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Fluorescent In Situ Hybridization of the Telomere Repeat Sequence in Hamster Sperm Nuclear Structures

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Abstract The flat, hooked-shaped architecture of the hamster sperm nucleus makes this an excellent model for in situ hybridization studies of the three dimensional structure of the genome. We have examined the structure of the telomere repeat sequence $(TTAGGG)_n$ with respect to the various nuclear structures present in hamster spermatozoa, using fluorescent in situ hybridization. In fully condensed, mature sperm nuclei, the telomere sequences appeared as discrete spots of various sizes interspersed throughout the volume of the nuclei. While the pattern of these signals was non-random, it varied significantly in different nuclei. These discrete telomere foci were seen to gradually lengthen into linear, beaded signals as sperm nuclei were decondensed, in vitro, and were not associated with the nuclear annulus. We also examined the relationship of telomeres to the sperm nuclear matrix, a residual nuclear structure that retains the original size and shape of the nucleus. In these structures the DNA extends beyond the perimeter of the nucleus to form a halo around it, representing the arrangement of the chromosomal DNA into loop domains attached at their bases to the nuclear matrix. Telomere signals in these structures were also linear and equal in length to those of the decondensed nuclei, and each signal represented part of a single DNA loop domain. The telomeres were attached at one end to the nuclear matrix and extended into the halo. Sperm nuclear matrices treated with Eco RI retained the telomere signals. These data support sperm DNA packaging models in which DNA is coiled into discrete foci, rather than spread out linearly along the length of the sperm nucleus.

Key words: telomere repeat sequences, sperm nuclei, genome, residual nuclear structure, DNA

The mammalian spermatozoa is a unique model for the study of eukaryotic DNA organization. Sperm DNA is biologically inert in that there is no active RNA transcription or DNA replication [Stewart et al., 1984]. This simplifies structural studies of tertiary DNA organization because the large proteinaceous complexes that are necessary for these processes are absent in sperm. Sperm DNA is also highly compacted [Fawcett, 1970; Lalli and Clermont, 1981] both to facilitate transport of the haploid genome to another organism and to protect it during the transit. In mammalian spermatozoa, this condensation is accompanied by the replacement of the somatic cell DNA binding proteins, the histones, by the much more basic, sperm specific protamines [Coelingh and Rozijn, 1975; Herskovits and Brahms, 1976; Bellvé et al., 1975]. The condensation limits the structural components of the sperm nucleus to the bare minimum necessary for structural organization.

Previous work has demonstrated that sperm DNA is organized into DNA loop domains attached at their bases to a sperm nuclear matrix [Ward et al., 1989], and these attachment sites are by specific sequences [Ward and Coffey, 1990; Kalandadze et al., 1990]. This is similar to the three dimensional organization of DNA in somatic cells in which the DNA loop domains are closely correlated with function. Active genes are associated with the nuclear matrix, while inactive genes are not [Getzenberg et al., 1991; Cockerill and Garrard, 1986; Mirkovitch et al., 1987; Stief et al., 1989], and DNA replication occurs at a fixed site on the nuclear matrix at the base of the loop domains [Vaughn et al., 1990; Vogelstein et al., 1980]. That a specific arrange-

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ment of DNA on the nuclear matrix is preserved in the highly condensed sperm nucleus suggests that it is necessary for proper sperm function in an as yet unidentified way. One possibility is that the three dimensional organization of sperm DNA is necessary for proper embryologic development [Ward and Coffey, 1991].

In addition to the nuclear matrix, mammalian sperm nuclei contain a second structural element termed the nuclear annulus, located at the base of the nucleus, associated with the tail [Ward and Coffey, 1989]. This structure is unique to sperm cells, and functions as an anchor for the entire genome to a single point when the nucleus is completely decondensed. The purpose for this type of DNA anchoring is unknown, but it is possible that it serves as a DNA organization center during fertilization.

