Vol. 173, No. 1, 1990 BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS
November 30, 1990 Pages 20-25

SPECIFIC ORGANIZATION OF GENES IN RELATION TO THE SPERM NUCLEAR MATRIX+

W. Steven Ward*[1] and Donald S. Coffey[2]

[1]The Division of Urology, Robert Wood Johnson Medical School, New Brunswick, NJ

[2]The Department of Urology and The Oncology Center, Johns Hopkins University School of Medicine, Baltimore, MD

Received October 10, 1990

We have recently reported that mammalian sperm DNA, the most highly condensed and functionally inert eukaryotic DNA, is organized into DNA loop domains attached at their bases to a sperm nuclear matrix (Chromosoma, 98: 153, 1989). We report here the specific arrangement of genes within the sperm loop domains by measuring the proximity of six hamster genes to the sperm nuclear matrix. After restriction endonuclease treatment of sperm nuclear matrices, five genes were clearly not associated with the sperm nuclear matrix and only one, αA-crystallin, was within 2 Kb of the matrix. This suggests that sperm DNA is organized in a specific manner in relation to the sperm matrix.

Mammalian sperm DNA is very tightly packaged, being more than six fold more highly condensed than the DNA in mitotic chromosomes (1,2). This sperm DNA is packaged by protamines into linear arrays (2), thereby differing substantially from somatic cell DNA packaging by histones into coiled nucleosomes (3). We have previously demonstrated, however, that mammalian sperm DNA organization resembles that of somatic cells in that both are organized into DNA loop domains that are attached to a nuclear matrix (4,5). The sperm DNA loop domains differed from those of adult, somatic cells in that the sperm DNA loops were only 60% as large (4). Furthermore, unlike most other biological DNA known which is negatively supercoiled, sperm DNA is not supercoiled (4,6).

The DNA loop domains in somatic cells are very specifically organized by their attachments to the nuclear matrix (7-10), that is thought to organize the entire length of DNA, three dimensionally, throughout the nucleus (7,8,11,12). The specific attachment sites of the DNA loop domains to the nuclear matrix are directly related to function, since both active genes (9,10,13,14) and replication origins (15-17) have been shown to be associated with the nuclear matrix.

*To whom correspondence should be addressed.

+This work was supported by HHS Grant DK 22000.

