A Model for the Structure of Chromatin in Mammalian Sperm

ROD BALHORN
Lawrence Livermore National Laboratory, Biomedical Sciences Division, University of California, Livermore, California 94550

ABSTRACT DNA in mammalian, and most vertebrate sperm, is packaged by protamines into a highly condensed, biochemically inert form of chromatin. A model is proposed for the structure of this DNA-protamine complex which describes the site and mode of protamine binding to DNA and postulates, for the first time, specific inter- and intraprotamine interactions essential for the organization of this highly specialized chromatin. In this model, the central polyarginine segment of protamine binds in the minor groove of DNA, crosslinking and neutralizing the phosphodiester backbone of DNA while the COOH- and NH_2-terminal ends of protamine participate in the formation of inter- and intraprotamine hydrogen, hydrophobic, and disulfide bonds. Each protamine segment is of sufficient length to fill one turn of DNA, and adjacent protamines are locked in place around DNA by multiple disulfide bridges. Such an arrangement generates a neutral, insoluble chromatin complex, uses all protamine sulfhydryl groups for cross linking, conserves volume, and effectively renders the chromatin invulnerable to most external influences.

Our knowledge of the biochemical composition and structure of the interphase and mitotic chromosome in eucaryotes has increased dramatically during the last two decades. It is now clear that the structure and genetic activity of the genome is modulated by two distinct groups of chromosomal proteins. The histones, a well-defined group of basic proteins rich in arginine, lysine, and histidine, bind to DNA through hydrophobic and electrostatic interactions and package DNA into the form known as chromatin. The other chromosomal proteins, or nonhistone proteins, comprise a less well-defined, much larger group of neutral and acidic proteins. Included in this group are various structural proteins and all the enzymes and proteins that interact with DNA or participate in its replication or repair, RNA synthesis, or the regulation of genetic activity.

Chromatin Structure in Somatic and Sperm-cell Nuclei

Numerous experiments have shown that the DNA in somatic nuclei is packaged in discrete subunits, called nucleosomes, containing ~200 base pairs of DNA wound around a core of eight histone molecules (30, 54, 60). Linear arrays of these nucleosomes appear to be further coiled several times (4, 32) or radially looped and twisted (63, 79) to generate the chromosomal fibers often observed by light and electron microscopy. In contrast, we have learned comparatively little about the structure of chromatin in the nuclei of sperm. Although the DNA in the sperm of a few animal species, such as the sea urchin (43) and frog (44), appears to be packaged in nucleosomes in a fashion similar to that found in somatic chromatin, the DNA in other fish (38, 40), and certain insect (46, 62), echinoderm (80), and mammalian sperm (29, 45, 51, 57) appears to be packaged in a very different manner. The DNA in these cells is condensed into an almost crystalline state and, for all practical purposes, is biochemically inert.

Biochemical studies have revealed that the DNA in most vertebrate sperm is associated with only one type of protein, protamine. These proteins are small, only half the size of the core histones, and extremely basic—between 55 and 70% of the amino acids in protamine are arginine (8, 25, 42). Electrophoretic and chromatographic analyses of these proteins have shown that the sperm of certain animals contain multiple protamine amino acid sequence variants and that the number of these variants differs from species to species. The sperm of bulls (24), rams (55, 56), and rats (20, 50), for example, contain only one protamine variant, whereas mouse sperm have two (6, 9) and the sperm of humans (20, 78) and certain fish (3) contain three different protamines.

Light and electron microscopy studies have demonstrated that the nucleus of the spermatid in these species undergoes a number of visible changes in nuclear shape and degree of

THE JOURNAL OF CELL BIOLOGY · VOLUME 93 MAY 1982 298-305

chromatin condensation during the normal course of differentiation (10, 22, 28, 81). The diffuse chromatin characteristic of genetically active cells is transformed into a highly condensed, inactive state in the mature sperm. Concomitant with these visible changes in chromatin organization, the histone and nonhistone proteins are removed from the DNA and replaced for a period of time by several transition proteins. These proteins are subsequently replaced by protamine during the final stages of spermatid maturation, chromatin reorganization and condensation (11, 12, 14, 35, 36, 48, 56, 61, 71, 76, 78, 85).

Comparisons of the amino acid sequences of several fish (1–3, 15, 16, 41) protamines reveal that these proteins are composed, almost totally, of arginine with occasional interspersed residues of serine, threonine, glycine, phenylalanine, valine, or proline. Although comparisons of the amino acid sequence of bull protamine (24) and the partial sequences of rat (50), boar (69), ram (69), stallion (69), human (34), and mouse (9) protamine indicate that the homology between various mammalian protamines is extensive, the primary structure of fish and mammalian protamines differs considerably. Mammalian protamines all contain a centrally located polyarginine stretch very similar in size to the entire length of the fish protamine, with additional nonbasic amino acid residues located in the COOH-terminal and NH_2-terminal "tail" fragments. In addition, all mammalian protamines contain numerous cysteine residues, and these amino acids are used to generate disulfide cross-links between adjacent protamine molecules during the final stages of chromatin maturation (7, 17, 21, 65, 82) as the sperm leaves the testis and traverses the epididymis.

Recent efforts in this laboratory to define the biochemical composition of sperm chromatin have revealed results which indicate that the DNA in mouse and certain other mammalian sperm must be packaged in a fashion very different from that found in somatic chromatin, a finding that is consistent with the conclusions of circular dichroism (86) and electron microscopy studies (45, 57, 58). Determinations of the DNA and protamine content of the mouse sperm nucleus (77), and the volume of the nucleus into which this material must be packed (94), make it clear that the DNA in these sperm can not be packaged in nucleosomes. The packaging of the 3.3 pg of DNA into nucleosomes would require over twice the available volume of the sperm nucleus. In addition, since the volume required by the DNA alone is equal to the entire volume available within the nucleus, it becomes evident that the 3 pg of protamine bound to DNA in sperm chromatin must occupy very little additional volume. Thus a significant portion of the protein must lie within the grooves of DNA. Our studies (77), as well as those of others (96), have also shown that the overall charge on the DNA-protamine complex in mouse and bull sperm must be essentially neutral. Calculations using the ratio of protamine to DNA in the sperm nucleus and the amino acid sequence or composition of the protamines indicate that the protamine complement of sperm chromatin supplies sufficient charge to neutralize completely the phosphodiester backbone of DNA. As a consequence of this neutralization, the DNA is condensed into a highly compact, relatively insoluble, and metabolically inert particle of genetic information.

A Model of the DNA-Protamine Complex

Numerous models have been proposed for the binding of protamine to DNA in sperm (31, 74, 86, 89, 93, 95). Some of these models deal only with very specific aspects of the interactions between DNA and protamine (27, 92), while others actually use partial or complete sequences of the fish protamines in describing the organization of the DNA-protamine complex in sperm chromatin (31, 74, 86, 89, 93, 95). Although it has been known for some time that the protamines of fish and mammals differ both in size and amino acid sequence, it is generally assumed that the mechanism of chromatin organization in the sperm of these organisms is similar. We know, however, that mammalian protamines contain large amounts of cysteine, whereas protamines of fish are devoid of this amino acid. The cysteine-containing protamines are ultimately cross-linked to each other through disulfide bonds (7, 17, 21, 65, 82), locking the protein around DNA. But the precise manner in which this cross-linking is accomplished remains unknown.

