its associated lipid may be one of the factors that retards the ability of these fiber tracts to sustain effective sprouting and axon elongation.

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15. Two steps and their rates have to be considered: (i) receptor-mediated uptake [via the apo-B,E(LDL) receptor] and (ii) fast axonal transport. Ferritin-LDL uptake by normal human fibroblasts into coated pits takes 3 minutes, and within 6 to 8 minutes LDL can be seen in lysosomes [M. S. Brown and J. L. Goldstein, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 3330 (1979)]. An in vivo rate of 2.7 μm per second for fast axonal transport in the rabbit visual system has been determined [T. Lorenz and M. Willard, *ibid.* **75**, 505 (1978)]. At this rate, particles could travel 810 μm in 5 minutes or ~160 μm per minute. Factoring in the delay of 3 to 6 minutes for uptake and compartmentalization, a particle could travel from 0 to 320 μm in 5 minutes, with 80% of the material able to travel less than 160 μm.
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Sequence-Specific Packaging of DNA in Human Sperm Chromatin

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The DNA in human sperm chromatin is packaged into nucleoprotamine (~85%) and nucleohistone (~15%). Whether these two chromatin fractions are sequence-specific subsets of the spermatozoon genome is the question addressed in this report. Sequence-specific packaging would suggest distinct structural and functional roles for the nucleohistone and nucleoprotamine in late spermatogenesis or early development or both. After removal of histones with 0.65*M* NaCl, exposed DNA was cleaved with Bam HI restriction endonuclease and separated by centrifugation from insoluble nucleoprotamine. The DNA sequence distribution of nucleohistone DNA in the supernatant and nucleoprotamine DNA in the pellet was compared by cloning size-selected single-copy sequences and by using the derived clones as probes of nucleohistone DNA and nucleoprotamine DNA. Two clones derived from nucleohistone DNA preferentially hybridized to nucleohistone DNA, and two clones derived from nucleoprotamine DNA preferentially hybridized to nucleoprotamine DNA, which demonstrated the existence of sequence-specific nucleohistone and nucleoprotamine components within the human spermatozoon.

DURING THE FINAL STAGES OF spermatogenesis in placental mammals, the histones associated with DNA in spermatid chromatin are replaced by smaller, extremely arginine-rich protamine molecules. As a result, the nucleosomal-based packaging of DNA characteristic of somatic chromatin is transformed into a highly condensed form of chromatin referred to as nucleoprotamine. In most mammals, this displacement is generally thought to be complete. In humans, however, approximately 15% of the histones remain associated with sperm DNA (*1*). Because the semen of normal human males contains large numbers of abnormal sperm types, it has been difficult to positively link histone with the packaging of chromatin in normal, mature sperm.

The nucleoprotamine complex is structurally distinct from nucleohistone (*2*), insoluble in buffers of physiological ionic strength, transcriptionally inactive (*3*), and resistant to enzymatic digestion (*4*). Histones can be quantitatively extracted from human sperm chromatin with a mixture of 8*M* urea and 0.2*M* NaCl without an accompanying release of DNA, which suggests that nucleohistone is present in each sperm nucleus and is bounded by regions of nucleoprotamine (*5*). Using blot hybridization techniques, we demonstrated that DNA is packaged in the two chromatin fractions in a sequence-specific manner, confirming the presence of sequence-specific nucleohistone and nucleoprotamine components in the spermatozoon.

Sperm previously stored at −20°C (*6*) were separated from semen by washing with 100 m*M* NaCl, 10 m*M* tris (*p*H 8.0), and 1 m*M* EDTA containing 0.1 m*M* phenylmethylsulfonyl fluoride (PMSF). Chromatin was isolated without sonication by solubilizing the tail, acrosome, and nuclear membrane with mixed alkyltrimethylammonium bromide (Sigma M7635) (*7*). To determine the NaCl concentration necessary for histone extraction without disrupting nucleoprotamine, we extracted the chromatin with increasing NaCl concentrations and analyzed the dissociated proteins in Triton-acid-urea (TAU) gels. As shown in Fig. 1A, histones began dissociating from sperm chromatin at 0.6*M* NaCl. Protamines were not released at NaCl concentrations up to at least 0.9*M*. The ease with which histones were removed from sperm chromatin in this study differs from an earlier report that

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histones are not removed by 2*M* NaCl from sperm heads not treated with micrococcal nuclease (*4*). The extraction profile we observed correlates more closely with the histone extraction profile of *Physarum polycephalum* active ribosomal DNA (rDNA) chromatin in which histones H3 and H4 are extracted by low concentrations of NaCl (*8*).