In this work, we have used the mammalian telomere repeat sequence $(TTAGGG)_n$ to further explore sperm DNA organization by the nuclear matrix and the nuclear annulus. This repeated sequence was isolated from human telomeres [Moyzis et al., 1988] and was shown to be present in at least 100 vertebrate species, including the species used in this study, the Syrian golden hamster [Meyne et al., 1990]. While the repeated sequence was also present in other sites in the hamster, the great majority of the signal was associated with chromosomal telomeres. The length of the telomeric repeat decreases with age [Hastie et al., 1990] and is longest in sperm DNA [de Lange et al., 1990; Allshire et al., 1989; Cross et al., 1989]. We examined the localization of the telomeric repeat with respect to the various sperm nuclear structures previously identified, and compared this with somatic cell nuclear matrices from the same species.

MATERIALS AND METHODS

Preparations of Sperm Nuclear Structures

For preparations of sperm nuclear matrices with DNA intact, hamster sperm nuclei were prepared from SDS washed spermatozoa, as described [Ward et al., 1989] with some modifications. Briefly, caudae epididimydes were dissected and the caudal fluid teased out with a pair of forceps. The spermatozoa containing fluid was homogenized in a Dounce homogenizer with 50 mM Tris, pH 7.4, and 0.5% SDS, to separate the heads from the tails. The suspension was layered onto a two layer sucrose step gradient in a Beckman SW-28 tube. The bottom layer contained 2.0 M Sucrose, 0.075 g/ml CsCl, 25 mM Tris, pH 7.4, and the second layer contained the same solution without CsCl. The three layer step gradient was centrifuged at 25K rpm, × 1.5 h. The pelleted nuclei were extracted with 2 M NaCl, 10 mM dithiothreitol (DTT), 25 mM Tris, pH 7.4 for 30 min on ice and then 10 min at 37°C. The extracted nuclei were put onto microscope slides at 4°C, and incubated for 20 min in the cold. The slides were then washed in 10 mM Tris, pH 7.4, and dried o/n at room temperature. The slides were then fixed by incubating in ethanol for 20 min then in two washes of 3:1 methanol:acetic acid for 20 min on ice. They were then dried and used immediately for in situ hybridization.

For sperm nuclear matrices that contained only matrix associated DNA, nuclei were prepared as above, and then treated with Eco RI, as described [Ward and Coffey, 1991]. Briefly, salt extracted nuclei were centrifuged, resuspended in reaction buffer and treated with Eco RI (0.5 U/μl) for 2 h at 37°C. The digested matrices were then centrifuged, and washed once with 2 M NaCl, 25 mM Tris, pH 7.4, and centrifuged again. These matrices, which contained less than 5% of the total DNA, were then fixed, and hybridized as described in the next section, In Situ Hybridization.

Sperm nuclei prepared from SDS washed spermatozoa were also extracted with 300 mM $CaCl_2$ and 10 mM DTT in order to expose the DNA to the fluorescent probes while retaining the shape of the nucleus [Bellvé et al., 1992]. This extracts only one form of the protamine that allows the nuclei to remain intact while exposing the condensed DNA to probes.

For decondensed nuclei, nuclei were prepared from spermatozoa that were washed in the nonionic detergent NP-40 as described [Ward and Coffey, 1989]. Briefly, spermatozoa were extracted from caudae epididymides and sonicated in 50 mM Tris, pH 7.4, to break off the tails. A three layer step gradient was then made in Beckman SW-28 tubes, with 2.82 M CsCl (density = 1.35 g/ml), 25 mM Tris (pH 7.4), 5 mM $MgCl_2$, 0.25% (v/v) on the bottom, 2.2 M sucrose, 50 mM Tris, 5 mM $MgCl_2$ (pH 7.4) in the second layer, and the sonicated sperm suspension in the top. The tubes were centrifuged at 20.5K rpm × 45 min, and the pelleted nuclei were suspended in 2 M NaCl, 25 mM Tris, pH 7.4. These nuclei were decondensed with 10 mM DTT, and placed on microscope slides and fixed as described previously.

