8. The DNA-Binding Unit in Sea Urchin Spermatogenous Histones. I

A New DNA-Binding Unit in H2B and H1^{*})

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Introduction. Identification of the nucleic acid-binding unit in the protein structure and further investigation into the mode of its binding will afford a key to the understanding of gene regulation. Three different kinds of DNA-binding unit have been proposed. One is the α -helix represented by the helix-turn-helix structure of prokaryotic repressors,^{1),2)} gene activator protein³⁾ and eukaryotic homeotic gene products.⁴⁾ Another is the antiparallel β -sheet found in G5BP⁵⁾ and DBP II.⁶⁾ The other is Zn-finger shown in transcription factor IIIA,⁷⁾ yeast gene activator proteins,⁸⁾ segmentation gene products⁹⁾ and steroid receptors.¹⁰⁾⁻¹⁴⁾

N-terminal regions of sea urchin spermatogenous histones, H1 and H2B, do not contain any units mentioned above. High frequency of Pro and lack of both His and Cys exclude the possibility of utilizing α -helix or Zn-finger, respectively, as the DNA-binding unit. β -Sheet also seems unqualified. Therefore, it is expected that these histones have a new type of DNA-binding unit. In the meantime, von Holt *et al.*¹⁵⁾ determined the primary structure of H1 and H2B, emphasizing extended pentapeptides kinked at Pro residue.

Methods and materials. The comparison of the primary structures of H2B's was carried out by modifying the table of Iwai and Kasai¹⁶⁾ and newly published data.^{17),18)} The primary structures of H1's were derived from the report of von Holt.¹⁵⁾ H2B's of various species were tabulated in Fig. 1a, classified in four groups of lower eukaryotes, higher eukaryotes, sea urchin scmatic cells and sea urchin sperm cells. The white and shadowed regions correspond to the conserved and diversified regions, respectively. The structures of N-terminal residues of H2B and H1 were compared following my viewpoint and tabulated in Fig. 1b. The repeating unit composed of the tetrapeptide was boxed; Arg residues were regarded as equivalent to Lys residues and a slight deviation from the standard was ignored.

Histones including spermatogenous and testicular H2B's were extracted with 0.4 N H₂SO₄ from testes of the sea urchin, *Temnopleulus hardwicki*. Histones were fractionated by DNA affinity chromatography. After loading histones, DNA ccolumn (Pharmacia) was washed with 8 M urea containing stepwisely increasing concentrations of NaCl (0.1 M to 0.6 M, lanes 1 to 6 in Fig. 2) to elute the loaded proteins.

The secondary structure prediction was carried out according to Chou and Fasman.¹⁹⁾

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Results and discussion. The diversity of the primary structures of histone H2B is limited to the N-terminal region as shown in Fig. 1a. This region of sea urchin spermatogenous H2B (Fig. 1a, 17–22) is longer than and distinct from those of other H2B's. Spermatogenous H1's have also longer N-termini than usual H1's.¹⁵⁾

Hereby I propose that these long N-termini of both histones are composed of five to six repeats of "Ser-Pro-Lys (Arg)-Lys (Arg)" residues (Fig. 1b). The unit of the repeat is neither Lys-Lys-Ser-Pro²⁰⁾ nor a pentapeptide starting from Pro.¹⁵⁾ If N-terminal parts of H1 and H2B are compared on the basis of the viewpoint as boxed in Fig. 1b, they are essentially the same, although some interunit insertion of Gly, Ala or Ser and a little incompleteness, resulted from substitution of either of seryl, threonyl and alanyl residues for lysyl residues or by the intraunit insertion of either of them, can be seen. This is quite unexpected, because H1 and H2B are the molecules derived from different species and have no similarities in the rest region.

The SPKK binds to DNA as inferred from the result of DNA affinity chromatography (Fig. 2). Spermatogenous H2B which has N-terminus composed of six SPKK residues was released at 0.5 M NaCl, while testicular H2B which belongs to the third group in Fig. 1a does not have such a repeat and released at 0.4 M NaCl together with other core histones. Since the difference of the two molecules in their primary structures is limited to only N-terminal region, the difference in affinity for DNA may reflect the difference in N-termini. Spermatogenous H1 binds to DNA most tightly. It should be noted that a similar sequence, trimethyl Ala-Pro-Lys-Lys, is present in N-terminal DNA binding regions of other H2B's.²¹⁾

The number of the amino acid residues of the unit, four, is that of β -turn, and the result of secondary structure predictions strongly suggests that SPKK forms β -turn in this sequence. This may be due to the facts that Pro has the highest tendency to become the second residue of the turn among the 20 amino acid residues and that Ser is one of the amino acid residues that can be found most frequently at the first position of the turn. Even when two units are combined like SPKKSPKK, other structural unit seems improbable, because the frequency of Pro at the second position, 0.272, is almost four times that at other positions, which is less than 0.074. Lewis *et al.*²²⁾ have pointed out that slightly hydrophilic residues facilitate the turn formation.

These statistical suggestions are justified if the Baker and Hubbard's collection of hydrogen bondings found in proteins²³⁾ is taken into consideration.

There are two kinds of additional hydrogen bondings which probably stabilize β -turn in proteins. One is the bond between OH of *i*-th Ser, Thr or Asx and NH of *i*+2-th. The other is that between OH of *i*+3-th Glx and NH of *i*-th. The side chain-main chain interaction of the former type can form a turn different from β -turn and called "Asx turn" by Rees *et al.*²⁴⁾ Alber *et al.*²⁵⁾ replaced the *i*-th amino acid of Asx turn of lysozyme with other amino acids and analyzed structural changes in atomic resolution and their effect on the stability of the protein. Asp, Asn, Ser and Thr form a similar Asx turn and the stabilities of those artificial proteins are similarly high. Many examples of β -turns which are stabilized by such a side chain-main chain interaction involving Ser at *i*-th positions can be seen in that collection.²³⁾

So I conclude that this unit composed of SPKK makes the turn stabilized by two hydrogen bondings, between *i*-th CO and *i*+3-th NH, and between *i*-th

OH of Ser and $i+2$ -th NH. This structure is the combination of β -turn and Asx turn using the same residue at i -th position. The i -th residue is limited to Asx, Glx, Cys, Thr and Ser like Asx turn, but Ser, Thr and Asx are more suited for these positions in view of the statistical consideration¹⁹⁾ and amino acid substitution experiment.²⁵⁾ The essence of this turn is i -th hydrophilic side chain offering hydrogen bond acceptor and $i+1$ -th Pro rotating around the next NH, the hydrogen bond donor. The i -th and $i+1$ -th residues enable both the acceptor and the donor to form the second hydrogen bonding, in addition to the first hydrogen bonding of β -turn.

Incomplete insertion in the repeating units can be seen in the first unit of the N-termini of H1's and more frequently in those of H2B's. These units may form turns of SPXK type, using a hydrophilic amino acid residue or Ala as X. The high tendency to β -turn of N-terminal region was first suggested by Poccia,²⁰⁾ but the unit of β -turn was not uniquely decided and even KKSP was nominated as one of candidates; KKSP cannot form the β -turn because Pro lacks NH as a potential hydrogen bond donor.

Summary. A new unit of DNA-binding in protein different from α -helix, β -sheet and Zn-finger, was revealed by the analysis of primary structures of sea urchin histones. The primary structure of this unit was identified as Ser-Pro-Lys(Arg)-Lys(Arg) residues. Its secondary structure is probably the turn stabilized by two hydrogen bondings.

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