0006-291X/90 $1.50

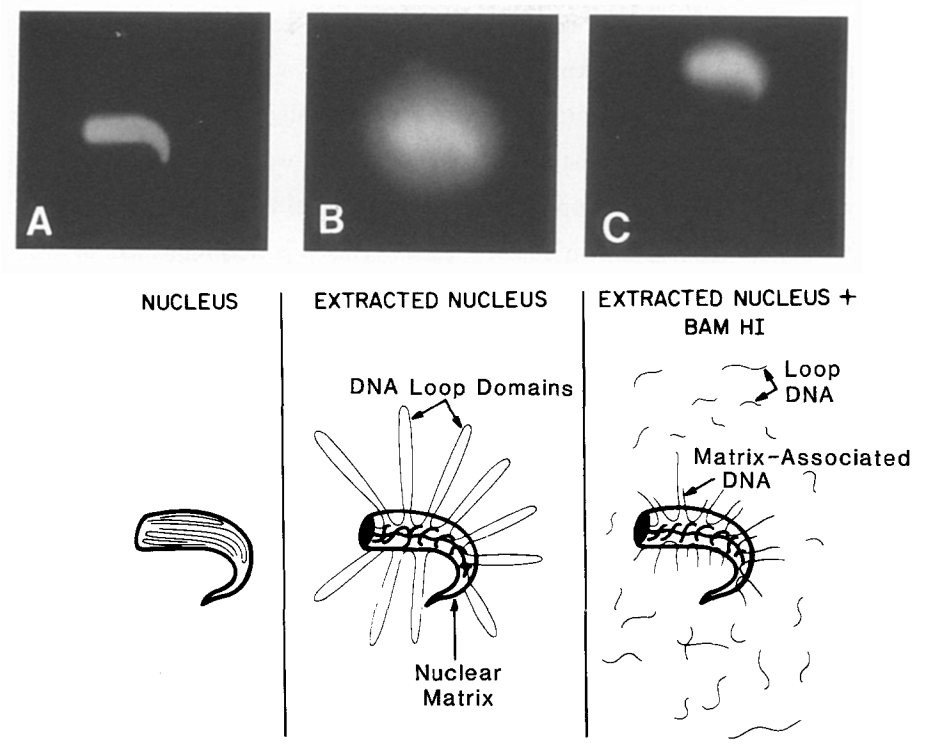

Figure 1. Treatment of Hamster Sperm nuclei with restriction endonucleases.
A comparison of various preparations of hamster sperm nuclei stained with DAPI (0.1 μg/ml) to reveal the DNA. The diagrams below each fluorescent photomicrograph illustrate the structure of the DNA loop domains in each figure. (A) An isolated hamster sperm nucleus. The loops are drawn as being packaged in straight, linearly compacted arrays because previous work has demonstrated that they are not supercoiled (2,3). The nucleus does not stain as brightly as the extracted nucleus in (B) because here the DNA is packaged too tightly to allow access of all the chromatin to the DAPI. (B) A sperm nucleus from which the protamines have been extracted to form a nuclear matrix. Staining with DAPI reveals the fluorescent halo of exposed DNA loop domains attached to the sperm nuclear matrix. The halo is 8 $\pm$ 1 μm wide, which corresponds to an average loop size of approximately 46 Kb. (C) A nuclear matrix that has been treated with Bam HI; the DNA that is released into solution is termed Loop DNA, while the DNA that remains associated with the nuclear matrix is termed Matrix-Associated DNA. The Matrix-Associated DNA represents 15% of the total DNA.

1A) were extracted with 2 M NaCl and 10 mM dithiothreitol to make sperm nuclear matrices that were devoid of protamines but retained their full complement of DNA. When stained with the DNA binding, fluorescent dye, DAPI, the extended loop domains formed a halos around the nuclear matrix (Figure 1B) (4). The DNA loop attached to the nuclear matrices were then digested with either Bam HI or Eco RI, and the fluorescent halo of DNA loop domains that surrounds the nuclear matrix (Figure 1B) became much smaller. This was because each loop domain was digested at specific sites by the enzyme, releasing a portion of its DNA into solution, termed Loop DNA. The diagrams under each fluorescent micrograph in Figure 1 illustrate how individual DNA loop domains behave under each condition. The insoluble Matrix-Associated DNA, the DNA that remained

Most of the specific attachment sites of DNA to the nuclear matrix have been located near genes or at replication origins, but sperm DNA is functionally inert, undergoing neither of these processes. Furthermore, sperm chromatin is structurally very different from somatic cell DNA, as described above. Given these differences, it was pertinent to address the question of whether sperm DNA loop domains were attached to the sperm nuclear matrix in a specific manner, as they are in somatic cells. This was accomplished by using six different hamster genes as probes for determining their association to the sperm nuclear matrix.

MATERIALS AND METHODS

Isolation of Hamster Sperm Nuclei: Hamster sperm nuclei were isolated as previously described (4). Briefly, fresh hamster cauda spermatozoa were washed in 0.5% SDS, which extracts the membranes and separates the heads from the tails, and the nuclei were separated by sucrose density gradient centrifugation.

Isolation of Matrix-Associated DNA and Loop DNA: To form the sperm DNA-nuclear matrix halo structure, isolated hamster sperm nuclei were extracted with 2 M NaCl, 10 mM dithiothreitol, and 25 mM Tris, pH 7.5, at 37°C for 30 min, and then centrifuged at 9,000 x g for 30 min. The pellet was resuspended in 20 volumes of 50 mM Tris, pH 8.0, 10 mM $MgCl_2$, making the final NaCl concentration 100 mM. The halo structures were then treated with restriction endonucleases as follows. One twentieth of the final volume of either Bam HI or Eco RI (10 U/μl) was added, and the suspension was incubated for 4 hr at 37°C. The digested nuclei were then diluted with one volume of 4 M NaCl, and incubated for an additional 10 min at 37°C. They were then centrifuged at 9,000 x g for 30 min. The supernatants, which contained the soluble Loop DNA were precipitated with 70% ethanol, and resuspended in 0.5 ml of 10 mM Tris, 1 mM EDTA, pH 8.0. The pellets, which contained the nuclear matrices and the Matrix-Associated DNA, were resuspended in 0.5 ml of 0.5% SDS, 100 mM Tris, pH 9.0, that contained 100 μg/ml proteinase K, and incubated overnight at 55°C. Both the Matrix-Associated DNA and the Loop fractions were then extracted with 1:1 (v/v) phenol/chloroform, and precipitated with 70% ethanol.

Southern Blots: To ensure complete digestion, the isolated DNAs were redigested with either Bam HI or Eco RI, depending on the enzyme that was used initially to isolated the DNA from the sperm nuclear matrices. The redigested DNA was used for Southern hybridization (18). GeneScreenPlus filters were used, and filters were probed and washed according to the manufacturer's specifications.

Probes: All probes used were full length cDNAs from the Syrian golden hamster. The α_1-adrenergic receptor gene was a kind gift of Dr. Robert Lefkowitz. The vimentin, αA-crystallin, and desmin probes were a kind gift of Dr. Hans Bloemendal. The 5S RNA gene was a kind gift of Dr. William R. Folk. The c-fos gene was a kind gift of Dr. Jeff Boyd and Dr. J. Carl Barrett.