In Situ Hybridization

Slides were prewarmed at 37°C in an incubator for 1 h, then dehydrated through 70%, 80%, and 95% ethanol on ice, for 2 min at each step, then dried completely using a hairdryer at low setting. The slides were then incubated at 75°C in 70% formamide to denature the DNA, and dehydrated again through successive cold ethanol washes, and dried once more. During this drying step, the probe was prepared. The double stranded $(TTAGGG)_4$ biotin labeled probed was obtained from Oncor. The probe was incubated at 37°C for 5 min, then denatured by incubation at 75°C (the Oncor probe is shipped in formamide to help denature it), and put immediately on ice until used. For hybridization, 10 μl of the probe was added to each slide, each slide was covered with a coverslip and sealed with rubber cement. These were incubated overnight at 37°C in an humidified incubator. The slides were washed by incubation in 35% or 45% formamide for 20 min at 40°C, and then in two washes of 2 × SSC (0.3 M NaCl, 0.03 M NaCitrate) for 4 minutes each, at 37°C, and finally in PBD ("phosphate buffered detergent" Oncor, Inc.) for 4 min at room temperature. The slides were detected with biotin avidin system according to the instructions in the kit from Oncor, Inc. [Cat# Stief et al., 1989 70-CF]. Briefly, 20 μl of Blocking Reagent 1 (PBD and goat serum) was added to each slide, and the slides incubated at room temperature for 5 min. The slides were then drained, and fluorescein-labeled avidin was added to each slide, and they were incubated for 20 min at 37°C in a humidifier chamber. The slides were then washed 3 times in PBD at room temperature for 2 min each wash. Blocking Reagent 2 (PBD and nonfat dry milk) was then added to each slide for 5 min. This was drained, and 20 μl of anti-avidin antibody (in blocking reagent 2) was added to each slide, and they were incubated for 20 min in a 37°C humidifier. The slides were then washed three times in 40 ml of PBD at room temperature for 2 min each. The slides were then stained with propidium iodide and examined under the microscope.