All four core histones were present in sperm chromatin with high levels of acetylation of H3 and H4 (note the presence of acetylated H3 and all four acetylated forms of H4, Fig. 1B, lane 2). Similar acetylation patterns have been observed from active rDNA *Physarum* chromatin (*9*) and from histones acetylated during spermatogenesis in trout (*10*). The identity of the protein denoted with a question mark in Fig. 1B has not yet been determined. Given the unusually high level of H2A compared to H2B in sperm chromatin, this protein may be an H2B sequence variant (possibly TH2B). The protein is not H1 on the basis of TAU-SDS two-dimensional gels. The number of histones and their relative amounts in sperm chromatin also differed from earlier reports that one primary histone is present in human sperm heads (*1, 4, 11*), but is in agreement with the findings of Gusse *et al.* (*12*) who reported the presence of histones H2A, TH2B, H3, and H4, but not H1, in highly purified sperm nuclei.

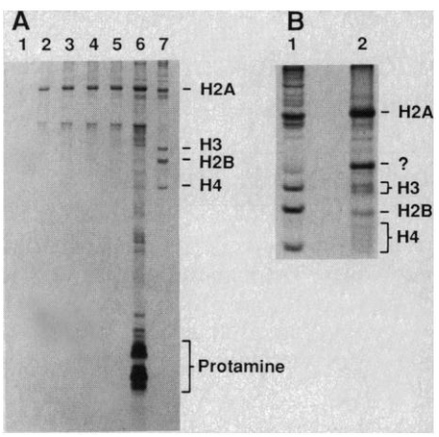

Fig. 1. Salt extraction and partial identification of human sperm histones. (**A**) Five aliquots of human sperm chromatin (each containing 0.5 mg of DNA) were individually extracted with 1 ml of 0.5*M*, 0.6*M*, 0.7*M*, 0.8*M*, or 0.9*M* NaCl in 10 m*M* tris buffer (*p*H 8.0) containing 0.1 m*M* PMSF on ice for 15 minutes. After centrifugation, the supernatants were removed, acidified by adding 1/9 volume 3*M* sulfuric acid, cooled on ice for 1 hour, and centrifuged again. The supernatants were concentrated in 8*M* urea, 0.45*N* acetic acid, and 0.1 m*M* PMSF by means of Centricon 10 microconcentrators (Amicon). The NaCl extractions (lanes 1 to 5) were run on a 22-cm 18% TAU gel (*16*) along with an acid-extract of sperm chromatin (lane 6) and HeLa cell histone standards (lane 7). Protamines were not detected in any of the NaCl extractions after Coomassie blue staining. (**B**) Analysis of the histone region of a TAU gel indicates all four core histones are present in sperm chromatin including acetylated H3 and all four acetylated forms of H4 (lane 2). The identity of the protein denoted with a question mark has not yet been determined. Lane 1 contains HeLa cell histone standards.

After removing the histones with 0.65*M* NaCl (Fig. 2, lane 2, and Fig. 3, lanes 1), the chromatin was washed with Bam HI buffer [6 m*M* tris (*p*H 7.9), 6 m*M* $MgCl_2$, 150 m*M* NaCl, in Fig. 2, lane 3, and Fig. 3, lanes 2], restricted with Bam HI at a concentration of 5000 units per milligram for 1.5 hours at 37°C, and centrifugated at 9000*g* for 2 minutes. The supernatant (Fig. 2, lane 4, and Fig. 3, lanes 3) was removed, and the pellet reextracted with 0.65*M* NaCl (Fig. 2, lane 5, and Fig. 3, lanes 4). The pellet and each supernatant were divided into halves for protein extraction or DNA purification.

No protamines were detected in any of the supernatants (Fig. 2, lanes 2, 3, 4, and 5). Two concentrations of protein from the pellet are shown in Fig. 2: a low concentration (lane 6) that resolves the protamine bands and a high concentration (lane 7) corresponding to an in vivo ratio of protamine to the histones in lane 2. Comparing lanes 2 and 7 in Fig. 2, one sees that histones are almost quantitatively removed by the first NaCl extraction.