By using available data on the composition of the mouse (70) and bull (5) sperm nucleus, amino acid sequence data on fish (1–3, 15, 16, 41) and bull (24) protamine, and a great deal of the experimental data on sperm chromatin structure reported in the literature, we have devised a model that describes not only the manner in which protamines bind to DNA, but also suggests a mechanism for the cross-linking of protamines through the formation of multiple inter- and intraprotamine disulfide bonds.

As a first step in the development of this model, calculations regarding the width of the DNA grooves required for proper protamine binding were used to rule out possible sites of binding. Although it is not widely accepted that protamine binds in either the minor or major groove exclusively, x-ray diffraction studies provide the most convincing evidence that the protamine must bind in the minor groove of DNA (31, 90). Certainly, modeling studies demonstrate that the major groove is several angstroms too wide to allow simultaneous interactions between the arginine residues of protamine and the phosphates on both DNA strands when the protein adopts either an α-helix or extended conformation—a feature necessary for proper charge neutralization. However, proteins in either conformation can easily bind to both phosphate chains if the protein lies in (or slightly above) the minor groove.

PROTAMINE: α-HELIX OR RANDOM-COIL CONFORMATION? Although there is evidence that protamine can adopt an α-helical conformation upon binding to tRNA (95), infrared data on sperm in deuterium oxide indicate that protamine does not adopt such a conformation upon binding to DNA (13). If protamine were to adopt an α-helical conformation upon binding to DNA as suggested by Warrant and Kim (95), calculations indicate that the charged region of the protein (the central 24-amino-acid segment containing 20 arginine, 2 cysteine, and single phenylalanine and glycine residues) could span only half (36×10^{-4} μm) the DNA length covered by the protamine molecule (77×10^{-4} μm) in bull sperm chromatin. Considering the distance between adjacent phosphates in the phosphodiester backbone (7.1 Å), the linear rise of the α-carbon atom per amino acid residue in α-helical proteins (1.5 Å/residue), and the reach of the arginine side chain (6.8 Å), only two out of every four arginines would be able to bind to the phosphates along the periphery of the minor groove. The other two arginine side chains would be directed out away from the groove. To effect proper protamine-DNA charge neutralization under these conditions, the free arginine side chains could interact with (and cross-link) neighboring strands of chromatin, but to do so, 50% of the DNA in the newly organized, precross-linked DNA-protamine complex would need to remain free of protamine to permit proper arginine-DNA phosphate interactions. Such organization seems highly unlikely and there appears to be little, if any, evidence to support its existence.

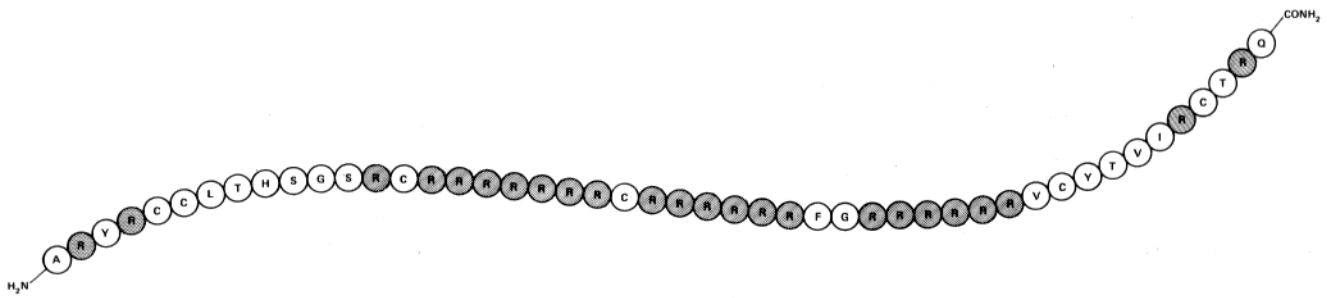


FIGURE 1 Amino acid sequence of bull protamine. The sequence is taken from Coelingh, et al. (24). The single letter nomenclature for amino acids follows the IUPAC-IUB rules (26): A, ala; C, cys; F, phe; G, gly; H, his; I, ile; L, leu; Q, glu; R, arg; S, ser; T, thr; V, val; Y, tyr. Arginine residues are shaded to show their distribution within the amino acid sequence.

If the entire amino acid sequence of protamine were to adopt an extended conformation and bind in the minor groove of DNA, however, the total length of minor groove required to accommodate the quantity of protamine present in a mouse or bull sperm (4.83 m and 6.03 m, respectively) would be approximately two to three times that actually present in these sperm (2.10–2.35 m, as calculated from their DNA content). If, on the other hand, only the central polyarginine segment (e.g., residues 16 through 36 in bull protamine) bound in or to the groove, the length of groove required to accommodate the total complement of protamine (2.3 m) would be just that available. Each protamine segment would be of sufficient length to fill the minor groove in one turn of DNA. The C-terminal and N-terminal residues, or "tails", in bull, mouse, and rat protamine would then be available for intra- and interprotamine interactions through hydrogen bonds or disulfide cross-links, or for hydrogen bonding to bases within the grooves.

CHEMICAL STRUCTURE OF THE DNA-PROTAMINE COMPLEX: Because insufficient amino acid sequence data are available for modeling mouse protamine binding to DNA, I have used the only available complete sequence of a mammalian protamine, that of bull protamine (Fig. 1), to describe the inter- and intraprotamine interactions involved in the packaging of sperm DNA. Since the known partial sequences of the predominant mouse (9) and several mammalian (34, 50, 69) protamines differ very little from the sequence of bull protamine, it seems likely that similar interactions occur in the sperm chromatin of all mammals.

One of the fundamental features of this model that applies to the binding of all protamines to DNA is that the central polyarginine sequence (or the entire protamine sequence in fishes) adopts an extended conformation and binds to DNA in the minor groove in a manner similar to that described by Feughelman et al. (31). To allow optimal interaction between all the arginines in protamine and all the phosphates in DNA, the polyarginine segment is not positioned down in the minor groove but lies half in and half out of the groove (Fig. 2), with the axis of the α-carbon chain ~10 Å from the DNA helix axis. Because of the planar nature of the peptide bond, the guanidino groups of adjacent arginines project from opposite sides of the molecule and interact with the O^2 and O^3 oxygens of the phosphates on opposite DNA strands, thereby cross-linking the two DNA strands through two hydrogen bonds and electrostatic interactions as proposed by De Santis et al. (27). As a result of this cross-linking, the O^2 oxygen is pulled slightly inward toward the center of the groove, reducing slightly the O^2-P-O^3 bisector angle (the angle formed between a line bisecting the O^2-P-O^3 bond angle of the phosphodiester backbone and the axis of the α-helix of DNA) and converting the normal