RESULTS

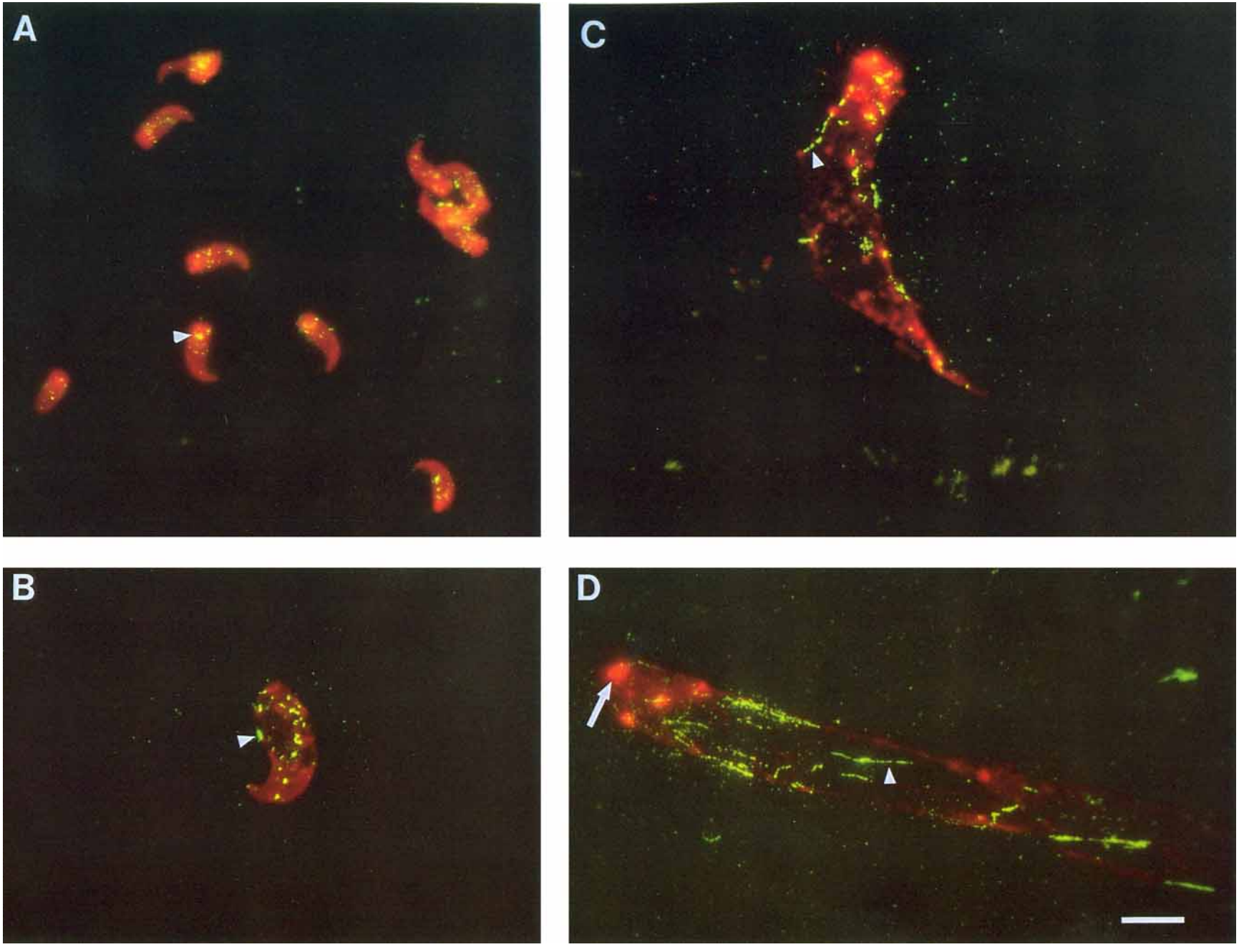
Condensed Sperm Nuclei

We first examined the location and appearance of the telomere signal in the fully condensed hamster sperm nucleus. In situ hybridization using mature sperm nuclei is difficult because the sperm DNA is so highly condensed [Fawcett, 1970; Lalli and Clermont, 1981; Wyrobek et al., 1990] and many investigators have developed methods for partially decondensing the nuclei before hybridizing probes [Wyrobek et al., 1990]. We treated hamster sperm nuclei with 300 mM $CaCl_2$ before in situ hybridization to extract a small portion of the protamines [Bellvé et al., 1992]. This procedure does not distort the shape of the nuclei, but exposes the chromatin enough for the probe to hybridize to the sperm DNA. Using this method, the telomere probe $(TTAGGG)_n$ gave a pattern of localized foci within the mature sperm nuclei (Fig. 1A). The spots were most prominent in the basal area of the sperm nucleus and few were seen in the hooked portion. There were no signals localized to the implantation fossa where the nuclear annulus is located. The pattern of telomere signals in fully condensed sperm nuclei varied in each nucleus.

Decondensed Sperm Nuclei

We next examined the appearance and location of telomeres in hamster sperm nuclei as they decondensed. Hamster sperm nuclei prepared from spermatozoa that were not washed in SDS decondensed when the protamines were fully extracted with 2 M NaCl and 10 mM DTT [Ward and Coffey, 1989]. Nuclei at various stages of decondensation were mounted on slides and hybridized to the telomere probe (Fig. 1B–D). At early stages of decondensation, the localized foci of hybridization signals became larger spots (Fig. 1B). The hooked shape of the nucleus could still be seen, although the entire nucleus became larger as it started decondensing (Figs. 1A–D are all at the same magnification). At later stages the signals became more linear (Fig. 1C) and at complete condensation, the telomere signals became long stretches of signals with a beaded appearance (arrowhead, Fig. 1D). This beaded appearance of the telomere signal is similar to the satellite DNA I sequences in synaptonemal complexes seen by Moens and Pearlman [Moens and Pearlman, 1989].