The DNA in the supernatants was purified by standard procedures (*13*), but purification of the nucleoprotamine (NP) DNA required pellet solubilization, protamine complexation with polyglutamic acid, and extensive extraction with chloroform (*14*). Purified NP DNA was dissolved in 10 m*M* tris (*p*H 8.0) with 0.1 m*M* EDTA (TE) at 1 mg/ml. Supernatant DNAs were dissolved in 1/10 the volume of TE used for the NP DNA. Equal volumes of the supernatant and NP DNAs were restricted with Bam HI and separated according to size on agarose gels along with NP DNA that was not restricted after purification. The DNA was then transferred to nitrocellulose membrane or extracted from the gel for cloning.

DNA fragments ranging from 3 to 0.8 kb were selected and cloned into the Bam HI site of pUC18 plasmid. Recombinant clones were screened with total human DNA labeled with ^{32}P by nick translation to eliminate clones containing moderately and highly repetitive sequences. To select single-copy sequences, we used the clones as probes of human placental DNA. The clones that hybridized to a single restriction fragment with approximately single-copy intensity were then used as probes of the fractionated nucleohistone (NH) DNA and NP DNA.

Results of probing with two clones derived from NH DNA and two clones derived from NP DNA are shown in Fig. 3.

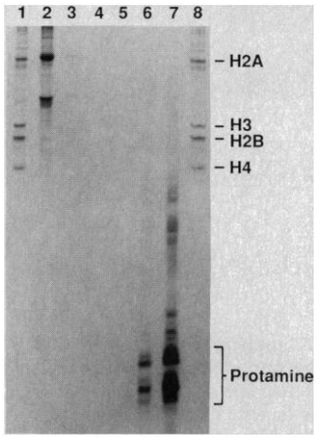

Fig. 2. TAU gel of proteins from sperm chromatin fractionated by NaCl extraction and Bam HI restriction. The histones were removed by 0.65*M* NaCl extraction (lane 2) as described in Fig. 1. The chromatin was washed in Bam HI buffer (lane 3), restricted with Bam HI, and centrifuged. The supernatant (lane 4) was removed, and the pellet was reextracted with NaCl (lane 5) and solubilized. All samples were acid-extracted, concentrated, and run on a 22-cm 18% TAU gel (see Fig. 1). Two concentrations of protein from the pellet were loaded: a low concentration (lane 6) to resolve the protamine bands and a high concentration (lane 7) corresponding to an in vivo ratio of protamine to the histones in lane 2. Lanes 1 and 8 are HeLa histone standards.

To facilitate comparison of hybridization intensities, we nick translated, mixed, and simultaneously used as hybridization probes a 2.5-kb NH clone and a 2.0-kb NP clone (Fig. 3A). The 2.5-kb NH clone hybridized more intensely to NH DNA (lane 3) than to NP DNA (lane 6). Conversely, the 2.0-kb NP clone hybridized more intensely to NP DNA (lane 6) than to NH DNA (lane 3). The probed filter was stripped of radioactive DNA and similarly hybridized with a mixture of a 1.8-kb NH clone and a 1.4-kb NP clone (Fig. 3B). Again the NH clone preferentially hybridized to NH DNA (lane 3), whereas the NP clone preferentially hybridized to NP DNA (lane 6). The absence of corresponding bands in NP DNA that was not rerestricted with Bam HI after DNA purification (Fig. 3, A and B, lanes 5) provides additional evidence for the protection of Bam HI cleavage sites by protamine binding.

The use of two sets of cloned DNA fragment lengths reduces the likelihood that these results were generated by a simple DNA fragment size artifact. In other control experiments attempts were made to restrict nucleohistone without removing the histones. As expected, Bam HI did not cleave the NH DNA. Although the presence of histones on DNA would be expected to interfere with Bam HI cleavage, we cannot rule out the possibility that other factors