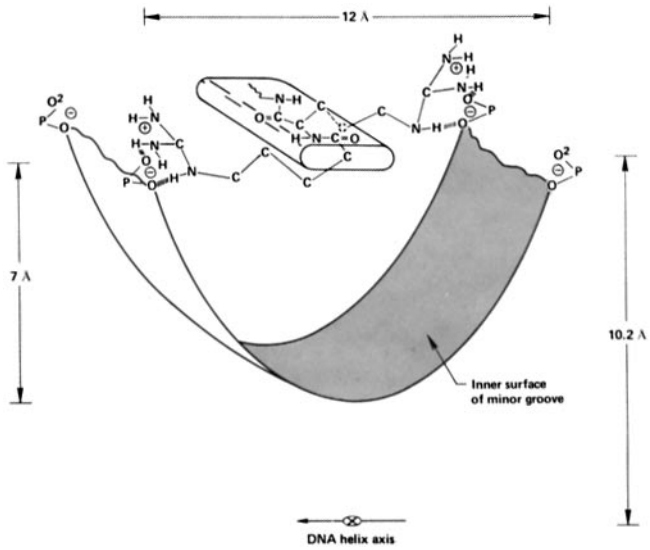


FIGURE 2 Position of polyarginine segment of protamine in minor groove of DNA. The polyarginine segment lies in the minor groove with the axis of the α-carbon chain ~10 Å from the axis of the DNA helix. Adjacent arginines interact with the phosphates in opposite strands of DNA through both hydrogen bonds and electrostatic interactions. As the protamines cross-link the two DNA strands, the O^2 oxygen is pulled inward toward the center of the minor groove, converting the normal B form of DNA to the B* form.

B form of DNA to the modified B form (B*) observed in DNA-protamine complexes (37).

With the distance between adjacent phosphates along a DNA strand being ~7.1 Å and the distance between the α-carbons of adjacent amino acids (and arginine side chains) 3.8 Å, the polyarginine segment can bind to and neutralize all but two of the phosphates in one turn of DNA (Fig. 3). These two phosphates interact later with arginine residues in the N-terminal tail fragment. The phenylalanine and glycine residues loop upward and out of the groove slightly, as proposed by Pardon and Richards (74), allowing the arginines in the C-terminal end of the polyarginine segment to move closer to the phosphates.

After the synthesis of protamine, and before its deposition on DNA, the serine and threonine residues in protamine are phosphorylated (39, 64, 66, 67). As a result of the position of these phosphorylated, negatively charged amino acids and the paucity of arginine residues in the C-terminal and N-terminal tail fragments, these regions do not bind along the groove of DNA, but the N-terminal tail (residue 1–15 in bull protamine) bends back, up and over the bound polyarginine segment (Fig. 4), and the two arginine residues near the N-terminus (residues

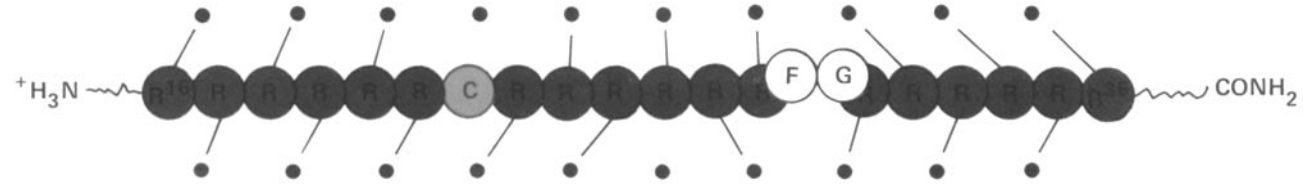
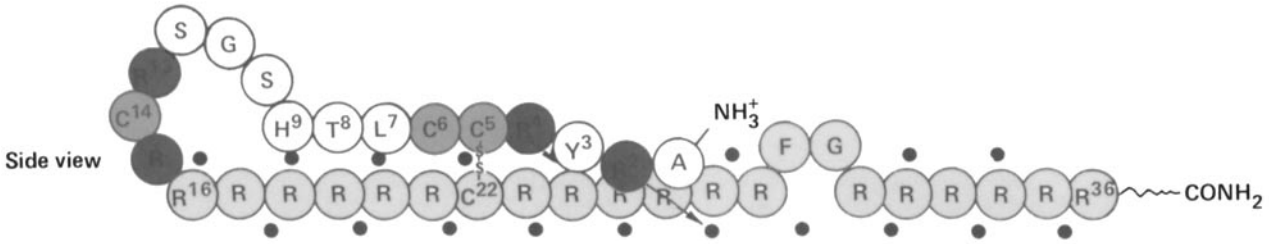


FIGURE 3 Binding of the polyarginine segment of bull protamine to phosphates in DNA. Solid circles represent phosphates in one turn of a DNA helix. All but two of the phosphates are bound by the polyarginine segment. These two bind to arginine residues in the N-terminal tail of protamine. The phenylalanine and glycine residues loop upward and out of the groove slightly, allowing the arginines in the C-terminal end of the polyarginine segment to move closer to the phosphates.



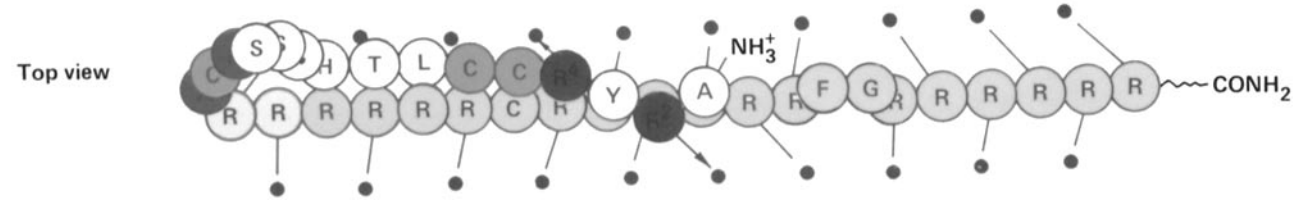


FIGURE 4 Interaction of N-terminal segment of protamine with DNA and the central polyarginine segment of the molecule. Solid circles represent phosphates in one turn of a DNA helix. Distances between adjacent phosphate residues (7.1 Å) and α-carbon atoms (3.8 Å) are drawn to scale. The N-terminal tail (residue 1–15 in bull protamine) bends back, up, and over the DNA-bound polyarginine segment. The two arginines near the N-terminus bind to the remaining free phosphates in DNA. The serine and threonine residues are dephosphorylated and a disulfide bridge forms between cysteines 5 and 22.

2 and 4) bind to the two remaining unbound phosphates of the DNA. After the binding of these arginines, the serine and threonine residues may then be dephosphorylated. As a result of this binding, cysteine 5 is brought close enough to cysteine 22 to allow the formation of a disulfide bridge. Cysteine 38 in the C-terminal end of an adjacent protamine molecule can then react with cysteine 14 to form a second disulfide cross-link (Fig. 5). Various hydrogen bonds (arginine 46-tyrosine 3, threonine 40-histidine 9, and tyrosine 39-arginine 13) and hydrophobic interactions (isoleucine 42-leucine 7) subsequently position the C- and N-terminal peptides of consecutive protamines adjacent to one another, permitting the formation of a third disulfide bridge between cysteine 6 in the N-terminal tail of one protamine and cysteine 44 in the C-terminal tail of the adjacent molecule. In this manner, each protamine in mammalian sperm is cross-linked to the next by disulfide bridges (Fig. 6), locking the protamine around the DNA.