When sperm nuclei are completely decondensed, the localization and function of the nuclear annulus can be easily identified. Figure 1D is a micrograph of a single hamster sperm nucleus that is completely decondensed so that no nuclear structures remain except the nuclear annulus (arrow, Fig. 1D), and all the DNA (stained red by propidium iodide) is anchored to


A
C
B
D

the annulus. In this and all other examples the telomere signals were never associated with the nuclear annulus, and when isolated nuclear annuli were probed with the telomeric repeat, no signal was seen (data not shown). These data suggest that the DNA that binds to the annulus is not telomeric.

Telomeres in Sperm DNA Loop Domains

We next determined the relationship of sperm telomeres to the sperm nuclear matrix. Figure 2 demonstrates in situ hybridization of the $(TTAGGG)_n$ repeated sequence with hamster sperm nuclear matrices that retain the full complement of DNA. Sperm nuclei were prepared from SDS-washed spermatozoa so that when the protamines were extracted with 2 M NaCl and 10 mM DTT the nuclear matrices remained intact [Ward et al., 1989]. This extraction results in a halo of DNA surrounding the nucleus, as can be seen in Figure 2A. This halo represents the sperm DNA loop domains extending out of the nucleus, but attached at their bases to the nuclear matrix [Ward and Coffey, 1990; Kalandadze et al., 1990].

Hybridization of the telomere repeat to these nuclear matrices resulted in linear beaded signals (Fig. 2B) identical in appearance and length to those seen in the fully decondensed nuclei (Fig. 1D, all micrographs are at the same magnification). In both cases, the DNA is unbound by protamine and free to extend linearly into solution. In the decondensed nucleus (Fig. 1D) the DNA is very long, being anchored only by the nuclear annulus at one point. In the nuclear matrix, the DNA is present in loop domains with an average length of 47 kb [Ward et al., 1989]. Therefore, an in situ hybridization signal with a DNA target of less that 23 kb (i.e., half a loop domain) are likely to appear fully extended. Each of the linear signals associated with the sperm nuclear matrix corresponds to a single DNA loop domain. The linear telomeric signals appear to initiate at the periphery of the nucleus (Fig. 2B) and then extend out into the DNA halo that surrounds the matrix (the halo of the same nucleus is shown more clearly in Fig. 2A). Some signals appear not to be associated with the sperm nuclear matrix, but most clearly have one end associated. None of the signals were seen to bend at the periphery of the halo, as if they were in the top part of the loop.

In support of a nuclear matrix attachment site for telomere signals in hamster spermatozoa, nuclear matrices were treated with Eco RI before fixation and in situ hybridization. By this treatment, all DNA that is part of a loop domain located distal to the restriction site closest to the nuclear matrix (greater than 95%) is released into solution. Only the DNA most closely associated with the nuclear matrix, at the base of the loop, remains attached to the structure. The telomere repeat sequence does not contain a restriction site for Eco RI, so if it is attached to the nuclear matrix the signal should remain associated with the structure after Eco RI treatment. As shown in Figure 2C, Eco RI treatment removed most of the DNA so that the matrices no longer contained a halo of fluorescence; however, the telomere signals remained associated with the Eco RI digested matrices (Fig. 2D). The telomere signals on the digested matrices do not appear as linear, beaded strands as they do in the decondensing nuclei (Fig. 1D) or in the halo structures (Fig. 2B). This is probably because so little DNA is present (less than 5%) that many non-specific binding sites on the nuclear matrix are available for the matrix associated DNA to bind. Also, the presence of less DNA offers less electrostatic repulsion of neighboring DNA that forces the DNA loop domains into the more uniform halo structure when all the DNA is present.

