 4.0 kb fragments have not yet been characterized. The intensities of hybridization of these H2B-specific probes with the 12 kb EcoRI fragment carrying H2B-Ⅳ and H2B-Ⅴ from the lung and sperm were about one quarter

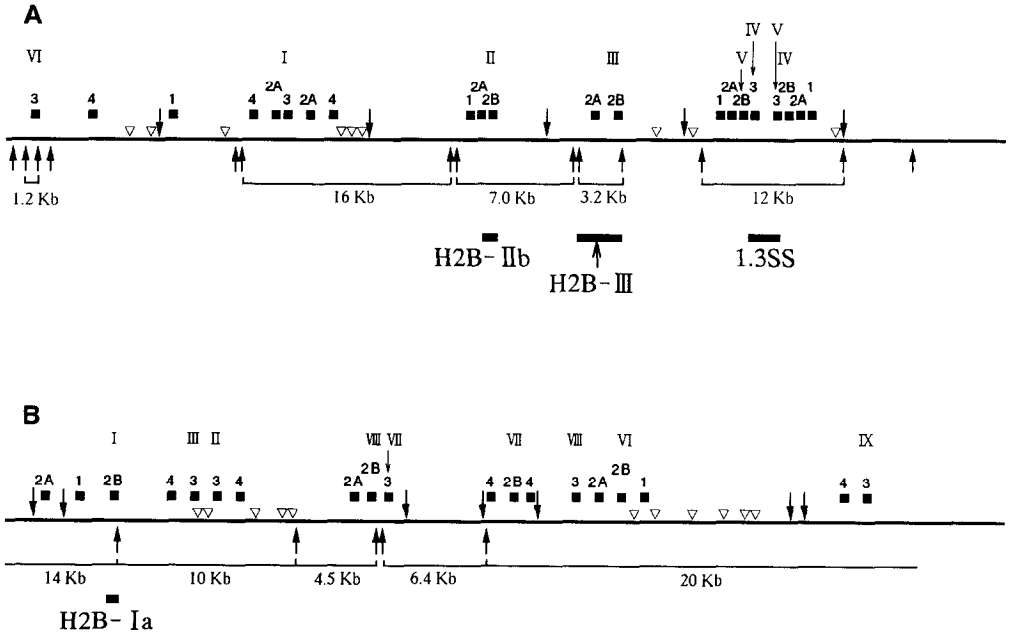

Fig. 1. Organizations of the chicken histone genes and locations of H3-specific and H2B-specific probes. The two major histone gene clusters A and B reported (3, 5, 11) are shown with slight modifications. Locations of known histone genes in the clusters are indicated by blackened squares. 1, 2A, 2B, 3, and 4 indicate histones H1, H2A, H2B, H3, and H4, respectively. The eight H2B genes and nine H3 genes are numbered Ⅰ to Ⅷ, and Ⅰ to Ⅸ, respectively. EcoRI fragments carrying the H2B and H3 genes are indicated by their lengths in kilobase pairs (kb). The locations of probes H2B-Ⅰa, H2B-Ⅱb, H2B-Ⅲ, and 1.3SS are indicated by bold underlines. Cleavage sites: upward arrows, EcoRI; downward arrows, BamHI; open downward arrowheads, Hind Ⅲ.