Role of the DNA-Protamine Complex in DNA Packing, Conformation, and Reactivity

Upon complete neutralization of the phosphodiester chains, normal electrostatic repulsion between neighboring DNA segments is eliminated and the molecules may be tightly packed together. To minimize the volume required for packing, the portion of the protamine protruding from the minor groove, including the C-terminal and N-terminal tails interacting above the polyarginine segment, is positioned in the major groove of adjacent molecules of DNA (Fig. 7). The two remaining arginine residues in the N-terminal tail and the single arginine in the C-terminal tail may then hydrogen bond to sites inside the major groove.

The binding of protamine to DNA in this manner is consistent with the x-ray diffraction data and associated modeling calculations which indicate that the protein must be bound to only one of the two grooves, the minor groove (31, 90). By requiring only arginine-phosphate interactions for binding, the protamines could begin replacing other chromatin proteins at any point along the DNA molecule and then proceed along the length of DNA irrespective of base sequence. As proposed by De Santis (27), the multiple hydrogen bonds and electrostatic interactions between arginine and the phosphate oxygens would explain the high affinity of arginine for phosphate. Since this affinity is significantly greater than that observed between lysine or histidine and phosphate (70, 72, 84), the simple

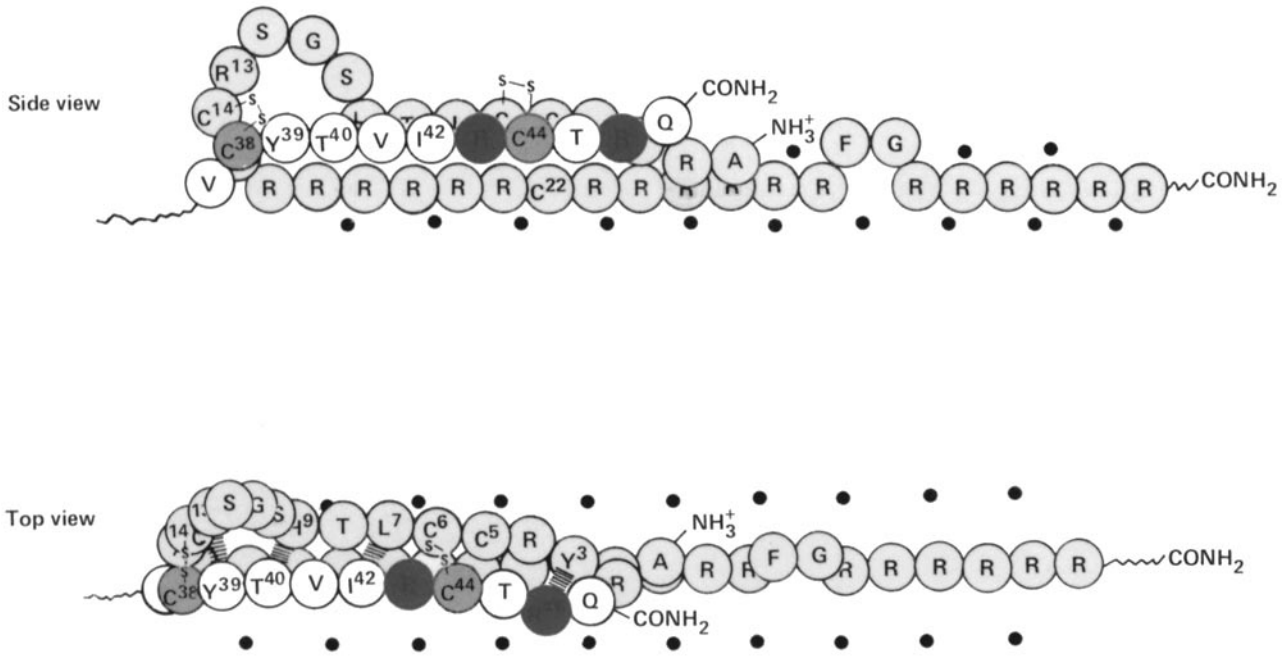


FIGURE 5 Binding between adjacent protamine molecules in the DNA-protamine complex. Cysteine 38 in the C-terminal end of one molecule reacts with cysteine 14 in the adjacent molecule to form a disulfide bridge. Various hydrogen bonds (arginine 46-tyrosine 3, threonine 40-histidine 9, tyrosine 39-arginine 13) and hydrophobic interactions (isoleucine 42-leucine 7) subsequently position the C- and N-terminal peptides of consecutive protamines adjacent to one another, permitting the formation of a third disulfide bridge between cysteine 6 in the N-terminal tail of one protamine and cysteine 44 in the C-terminal tail of the adjacent molecule. *Side View*: View of protamine bound to a linear representation of the minor groove from an observation point slightly above and perpendicular to the length of the groove. *Top View*: Binding as observed from directly above a linear representation of the minor groove.

deposition of the protamines in or near the minor groove during spermatid differentiation should be adequate to loosen and ultimately displace the transition proteins and residual histones, which appear to bind through hydrophobic and electrostatic interactions in the major groove (73–75, 83, 97) or along the outside of the phosphodiester backbone of DNA (59).

Upon binding to DNA, the protamine molecule maintains its extended conformation, consistent with infrared and amide deuteration studies (13, 37). As adjacent arginine residues alternately bind to opposite DNA strands, the phosphodiester chains are cross-linked along the entire length of DNA. As a result of this cross-linking, the DNA would be prevented from making the normal B-to-A or B-to-C conformation changes that are observed when the hydration of free DNA is decreased, transitions that do not appear to be allowed in DNA-protamine or DNA-polyarginine complexes (37, 90). This cross-linking might also, as described above, reduce the O^2-P-O^3 bisector angle by ~4° and convert the DNA from the normal B form observed in solution to the modified B form found in sperm chromatin and protamine-DNA complexes (37).

After the binding of protamine to the minor groove and the insertion of part of the protamine into the major groove as described, both grooves would still remain relatively empty and easily accessible to small molecules. This would explain the similar reactivity of the N^7 of guanine in the major groove and the N^3 of adenine in the minor groove of sperm chromatin in the presence of small alkylating agents (68). The partial filling of the major groove by protamine and possible hydrogen bonding between the protamine tail fragments and bases within this groove might also explain the slight reduction in guanine alkylation observed in the major groove at low temperatures. At low temperatures, the presence of the protein might hinder, slightly, the diffusion of the agents into the site for reaction.