Fig. 1. Telomere specific in situ hybridization of condensed and decondensing hamster sperm nuclei. A probe for the telomere repeat $(TTAGGG)_n$ was hybridized to isolated hamster sperm nuclei at various stages of decondensation. All figures (**A–D**) are shown at the same magnification. A: Nuclei treated with 300 mM $CaCl_2$ and 10 mM DTT to decondense the chromatin just enough to allow hybridization. These nuclei are the same size and shape as fully condensed sperm nuclei. The telomere signals appear as yellow spots in the nuclei because the green (FITC) telomere signal is combined with the red (PI) DNA signal. B: A nucleus decondensed with 2 M NaCl and 10 mM DTT to begin complete decondensation. The telomere signals now appear green (arrowhead) because the PI stained DNA (red) is starting to dissipate. C: A nucleus at a more fully decondensed stage. The telomere signals are beginning to lengthen (arrowhead). D: Part of a fully decondensed sperm nucleus. The telomere signals are now fully decondensed into linear, beaded strands (arrowhead). (arrow, the nuclear annulus). Bar = 10 μm.

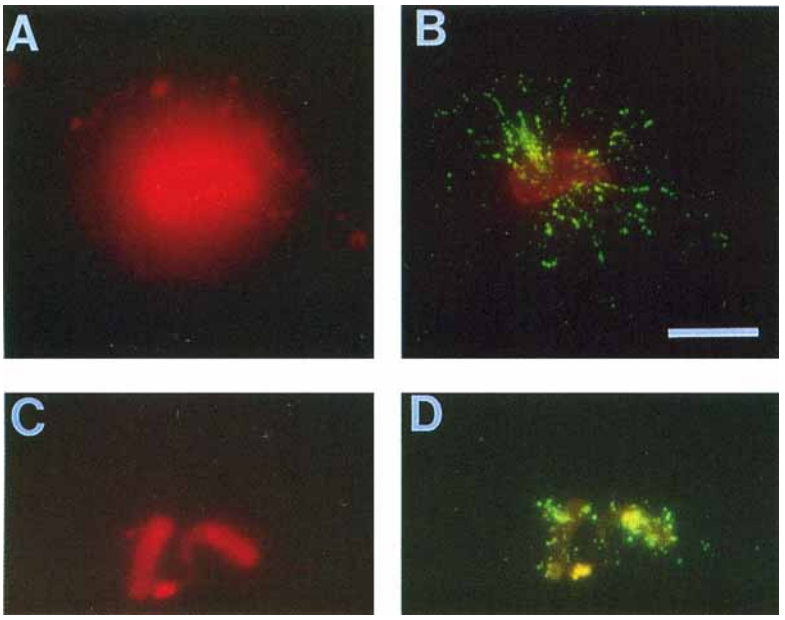


Fig. 2. Telomere association with the sperm nuclear matrix. Hamster sperm nuclear matrices with their entire complement of DNA were prepared and hybridized to the telomere specific repeat $(TTAGGG)_n$ probe. **A:** A single sperm nuclear matrix stained with the fluorescent dye propidium iodide to visualize the DNA. A halo of fluorescence surrounds the visible outline of the sperm nuclear matrix. The halo represents DNA loop domains attached at their bases to the nuclear matrix. The sperm nuclear matrix retains the original size and hooked shape of the nucleus. **B:** The same nucleus as in A, photographed with a green filter. The nucleus was hybridized to the telomere probe (green), and some of the DNA staining remains visible (red). The telomeres appear as linear, beaded signals eminating from the sperm nuclear matrix. **C:** Two sperm nuclear matrices that have been digested with Eco RI, hybridized to the telomere repeat probe (green) and stained with propidium iodide. **D:** The same two nuclei as in C photographed with the green filter. Teleomere signals are condensed back onto the nuclear matrix, but are still present. All figures are shown at the same magnification as all panels in Figure 1. Bar = 10 μm.