RESULTS

To test for the specific organization of genes within sperm loop domains, we isolated Matrix-Associated DNA and Loop DNA from restriction endonuclease treated sperm nuclear matrices, as follows (Figure 1). Detergent washed hamster sperm nuclei (Figure

associated with the nuclear matrix after restriction endonuclease digestion, could then be easily separated by centrifugation from the Loop DNA that remained in solution. Approximately 15% of the total DNA remained associated with the sperm nuclear matrix after digestion with Bam HI, and 5% of the total remained with the nuclear matrix after Eco RI digestion.

Loop or Matrix-Associated DNAs were further purified by protease digestion and phenol extraction, redigested with the same restriction enzyme used in the original digestion, and used for Southern hybridizations using six different hamster genes as probes (18). These genes were chosen because they were cloned from the same species, Syrian golden hamster. The six genes recognized eight different bands on Southern blots, depending on the restriction endonuclease used to digest the DNA (Figure 2). The amount of each gene was determined on a per μg DNA basis; each lane contained 10 μg of either Loop DNA (L) or Matrix-Associated DNA (M). Four of the six genes, the α_1-adrenergic receptor, desmin, vimentin, and c-fos, were clearly absent from Matrix-Associated DNA and found only in the Loop DNA fractions. Of the remaining two genes tested, the 2 Kb Bam HI fragment of the 5S RNA gene was not associated with the nuclear matrix while the 12 Kb Eco RI fragment was. Since the coding region of the 5S RNA gene represents only 800 bp of the Bam HI 2 Kb fragment (19), this gene may be as much as 12 Kb from a matrix attachment site.

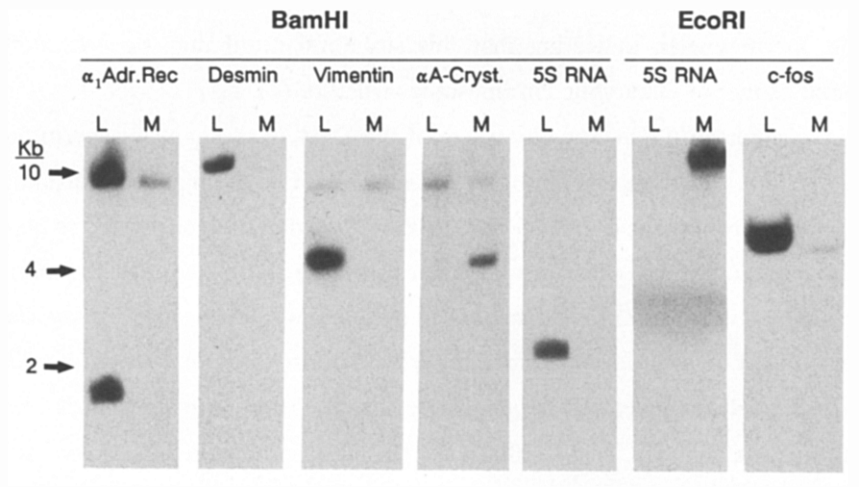

Figure 2. Southern Blot showing the Distribution of genes between the Loop DNA and Matrix-Associated DNA.

Six genes were used as probes for the specificity of sperm DNA loop domain arrangement. Isolated Loop and Matrix DNAs prepared as described in Methods were redigested with the same restriction endonuclease used to treat the nuclear matrices. Each lane contains the same amount (10 μg) of either Loop (L) and Matrix-Associated DNA (M) from nuclear matrices treated with either Bam HI or Eco RI were probed with six different DNA probes, using standard techniques. Of all the eight bands detected with these probes, only two, the 4 Kb Bam HI band of the αA-crystallin gene and the 12 Kb band of the 5S RNA gene, were enriched with the Matrix-Associated DNA (M).

Thus, only one gene of the six tested, the αA-crystallin gene, is within 2 Kb of the sperm nuclear matrix.

Of the eight genomic bands recognized by the six probes used in this study, only two, the 4 Kb Bam HI fragment of the αA-crystallin gene, and the 12 Kb Eco RI fragment of the 5S RNA gene were associated with the matrix (Figure 2). All other bands, ranging from 0.8 Kb to 10 Kb in size, were found in the loop fraction. The range in sizes is important because it means, for example, that the 4 Kb fragment of the αA-crystallin gene is not non-specifically binding to the nuclear matrix because of its size. Both larger and smaller fragments (such as the 10 Kb and the 0.8 Kb Bam HI fragments of the α_1-adrenergic receptor gene) wash off the matrix, completely. Since any one band is either in the Loop DNA fraction or in the Matrix-Associated DNA fraction, the attachment sites of DNA loop domains to the sperm nuclear matrix must be specific, not random.