Since only the central polyarginine segment binds to DNA, one protamine molecule is accommodated per turn of DNA and the length of DNA required for binding the amount of protamine determined biochemically to be present matches precisely the length of DNA available in the sperm head. The presence of the C-terminal and N-terminal peptide "tails" would appear to be a refinement of sperm chromatin packaging afforded only to mammals or higher vertebrates. In these animals the C- and N-terminal ends of the protamine allow the individual protamines to be cross-linked around DNA through the formation of inter- and intraprotamine disulfide bridges. Such cross-linking is consistent with the observation that protamine in mammalian sperm cannot be dissociated from DNA with acid or high concentrations of salt without first reducing the disulfide cross-links (49, 52, 53, 65, 66). Once these cross-links form in mature sperm, the binding of intercalating dyes, such as actinomycin D, is severely restricted, because such binding requires distortion of the DNA helix and the separation of adjacent base pairs at the site of intercalation (87, 88). In the cross-linked chromatin of mature sperm, the binding (intercalation) of these dyes must be restricted to a limited number of sites located between the regions of DNA bound by the polyarginine segments of adjacent protamine molecules.

In fish protamine, these C- and N-terminal peptide segments are absent, but the protamine binds to DNA in a fashion similar to that observed in mammalian protamines containing cysteine, neutralizing the phosphodiester chains, and allowing the DNA to be condensed and packaged in a small volume. In these protamines, the arginines that bind to the phosphodiester

backbone of DNA span the entire length of the molecule. Interspersed groups of two or more nonarginine amino acids loop out away from the groove (Fig. 8) in a manner similar to that described for the phenylalanine and glycine residues of bull protamine. This permits the short polyarginine segments (4–6 residues in length) to be positioned adjacent to one another, thereby neutralizing every phosphate residue in one turn of DNA. Because adjacent protamines are not cross-linked by disulfide bridges in fish sperm chromatin, these proteins are readily dissociated from DNA by extraction with salt or acid.

The specific cysteine interactions proposed in this model for inter- and intraprotamine disulfide bond formation require the involvement of all the cysteine residues in disulfide bridges. The function of the rare cys-cys sequence, as in other proteins containing this sequence (18, 19, 33, 47, 91), is to form disulfide bridges with other cysteine residues, linking three peptide segments in close proximity. The structure of the common peptide bond does not allow a disulfide bridge between adjacent cysteine residues. The proposed protamine tertiary structure is also stabilized further by several specific hydrogen bonds and hydrophobic interactions.

This model also suggests a function for protamine phosphorylation. By increasing the overall negative charge on the C-terminal and N-terminal tails of the mammalian protamines or decreasing the net positive charge in certain regions of fish protamine through the phosphorylation of the serine and threonine residues, these regions of the protein would be repelled by the phosphodiester backbone of DNA. In mammalian

FIGURE 6 Cross-linking of protamine molecules in the DNA-protamine complex. After the dephosphorylation of serine and threonine residues, each protamine molecule is cross-linked to the next through multiple disulfide bridges, locking the protamine around the DNA.

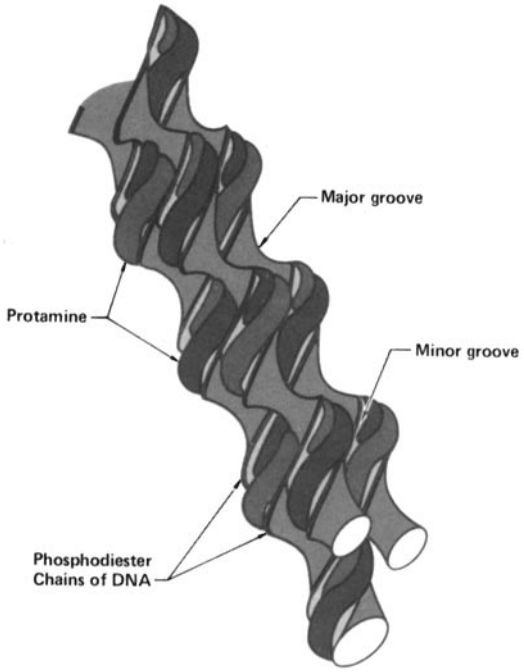


FIGURE 7 Packing of DNA molecules in chromatin. Upon complete neutralization of the phosphodiester chains, normal electrostatic repulsion between neighboring DNA segments is eliminated and the molecules may be tightly packed together. To minimize the volume required for packing, the portion of the protamine protruding from the minor groove, including the C-terminal and N-terminal tails interacting above the polyarginine segment, is positioned in the major groove of adjacent molecules of DNA. The two remaining arginine residues in the N-terminal tail and the single arginine in the C-terminal tail may then hydrogen bond to sites inside the major groove.

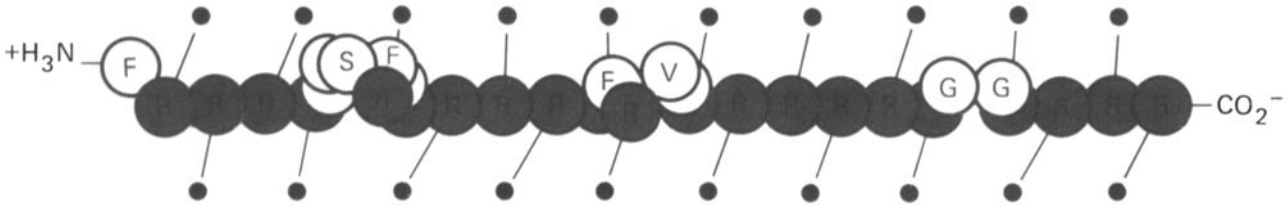


FIGURE 8 Binding of salmon protamine to the minor groove of DNA. The position of salmine in the minor groove and interactions between adjacent arginines and the phosphates on opposite strands of DNA are similar to those described for bull protamine. Three peptide segments, S-S-S-R-F-V, F-R-V-S, and G-G, loop up above the groove and allow the four polyarginine segments in salmine to move into position adjacent to one another and bind to and neutralize every phosphate residue in one turn of DNA. Similar modes of binding can be postulated for the other sequenced fish protamines, clupine, thynnin, and iridine.

sperm, these segments of protamine would never bind to DNA. The "tails" would move away from the DNA, closer to the peptide segments with which they will ultimately interact and out of the way of the next incoming protamines. In fish sperm, the polyarginine segments adjacent to regions containing the phosphorylated residues would bind later, after dephosphorylation.

CONCLUSION

I have described a model of sperm chromatin structure that appears consistent with most of the existing experimental data on chromatin in intact mammalian sperm and reconstituted DNA-protamine complexes. Several features of this model, including the notion that protamine binds to DNA in an extended conformation, the location of the protein in the minor groove, specific interactions between the arginines in protamine and the phosphate oxygens in DNA, and the juxtapositioning of the major and minor grooves on adjacent DNA molecules in condensed sperm chromatin have been extracted from various earlier studies and models and thus are not entirely new. But, by combining these features with new information on protamine-DNA stoichiometry in mammalian sperm, the partial or complete sequences of several mammalian protamines, information regarding interprotamine cross-linking through disulfide bridges, and the effect of this cross-linking on dye binding to sperm chromatin and on the dissociation of protamine from DNA, I have been able to propose a coherent model that describes not only the binding of both fish and mammalian protamines to DNA but also suggests a specific mechanism for cross-linking the protamines and further stabilizing the final chromatin complex in the mature mammalian sperm. The specifics of the model suggest a function for protamine phosphorylation, describe how the B conformation of DNA in solution may be converted to the B* conformation upon protamine binding, and suggest explanations for the observed reduction in Actinomycin D binding to sperm chromatin and for the unexpected reactivity of the bases in the major and minor grooves of sperm DNA to various alkylating agents.