Telomere Lengths

The lengths of various telomere repeats units have been measured by Southern blots and were estimated in human spermatozoa to be greater than 22 kb by Hastie et al. [1990] and between 10 to 14 kb by de Lange et al. [1990]. The completely linearized strands of DNA present in the decondensed sperm heads allow for the measurement of individual telomere hybridization signals directly. We measured the lengths of several telomere signals such as the one shown by the arrowhead in Figure 1D, and found a wide variation in length, from as high as 42 kb to as small a 10.5 kb. Two signals were greater than 50 kb, but their exact lengths were uncertain.

DISCUSSION

In this study we have used fluorescent in situ hybridization of a repeated subset of the genome localized to a specific chromosomal structure, the telomere, to extend our previous studies of the organization of the entire sperm genome DNA [Ward and Coffey, 1991] to more specific chromosomal loci. The telomere repeat $(TTAGGG)_n$ is particularly useful in this endeavor because it recognizes several strands of DNA that in sperm nuclei are large [up to 14 kb, de Lange et al., 1990]. Thus, individual strands of specific sequences of DNA are easily recognizable, and their relationships to nuclear structures can be easily discerned. The hamster sperm nucleus is a useful model for studying the relationship between nuclear structures and DNA organization because the nucleus can be manipulated biochemically to form several types of structures. The nuclei may be decondensed so that the DNA packaging is gradually unfolded, and this can be examined at several stages (Fig. 1), and the decondensing nuclei retain a structural landmark, the nuclear annulus, which serves to orient the DNA fibers. Hamster sperm nuclei will also form nuclear matrix-halo structures in which the DNA loop domains remain intact, attached at their bases to a sperm nuclear matrix, so that with fluorescent in situ hybridization, individual loop domains may be visualized. The hamster sperm nucleus therefore provides

a unique model with which to probe eukaryotic nuclear structure.

Mapping DNA Sequences Within the Sperm Nucleus

The flat, hook-shaped structure of the hamster sperm nucleus is an essentially two dimensional structure, whereas the spheroid shaped somatic cell nuclei are more three dimensional. Thus, mapping studies of genetic elements within the nucleus that are difficult in somatic cells become more possible in sperm nuclei. Our result using the telomere probe in fully condensed nuclei indicated that the patterns of the telomere signals varied in each nucleus. This suggests that the chromosomes are not packaged into precise positions during spermiogenesis, but that there is a certain plasticity to the spacial arrangement of chromosomes within the hamster sperm nucleus. Individual DNA loop domains, however, are more precisely arranged with respect to the nuclear matrix. Each DNA loop domain, on average 47 kb for the hamster sperm nucleus, is associated with the nuclear matrix at a specific sequence [Ward and Coffey, 1990; Kalandadze et al., 1990]. These data suggest that the specificity of DNA organization within the sperm nucleus may be more specific at the molecular level than at the microscopic level.

Association of Telomeres With Sperm Nuclear Structures

In situ hybridization of the telomere repeat to sperm nuclear matrices demonstrated that the telomeres were extended into the individual loop domains that are associated with the nuclear matrix (Fig. 2B). One end of most of the linear signals usually appeared to be associated with the nuclear matrix, suggesting that the telomeric repeats might be very closely associated with a nuclear matrix binding region. This was confirmed by the continued association of the telomeres with nuclear matrices treated with a restriction endonuclease (Fig. 2D). It is interesting to speculate that telomerase [Shippen-Lentz and Blackburn, 1990] may increase the length of the telomeres while it is associated with the nuclear matrix, just as DNA replication is known to occur [Vaughn et al., 1990; Vogelstein et al., 1980]. This might result with one end of the telomere, the last to be synthesized, being associated with the nuclear matrix as in Figure 2B.

These results are consistent with those of de Lange [1992], who has recently demonstrated that in several human cell lines telomeres are associated with the nuclear matrix. de Lange also reported that when the telomeric repeats induced at chromosomal internal sites by transfection are not associated with the matrix, suggesting that its position at the end of the chromosome was necessary for its association with the nuclear matrix. Our results provide a visual corroboration to de Lange's conclusions, and further suggest that, at least in spermatozoa, only one end of the chromosome is attached to the nuclear matrix.