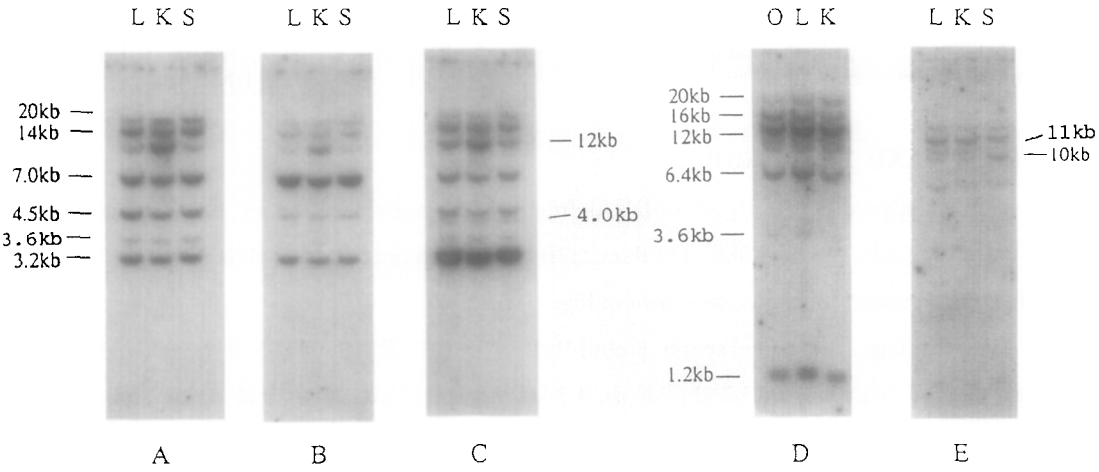

Fig. 2. Southern analyses of lung, kidney, oviduct, and sperm DNAs. Aliquots of 20 μg of EcoRI-digested DNAs from the lung (L), kidney (K), sperm (S), and oviduct (O) were separated by electrophoresis on 0.8% agarose gel, blotted onto nitrocellulose membrane filters, and hybridized as described (17, 18) with the following probes: A, H2B-Ⅰa; B, H2B-Ⅱb; C, H2B-Ⅲ; D and E, 1.3SS. Strongly hybridized bands are indicated by their lengths in kb.

of that with the fragment from the kidney; the other fragments in the three DNA preparations showed equal intensities of hybridization. These results are consistent with those reported in our previous paper, where the oviduct was used instead of the sperm (18).

Probe 1.3SS hybridized with seven discrete fragments of 1.2 kb, 3.6 kb, 6.4 kb, 10 kb, 12 kb, 16 kb, and 20 kb from the oviduct, lung, kidney, and sperm, and also with an 11 kb fragment from the oviduct, lung, and kidney (Fig. 2D and 2E). Six of these fragments corresponded to H3 gene-carrying EcoRI fragments belonging to the two gene clusters mentioned above. The 3.6 kb fragment may be from another region containing an H2A and an H3 gene (3, 11), because it also hybridized with probe H2B-Ⅲ carrying the H2A coding sequence (see Figs. 1 and 2C). The identity of the 11 kb fragment is unknown. Possibly it is related to the region carrying a variant H3 gene with introns previously reported (3, 11). The intensities of hybridization of the fragments bearing the H3 genes with probe 1.3SS were similar in the oviduct, lung, and kidney DNA preparations (Fig. 2D). Although the 10 kb fragment contains two H3 genes (H3-Ⅱ and H3-Ⅲ), its intensity of hybridization with the H3-specific probe was much lower than that of the 6.4 kb fragment containing an H3 gene. Surprisingly, in another experimental series involving sperm DNA, probe 1.3SS hybridized with the 10 kb EcoRI fragment carrying H3-Ⅱ and H3-Ⅲ from the lung and kidney DNAs with an intensity of about one quarter of that with the fragment from the sperm preparation (Fig. 2E). Conversely in the sperm preparation, the intensity of hybridization of the 11 kb fragment with the H3-specific probe was too low to be detectable although the pattern of other hybridization bands was similar to those in the other tissues.

H3-Ⅱ and H3-Ⅲ, which have inverted orientations (8), share a 5'- noncoding region of about 900 bp, the two genes being transcribed in opposite directions. H3-Ⅱ and H3-Ⅲ, like four other H3 genes sequenced, have been shown to encode the same amino acid sequence (4, 8, 10, 11). Thus, the regulation of expression of these two particular genes could be expected to be virtually the same manner. Our unpublished results, however, showed that the regulations of expression of H3-Ⅱ and H3-Ⅲ based on the 5' consensus elements are very different. In addition, the human H3 histone subfamily has been reported to contain at least three different protein variants (20). To elucidate whether the chicken H3 histone subfamily, like the H2B histone subfamily (5, 7), contains some different protein variants, we have again isolated and thoroughly sequenced several independent M13mp18 and mp19 phages carrying the H3 genes. Codon 113 of H3-Ⅲ in these experiments is ATG coding for Met (GenBank accession no. M61155) whereas that of the gene described previously is ATC coding for Ile (8). These findings, together with reported results (4, 8, 10, 11), indicate that the six H3 genes sequenced encode two different protein variants.