This document was prepared as an account of work sponsored by an agency of the United States Government. Neither the United States Government nor the University of California nor any of their employees, makes any warranty, express or implied, or assumes any legal liability or responsibility for the accuracy, completeness, or usefulness of any information, apparatus, product, or process disclosed, or represents that its use would not infringe privately owned rights. Reference herein to any specific commercial products, process, or service by trade name, trademark, manufacturer, or otherwise, does not necessarily constitute or imply its endorsement, recommendation, or favoring by the United States Government or the University of California. The views and opinions of authors expressed herein do not necessarily state or reflect those of the United States Government thereof, and shall not be used for advertising or product endorsement purposes.

Work performed under the auspices of the U.S. Department of Energy by the Lawrence Livermore National Laboratory under contract number W-7405-ENG-48, and the U.S. Environmental Protection Agency, EPA-IAG-AD-89-F-0-046-0.

Received for publication 18 September 1981, and in revised form 21 December 1981.

REFERENCES

1. Ando, T., and K. Suzuki. 1966. The amino acid sequence of the second component of clupeine. *Biochem. Biophys. Acta.* 121:427–429.
2. Ando, T., and K. Suzuki. 1967. The amino acid sequence of the third component of clupeine. *Biochem. Biophys. Acta.* 140:375–376.
3. Ando, T., and S. Watanabe. 1969. A new method for fractionation of protamines and the amino acid sequence of salmine and three components of iridine. *J. Protein Research* 1:221–224.
4. Bak, A. L., J. Zeuthen, and F. H. C. Crick. 1977. Higher order structure of human mitotic chromosomes. *Proc. Natl. Acad. Sci. U. S. A.* 74:1595–1599.
5. Balhorn, R. unpublished results.
6. Balhorn, R., B. L. Gledhill, and A. J. Wyrobek. 1977. Mouse sperm chromatin proteins: quantitative isolation and partial characterization. *Biochemistry.* 16:4074–4080.
7. Bedford, J. M., H. I. Calvin, and G. W. Cooper. 1973. The maturation of spermatozoa in the human epididymis. *J. Reprod. Fertil.* 18:199–213. (Suppl.)
8. Bellve, A. R., E. Anderson, and L. Hanley-Bowdoin. 1975. Synthesis and amino acid composition of basic proteins in mammalian sperm nuclei. *Dev. Biol.* 47:349–365.
9. Bellve, A. R., and R. Carraway. 1978. Characterization of two basic chromosomal proteins isolated from mouse spermatozoa. *J. Cell Biol.* 79:177a.
10. Bols, N. C., S. A. Boliska, J. B. Rainville, and H. E. Kasinsky. 1980. Nuclear basic protein changes during spermiogenesis in the Longnose skate and Spiny dogfish. *J. Exp. Zool.* 212:423–433.
11. Bols, N. C., and H. E. Kasinsky. 1977. On the diversity of sperm histones in the vertebrates: II. A cytochemical study of the basic protein transitions during spermiogenesis in the cartilagenious fish *Hydrolagus colliei. J. Exp. Zool.* 198:109–113.
12. Bouvier, D. 1977. Chemical aspects of histone acetylation and replacement in mouse spermatids at different stages of maturation. *Cytobiologie.* 15:420–438.
13. Bradbury, E. M., W. C. Prince, and G. R. Wilkinson. 1962. Polarized infrared studies of nucleoproteins, I: Nucleoprotamines. *J. Mol. Biol.* 4:39–49.
14. Branson, R. E., S. R. Grimes, Jr., G. Yonuschot, and J. L. Irvin. 1975. The histones of rat testis. *Arch. Biochem. Biphys.* 168:403–412.
15. Bretzel, G. 1972. Uber Thynin, das protamine des thunfisches. Die sequenz der komponente Y1. XII. Mittelung iber die struktur der protamine in der untersuchungsreike von E. Waldschmidt-Leitz und mitarbeitern. *Hoppe Seylers Z. Physiol. Chem.* 353:1362–1364.
16. Bretzel, G. 1973. Uber thynnin, das protamine des thunfisches. Die aminosäuresequenz von thynnin Z1. XIII. Mittelung über die struktur der protamine in der untersuchungsreike von E. Waldschmidt-Letiz und mitarbeitern. *Hoppe Seylers Z. Physiol. Chem.* 354:312–320.
17. Bril-Petersen, E., and H. G. K. Westenbrink. 1963. A structural protein as a counterpart of deoxyribonucleic acid in mammalian spermatozoa. *Biochim. Biophys. Acta.* 76:152–153.
18. Brown, J. R. 1976. Structural origins of mammalian albumin. *Fed. Proc.* 35:2141–2144.
19. Bundell, T. L., J. F. Cutfield, S. M. Cutfield, E. J. Dodson, G. G. Dodson, D. C. Hidgkin, D. A. Mercola, and M. Vijayan. 1971. Atomic position of rhombohedral 2-zinc insulin crystals. *Nature (Lond.).* 231:506–511.
20. Calvin, H. I. 1976. Comparative analysis of the nuclear basic proteins in rat, human, guinea pig, mouse, and rabbit spermatozoa. *Biochem. Biophys. Acta.* 434:377–389.
21. Calvin, H. I., and J. M. Bedford. 1971. Formation of disulfide bonds in the nucleus and accessory structures of mammalian spermatozoa during maturation in the epididymis. *J. Reprod. Fertil.* 13:65–75. (Suppl.)
22. Castellani, L., F. Chiara, and F. Cotelli. 1978. Fine structure and cytochemistry of the morpheogenesis of round-headed human sperm. *Arch. Androl.* 1:291–297.
23. Chou, P. Y., and G. D. Fasman. 1974. Prediction of protein conformation. *Biochemistry.* 13:222–245.
24. Coelingh, J. P., C. H. Monfoort, T. H. Rozijn, J. A. Gevers Leuven, R. Schiphof, E. P. Steyn-Parve, G. Braunitzer, B. Schrank, and A. Ruhfus. 1972. The complete amino acid sequence of the basic nuclear protein of bull spermatozoa. *Biochem. Biophys. Acta.* 285:1–14.
25. Coelingh, J. P., T. H. Rozijn, and C. H. Monfoort. 1969. Isolation and partial characterization of a basic protein from bovine sperm heads. *Biochem. Biophys. Acta.* 188:353–356.
26. Dayhoff, M. O. 1969. Atlas of protein sequence and structure. National Biomedical Research Foundation, Silver Spring, Maryland. Volume 4.
27. DeSantis, E. Forni, and R. Rizzo. 1974. Conformational analysis of DNA-basic polypeptide complexes: possible models of nucleoprotamines and nucleohistones. *Biopolymers.* 13:313–326.
28. Dooher, G. B., and D. Bennett. 1973. Fine structural observations on the development of the sperm head in the mouse. *Am. J. Anat.* 136:339–361.
29. Evenson, D. P., S. S. Witken, E. deHarven, and A. Bendick. 1978. Ultrastructure of partially decondensed human spermatozoal chromatin. *J. Ultrastruct. Res.* 63:178–187.
30. Felsenfeld, G. 1978. Chromatin. *Nature (Lond.).* 217:115–1220.
31. Feughelman, M., R. Langridge, W. E. Seeds, A. R. Stokes, H. R. Wilson, C. W. Hooper, M. H. F. Wilkins, R. K. Barclay, and L. D. Hamilton. 1955. Molecular structure of deoxyribonucleic acid and nucleoprotein. *Nature (Lond.).* 175:834–838.
32. Finch, J. T., and A. Klug. 1976. Solenoidal model for superstructure in chromatin. *Proc. Natl. Acad. Sci. U. S. A.* 73:1987–1901.
33. Fox, J., and A. T. Tu. 1979. Conformational analysis of snake neurotoxin by prediction from sequence, circular dichromism, and raman spectroscopy. *Arch. Biochem. Biophys.* 193:407–414.
34. Gaastra, W., J. Lukkes-Hofstra, and A. H. J. Kolk. 1978. Partial covalent structure of two basic chromosomal proteins from human spermatozoa. *Biochem. Genet.* 16:525–529.
35. Goldberg, R. B., R. Geremia, and W. R. Bruce. 1977. Histone synthesis and replacement during spermatogenesis in the mouse. *Differentiation.* 7:167–180.
36. Hart, R. G. 1970. A model of the chromosome. *In*: Advances in Biological and Medical Physics. J. H. Lawrence and J. W. Gofman, editors. Academic Press, Inc., New York. Vol. 12:139–161.
37. Herskovits, T. T., and J. Brahms. 1976. Structural investigations on DNA-protamine complexes. *Biopolymers.* 15:687–706.
38. Honda, B. M., D. L. Baillie, and E. P. M. Candido. 1974. The subunit structure of chromatin: characteristics of nucleohistone and nucleoprotamine from developing trout testis. *FEBS (Fed. Eur. Biochem. Soc.) Letters.* 48:156–159.
39. Ingles, C. J., and G. H. Dixon. 1967. Phosphorylation of protamine during spermatogenesis in trout testis. *Proc. Natl. Acad. Sci. U. S. A.* 58:1011–1018.
40. Inoue, S., and M. Fuke. 1970. An electron microscope study of deoxyribonucleoprotamines. *Biochim. Biophys. Acta.* 204:296–303.
41. Iwai, K., C. Nakahara, and T. Ando. 1971. Studies on protamines, XV. The complete amino acid sequence of the Z component of clupeine. Application of N leads to O acyl rearrangement and selective hydrolysis in sequence determination. *J. Biochim. (Tokyo).* 69:493–509.
42. Kawashima, S., and T. Ando. 1978. Deoxyribonucleoproteins of herring sperm nuclei. I. Chemical composition. *J. Biochem.* 83:1117–1123.
43. Keichline, L. D., and P. M. Wassarman. 1979. Structure of chromatin in sea urchin embryos, sperm, and adult somatic cells. *Biochemistry.* 18:214–219.
44. Kharchenko, E. P., and N. N. Nalivaeva. 1980. Analysis of structural characteristics of