The telomere repeats were not associated with the nuclear annulus, the structure that anchors all the DNA to one point when the sperm nucleus decondenses [Ward and Coffey, 1989]. This anchoring function might suggest that the annulus bound to a common chromosomal structural unit, such as the telomeres or centromeres. Previous in situ hybridization [Macgregor and Walker, 1973] and immunohistochemical [Haaf et al., 1990] studies have demonstrated that the centromeres are unlikely candidates for binding to the nuclear annulus because they are distributed throughout the sperm nucleus. Part of the reason that we used the telomere repeat as a probe was to determine if telomeres were associated with the nuclear annulus, and our data suggest that they are not (Fig. 1A–D). This suggests that another, as yet unidentified, sequence is associated with the annulus.

Beaded Appearance of the Telomeric In Situ Hybridization

The beaded appearance of the signal is similar to that seen by Moens and Pearlman for the centromeric DNA I satellite sequences [Moens and Pearlman, 1989]. These authors suggested that the beaded appearance corresponded to the periodicity of the satellite sequence for DNA I. The telomere repeat sequence is usually present as a continuous, uninterrupted sequence, but it is variable [de Lange et al., 1990; Brown et al., 1990]. Furthermore, spermatozoa have much longer telomeres than other cell types [de Lange et al., 1990; Allshire et al., 1989; Cross et al., 1989], and it is possible that the variation in length of the repeated sequence includes some interruptions in the repeat. An alternative explanation may be found in the extreme length of the target DNA, the telomere repeat sequence which in spermatozoa may be as large as 14 kb

[de Lange et al., 1990]. It is possible that the opposite DNA strand offers significant steric hindrance, and that the beaded appearance represents an equilibrium between the hybridization of the probe and the opposite DNA strand of the chromosome.

DNA in Mammalian Spermatozoa Is Coiled or Folded Within the Nucleus

Finally, the data in this study suggest that mammalian sperm DNA is coiled or folded in some manner in the fully decondensed nuclei. This is most readily seen when comparing the telomere hybridization signals in the fully condensed sperm nuclei (Fig. 1A) to that of the uncoiled DNA in the fully decondensed sperm nuclei (Fig. 1D) or in the nuclear matrix halo structures (Fig. 2B). By decondensing the nuclei, each telomere signal unfolded or uncoiled from a discrete spot (Fig. 1D) into a gradually more lengthened signal as the DNA was released from the sperm nucleus (Fig. 1B–D). The same phenomenon was noted when the DNA loop domains were unfolded on the nuclear matrix (Fig. 2B). This packaging might not have been predicted from the current model of protamine-DNA interaction in which the protamines bind to DNA in linear fashion along the minor groove [Balhorn, 1982]. Unlike models for somatic cell DNA packaging in which histone binding coils the DNA into nucleosomes [McGhee and Felsenfeld, 1980] and then into solenoids [Finch and King, 1976], the protamine binding model suggests no type of folding or coiling of the DNA [Balhorn, 1982]. The result that protamine bound hamster DNA is coiled into discrete foci suggests that protamines may confer upon the DNA some degree of folding. These results, and results from others [Allen et al., 1991], led to the proposal of a modified model of protamine-DNA binding that has recently been proposed [Ward, 1993]. In this model, each DNA loop domain is coiled into a toroidal shaped structure in which the sperm chromatin is tightly packaged, as previously described [Balhorn, 1982], but coiled slightly so that long stretches of DNA (47 kb) may be localized into discrete foci, as demonstrated in this work. The data are also consistent with other models [Koehler et al., 1983] in which sperm DNA is coiled into the sperm nucleus.

Regardless of which aspects of which models of DNA packaging will ultimately prove to be correct, the data in this work have demonstrated that large stretches of individual telomere repeat sequences (up to 42 kb in this work) are tightly compacted into discrete foci within the sperm nucleus. Furthermore, the data have presented direct visualization of the association of telomeres with the sperm nuclear matrix.

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