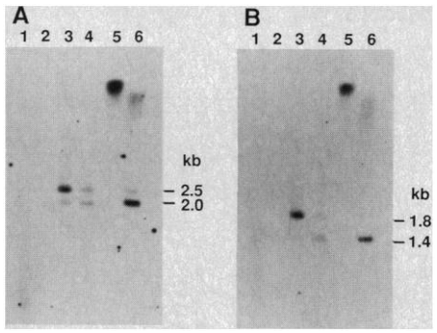

Fig. 3. Southern blot analysis of fractionated nucleohistone DNA and nucleoprotamine DNA from human sperm chromatin. After removal of histones (lanes 1), the chromatin was washed in Bam HI buffer (lanes 2), restricted with Bam HI, and centrifuged. The supernatant (lanes 3) was removed, and the pellet was reextracted with NaCl (lanes 4). DNA in the supernatants (lanes 1 to 4) and pellet (lanes 6) was purified and restricted with Bam HI as described in the text, separated according to size on a 1% agarose gel along with NP DNA that was not restricted after DNA purification (lanes 5), and transferred to nitrocellulose filter membrane. The filter was baked at 80°C for 2 hours and then prehybridized overnight in 5× Denhardt's solution, 3× saline sodium citrate (SSC), and 0.5% SDS with 0.2 mg/ml of salmon sperm DNA at 60°C (*13*). (**A**) A 2.5-kb NH clone and a 2.0-kb NP clone were labeled with ^{32}P by nick translation and simultaneously used as hybridization probes. After hybridization at 60°C for 16 hours, the filters were washed in 0.5× SSC, blotted dry, and autoradiographed. (**B**) The filter was stripped of radioactive DNA by washing in 0.1% SDS at 90°C for 5 minutes. A 1.8-kb NH clone and a 1.4-kb NP clone were used as hybridization probes as described above. In both (A) and (B) the NH clones preferentially hybridized to the NH DNA (lanes 3), and the NP clones preferentially hybridized to the NP DNA (lanes 6).

may also be involved. On occasion, Bam HI failed to cleave the DNA even after removal of the histones, indicating that factors other than histone protection may also contribute to the inhibition of NH DNA restriction.

We conclude that human sperm DNA is packaged into nucleohistone and nucleoprotamine in a sequence-specific manner. The nucleohistone component does not contain a full genomic equivalent, and therefore cannot be exclusively derived from contaminating somatic cells. Our results indicate the nucleohistone component is present in most (not necessarily all) ejaculated sperm. Thus, the bulk of the DNA is condensed and inactivated by protamines, but the remaining DNA is complexed with histones in a fashion that may resemble active rDNA chromatin (*8, 9*). Even though these results do not accurately quantitate the relative distribution of DNA in these two domains, the percentage of NH DNA solubilized by Bam HI digestion (10 to 15%) is consistent with that reported to be complexed with histones by others (*1*).

The biological significance of the nucleohistone component is unknown. The DNA complexed as nucleohistone could be functional either structurally or transcriptionally in the final stages of spermatogenesis or in the early stages of embryonic development. For example, the nucleohistone could designate initiation sites for chromatin decondensation, thus serving a structural role. Furthermore, paternal-specific gene products have been detected in early mammalian development by the two-cell stage (*15*), and this nucleohistone component could contain genes programmed for very early expression.

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Chemical Mimicry: Bolas Spiders Emit Components of Moth Prey Species Sex Pheromones

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Field studies have indicated that bolas spiders attract male moth prey, apparently by mimicking the odor of female moth sex pheromones. Three moth sex pheromone compounds, (*Z*)-9-tetradecenyl acetate, (*Z*)-9-tetradecenal, and (*Z*)-11-hexadecenal, were identified in volatile substances emitted by hunting adult female *Mastophora cornigera* spiders. These compounds are components of pheromone blends that attract some of this spider's moth prey species.

SPIDERS IN THE GENUS *Mastophora* ARE notable because of their minimal "bolas" web (Fig. 1a) and because they capture only one type of prey: male moths. Prey approach spiders from downwind and, in experiments, are attracted into traps that contain hunting spiders. Spiders can be hand-fed a wide variety of moths, but in the field they capture only males of a few species. This evidence suggests that spiders mimic the odor of the sex pheromones emitted by female moths (*1–5*).

We have identified three compounds, identical to sex pheromone components of some moth prey species, in volatile substances collected from hunting *M. cornigera*. To the best of our knowledge, this is the first chemical evidence to support the chemical mimicry hypothesis. Volatiles collected from different spiders varied in blend composition and ratio, which suggests that individuals may alter their blends or that different individuals may produce different blends. The compounds were not found in freshly spun webs, which indicates that they are emitted from an undetermined part of the spider's body. This study furthers our understanding of the chemical ecology of moth-attracting spiders and their possible influence on the evolution of chemical communication in moths.

Volatiles were collected from mated adult female *M. cornigera*. Twenty-four individuals were reared from egg cases laid by female spiders from San Diego, California

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