sperm chromatin in amphibians. *J. Evol. Biochem. Physiol. (Engl. Transl. Zh. Eval. Biokhim. Fiziol.).* 15:410–416.
45. Kierzenbaum, A. L., and L. L. Tres. 1975. Structural and transcriptional features of the mouse spermatid chromosome. *J. Cell Biol.* 65:258–270.
46. Kierszenbaum, A. L., and L. L. Tres. 1978. The packaging unit; a basic structural feature for the condensation of late cricket spermatid nuclei. *J. Cell Sci.* 33:265–283.
47. Kimball, M. R., A. Sato, J. S. Richardson, L. S. Rosen, and B. W. Low. 1979. Molecular conformation of erabutoxin b; atomic coordinates at 2.5 Å resolution. *Biochem. Biophys. Res. Commun.* 88:950–959.
48. Kistler, W. S., and M. E. Geroch. 1975. An unusual pattern of lysine rich histone components associated with spermatogenesis in rat testis. *Biochem. Biophys. Res. Commun.* 63:378–384.
49. Kistler, W. S., M. E. Geroch, and H. G. Williams-Ashman. 1973. Isolation and properties of small basic proteins from rat testis and epididymal spermatozoa. *J. Biol. Chem.* 248:4532–4544.
50. Kistler, W. S., P. S. Keim, and R. L. Heinrickson. 1976. Partial analysis of the basic chromosomal protein of rat spermatozoa. *Biochem. Biophys. Acta.* 427:752–757.
51. Koehler, J. K. 1966. Fine structure observations in frozen-etched bovine spermatozoa. *J. Ultrastruct. Res.* 16:359–375.
52. Kumaroo, K. K., G. Jahnke, and J. L. Irvin. 1975. Changes in basic chromosomal proteins during spermatogenesis in the mature rat. *Arch. Biochem. Biophys.* 168:413–424.
53. Lam, D. M. K., and W. R. Bruce. 1971. The biosynthesis of protein during spermatogenesis of the mouse: extraction, partial characterization, and site of synthesis. *J. Cell Physiol.* 78:13–24.
54. Lilley, D. M. J., and J. F. Pardon. 1979. Structure and function of chromatin. *Annu. Rev. Genet.* 13:197–233.
55. Loir, M., and M. Lanneau. 1975. An electrophoretic analysis of the basic nuclear proteins of ram spermatids. *Exp. Cell Res.* 92:509–512.
56. Loir, M., and M. Lanneau. 1978. Transformation of ram spermatid chromatin. *Exp. Cell Res.* 115:2311–243.
57. Lung, B. 1968. Whole-mount electron microscopy of chromatin and membranes in bull and human sperm heads. *J. Ultrastruct. Res.* 22:485–493.
58. Lung, B. 1972. Ultrastructure and chromatin disaggregation of human sperm head with thioglycolate treatment. *J. Cell Biol.* 52:197–186.
59. McGhee, J. D., and G. Felsenfeld. 1979. Reaction of nucleosome DNA with dimethyl sulfate. *Proc. Natl. Acad. Sci. U. S. A.* 76:2133–2137.
60. McGhee, J. D., and G. Felsenfeld. 1980. Nucleosome structure. *Annu. Rev. Biochem.* 49:1115–1156.
61. McMaster-Kaye, R., and J. S. Kaye. 1976. Basic protein changes during the final stages of sperm maturation in the house cricket. *Exp. Cell Res.* 97:378–386.
62. McMaster-Kaye, R., and J. S. Kaye. 1980. Organization of chromatin during spermiogenesis: beaded fibers, and loss of nucleosomal structure. *Chromosoma. (Berl.).* 77:41–46.
63. Marsden, M. P. F., and U. K. Laemeli. 1979. Metaphase chromosome structure: evidence for a radial loop model. *Cell.* 17:849–858.
64. Marushige, K., V. Ling, and G. H. Dixon. 1969. Phosphorylation of chromosomal basic proteins of maturing trout testis. *J. Biol. Chem.* 244:5953–5958.
65. Marushige, Y., and K. Marushige. 1974. Properties of chromatin isolated from bull spermatozoa. *Biochim. Biophys. Acta.* 340:498–508.
66. Marushige, Y., and K. Marushige. 1975. Transformation of sperm histone during formation and maturation of rat spermatozoa. *J. Biol. Chem.* 250:39–45.
67. Maurshige, Y., and K. Marushige. 1978. Phosphorylation of sperm histone during spermiogenesis in mammals. *Biochim. Biophys. Acta.* 518:440–449.
68. Mirzabekov, A. D., D. F. Sanko. A. M. Kolchinsky, and A. F. Melnikova. 1977. Protein arrangement in the DNA grooves in chromatin and nucleoprotamine in vitro and in vivo revealed by methylation. *Eur. J. Biochem.* 75:379–390.
69. Monfoort, C. H., R. Schiphof, T. H. Rosijn, and E. P. Steyn-Parve. 1973. Amino acid composition and carboxyl-terminal structure of some basic chromosomal proteins of mammalian spermatozoa. *Biochim. Biophys. Acta.* 322:173–177.
70. Murray, K., E. M. Bradbury, C. Crane-Robinson, R. M. Stephens, A. J. Haydon, and A. R. Peacocke. 1970. The dissociation of chicken erythrocyte deoxyribonucleoprotein and some properties of its partial nucleoproteins. *Biochem. J.* 120:859–871.