Results on the intensities of hybridization of the H2B and H3 genes are summarized in Tabel Ⅰ. In the 12 kb EcoRI fragment, the variable intensity of hybridization is limited to very narrow region surrounding and within the H2B-Ⅳ and H2B-Ⅴ coding sequences and does not extend over two genes each of H1, H2A, and H3 (18). The same is true for the 10 kb EcoRI fragment; that is, the variation does not extend over the entire fragment but is limited to the narrow region surrounding H3-Ⅱ and H3-Ⅲ. We are now studying the 11 kb fragment. The most likely of several possible explanations for these findings is that undefined structural changes occur to form a tight DNA structure in the regions surrounding the particular H2B genes or H3 genes in most tissues (or cells) other than the kidney or sperm. In the case of the

Table Ⅰ. Relative hybridization intensities of particular H2B and H3 genes

	Relative Hybridization Intensity		
	H2B-Ⅳ and H2B-Ⅴ in 12 kb fragment	H3-Ⅱ and H3-Ⅲ in 10 kb fragment	H3 gene in 11 kb fragment
Sperm	+	++++	±
Kidney	++++	+	+
Lung	+	+	+
Oviduct	+*	+	+

Relative hybridization intensities due to the H2B and H3 genes within the 12 kb, 10 kb, and 11 kb EcoRI fragments are presented.
*from a separate paper (18).

H2B genes, this possible structural change of H2B-V was partly responsible for its high mRNA levels in the kidney (18). These results indicate a tendency for increase in the level in the kidney of the remarkable H2B protein variant carrying Arg at position 30 (7). We are now studying the regulation of expression of H3-Ⅲ encoding an H3 protein variant containing Met at position 113.

REFERENCES

1. Ruiz-Carrillo, A., Affolter, M., and Renaud, J. (1983) J. Mol. Biol., *170*, 843-859.
2. Sugarman, B. J., Dodgson, J. B., and Engel, J. D. (1983) J. Biol. Chem., *258*, 9005-9016.
3. D'Andrea, R. J., Coles, L. S., Lesnikowski, C., Tabe, L., and Wells, J. R. E. (1985) Mol. Cell. Biol., *5*, 3108-3115.
4. Wang, S. W., Robins, A. J., D'Andrea, R. J., and Wells, J. R. E. (1985) Nucleic Acids Res., *13*, 1369-1387.
5. Grandy, D. K. and Dodgson, J. B. (1987) Nucleic Acids Res., *15*, 1063-1080.
6. Coles, L. S., Robins, A. J., Madley, L. K., and Wells, J. R. E. (1987) J. Biol. Chem., *262*, 9656-9663.
7. Nakayama, T. and Setoguchi, Y. (1991) Gene, *98*, 299-300.
8. Nakayama, T. (1991) Gene, *102*, 289-290.
9. Nakayama, T., Takechi, S., Ohshige, T., Kondo, K., and Yamamoto, K. (1991) Gene, *108*, 311-312.
10. Setoguchi, Y. and Nakayama, T. (1991) Nucleic Acids Res., *19*, 6327.
11. Engel, J. D., Sugarman, B. J., and Dodgson, J. B. (1982) Nature, *297*, 434-436.
12. Fletcher, C., Heintz, N., and Roeder, R. G. (1987) Cell, *51*, 773-781.
13. Lee, W., Mitchell, P., and Tjian, R. (1987) Cell, *49*, 741-752.
14. Kadonaga, J. T., Jones, K. A., and Tjian, R. (1986) Trends Biochem. Sci., *11*, 20-23.
15. Sturm, R. A., Dalton, S., and Wells, J. R. E. (1988) Nucleic Acids Res., *16*, 8571-8586.
16. Huh, N.-E., Hwang, I., Lim, K., You, K.-H., and Chae, C.-B. (1991) Nucleic Acids Res., *19*, 93-98.
17. Southern, E. M. (1975) J. Mol. Biol., *98*, 503-517.
18. Nakayama, T. and Setoguchi, Y. (1992) Nucleic Acids Res., in press.
19. Maniatis, T., Fritsch, E. F., and Sambrook, J. (1982) Molecular Cloning: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N. Y.
20. Wu, R. S., Tsai, S., and Bonner, W. M. (1983) Biochemistry, *22*, 3868-3873.