71. O'Brien, D. A., and A. R. Bellve. 1980. Protein constituents of the mouse spermatozoon II. Temporal synthesis during spermiogenesis. *Dev. Biol.* 75:405–418.
72. Ohlenbusch, H. H., B. M. Oliveira, D. Tuan, and N. Davidson. 1967. Selective dissociation of histone from calf thymus nucleoprotein. *J. Mol. Biol.* 25:299–316.
73. Olins, D. E. 1969. Interaction of lysine-rich histones and DNA. *J. Mol. Biol.* 43:439–460.
74. Pardon, J., and B. Richards. 1973. *In:* Subunits in Biological Systems, Part B. G. D. Fasman and S. W. Timasheff, editors. Marcel Dekker, Inc. New York.
75. Pietsch, P. 1969. Structural events in DNA transcription and replication. The influence of histones on in vitro reactions of actinomycin-D and phleomycin 909. *Cytobios.* 1:375–391.
76. Platz, R. D., S. R. Grimes, M. L. Meistrich, and L. S. Hnilica. 1975. Changes in nuclear proteins of rat testis cells separated by velocity sedimentation. *J. Biol. Chem.* 250:5791–5800.
77. Pogany, G., M. Corzett, S. Weston, and R. Balhorn. 1981. DNA and protein content of mouse sperm: implications regarding sperm chromatin structure. *Exp. Cell Res.* In press.
78. Puwaravutipanich, T. and S. Panyim. 1975. The nuclear basic proteins of human testis and ejaculated spermatozoa. *Exp. Cell Res.* 90:153–158.
79. Rattner, J. B., M. Goldsmith, and B. A. Hamkalo. 1980. Higher order organization of meiotic chromosomes. *J. Cell Biol.* 87(2, Pt. 2): 41 *a* (Abstr.).
80. Rocha, E., and L. Cornudella. 1976. Differential nuclease action on nuclei and chromatin from developing germ cells of the echinoderm *Holothuria tubulosa. Biochem. Biophys. Res. Commun.* 68:1073–1081.
81. Roosen-Runge, E. C. 1962. The process of spermatogenesis in mammals. *Biol. Rev. Camb. Philos. Soc.* 37:343–377.
82. Saowaros, W., and S. Panyim. 1979. The formation of disulfide bonds in human protamines during sperm maturation. *Experientia.* 35:191–192.
83. Shih, T. Y., and J. Bonner. 1970. Thermal denaturation and template properties of DNA complexes with purified histone fractions. *J. Mol. Biol.* 48:469–487.
84. Shih, T. Y., and J. Bonner. 1970. Template properties of DNA-polypeptide complexes. *J. Mol. Biol.* 50:333–344.
85. Shires, A., M. P. Carpenter, and R. Chalkley. 1975. New histones found in mature mammalian testis. *Proc. Natl. Acad. Sci. U. S. A.* 72:2714–2718.
86. Sipski, M. L., and T. E. Wagner. 1977. The total structure and organization of chromosomal fibers in eutherian sperm nuclei. *J. Biol. Reprod.* 16:428–441.
87. Sobell, H. M. 1973. The stereochemistry of actinomycin binding to DNA and its implications in molecular biology. *Prog. Nucl. Acid Res.* 13:153–190.
88. Sobell, H. M., and S. C. Jain. 1972. Stereochemistry of actinomycin binding to DNA. II. Detailed molecular model of actinomycin-DNA complex and its implications. *J. Mol. Biol.* 68:21–34.
89. Suau, P., and J. A. Subirana. 1977. X-ray diffraction studies of nucleoprotamine structure. *J. Mol. Biol.* 117:909–926.
90. Suwalsky, M., and W. Traub. 1972. An x-ray diffraction study of poly-L-arginine hydrochloride. *Biopolymers.* 11:2223–2232.
91. Tsernoglor, T., and G. A. Petsko. 1976. The crystal structure of a post-synaptic neurotoxin from sea snake at A resolution. *FEBS (Fed. Eur. Biochem. Soc.) Lett.* 68:1–4.
92. Wagner, K. G., J. Bode, K. Wehling, and L. Willmitzer. 1976. Sperm-specific proteins: interaction with DNA and chromatin and influence on phosphorylation thereon. *Studia Biophysica.* 55:39–48.
93. Wilkins, M. F. H. 1956. Physical studies of the molecular structure of deoxyribonucleic acid and nucleoprotein. *Cold Spring Harbor Symp. Quant. Biol.* 21:75–90.
94. Wyrobek, A. J., M. L. Meistrich, R. Furrer, and W. R. Bruce. 1976. Physical characteristics of mouse sperm nuclei. *Biophys. J.* 16:811–825.
95. Warrant, R. W., and S. H. Kim. 1978. Alpha-Helix-double helix interaction shown in the structure of a protamine transfer RNA complex and a nucleoprotamine model. *Nature (Lond.).* 271:130–135.
96. Yu, S. S., and H. J. Li. 1973. Helix-coil transition and conformational studies of protamine-DNA complexes. *Biopolymers.* 12:2777–2788.
97. Zubay, G. 1964. Nucleohistone structure and function. *In:* The Nucleohistones. J. Bonner, and P. O. P Ts'o, editors. Holden-Day, San Francisco. 95–107.