DISCUSSION

The data presented here indicate that the DNA loop domains present in the mammalian sperm nucleus are organized in a specific, non-random manner on the sperm nuclear matrix. Mammalian sperm DNA is too highly condensed to allow for nucleosome or solenoid organization of the chromatin, and so these structures were sacrificed in the evolutionary pressure that caused mammalian sperm DNA to become so condensed (1,2). But the specific organization of the DNA into loop domains attached to a nuclear matrix is still present in sperm nuclei, indicating that this structural motif may be one of the most fundamental aspect of eukaryotic chromosome structure (4).

We have reported the average loop size of the DNA in the hamster sperm nucleus to be 46 Kb (4), thus 23 Kb, or one half this loop size, would be the maximum distance a gene could be located from the sperm nuclear matrix. Although more genes need to be checked, one can now speculate that most genes appear not to be associated with the matrix. This is in agreement with current models that postulate that only active genes are associated with the nuclear matrix of somatic cells. Since there is no transcription in the sperm nucleus, this model predicts that no genes will be associated with the sperm nuclear matrix. In support of this model, Kalandadze, *et al.* (20) found that the permanent attachment site of the chicken α-globin is bound to the chicken sperm nuclear matrix, but the attachment site of the α-globin gene that is associated with transcription was not (20). The reason that the αA-crystallin gene is within 2 Kb of the sperm nuclear matrix may be that a similar type of organization may be present. Thus, one may speculate that there is a very fundamental type of loop attachment to the nuclear matrix that is not related to transcription that is necessary for chromosomal organization. This attachment site may be similar to the permanent attachment site of the α-globin gene described by Kalandadze, *et al.* (20). Even in the

somatic cell most of the genome is not transcribed, but it must all be organized, even in the inert DNA of spermatozoon. It is probable that the attachment sites in sperm are the most fundamental of all, and, therefore, the spermatozoon will provide a good model for studying nuclear matrix - DNA interactions.

Finally, two lines of evidence suggest that the specific organization of sperm DNA is important for its function. The first is that sperm chromatin structure has been shown to be an important factor in fertility of bulls (21). The second is that *Xenopus* oocyte extracts were demonstrated to be able to initiate and complete one full round of DNA synthesis when demembranated sperm heads were used as templates, but DNA synthesis of purified sperm DNA was at least 10 fold less efficient, suggesting that efficient DNA synthesis required the proper DNA organization that was maintained within the sperm nucleus (22). These data suggest that sperm DNA organization may be essential to sperm function. The data presented in this report represent the first steps towards the elucidation of this specific organization in mammalian spermatozoa.

REFERENCES

1. Pogany,G.C., Corzett.M., Weston,S. and Balhorn,R. (1981) Exp. Cell. Res., 136, 127-136.
2. Balhorn R. (1982) J. Cell Biol., 93, 298-305.
3. McGhee JD, Felsenfeld F. (1980) Ann. Rev. Biochem., 49, 1115-11156.
4. Ward,W.S., Partin,A.W. and Coffey,D.S. (1989) Chromosoma, 98, 153-159.
5. Vogelstein,B., Pardoll,D.M. and Coffey,D.S. (1980) Cell, 22, 79-85.
6. Risley,M.S., Einheber,S. and Bumcrot,D.A. (1986) Chromosoma, 94, 217-227
7. Nelson,W.G., Pienta,K.J., Barrack,E.R. and Coffey,D.S. (1986) Ann. Rev. Biophys. Chem., 15, 457-475.
8. Pienta,K.J., Partin,A.W. and Coffey,D.S. (1989) Cancer Res., 49, 2525-2532.
9. Mirkovitch,J., Gasser,S.M. and Laemmli,U.K. (1987) Philos. Trans. R. Soc. Lond. (Biol), 317, 563-74.
10. Cockerill,P.N. and Garrard,W.T. (1986) Cell, 44, 273-282.
11. Lichter,P., Tang,C-J.,C., Call,K., Hermanson,G., Evans,G.A., Housman,D. and Ward,D.C. (1990) Science, 247, 64-69.
12. Lawrence,J.B., Villnave,C.A. and Singer,R.H. (1988) Cell, 52, 51-61.
13. Robinson,S.I., Nelkin,B.D. and Vogelstien,B. (1982) Cell, 28, 99-106.
14. Stief,A., Winter,D.M., Stratling,W.H. and Sippel,A.E. (1989) Nature, 341, 343-345.
15. Van der Velden,H.M.W., van Willigen,G., Wetzels,R.H.W. and Wanka,F. (1984) FEBS Lett., 171, 13-16.
16. Vaughn,J.P., Dijkwel,P.A., Mullenders,L.H.F. and Hamlin,J.L. (1990) Nucleic Acids Res., 18, 1965-1969.
17. Opstelten,R.J.G., Clement,J.M.E. and Wanka,F. (1989) Chromosoma, 98, 422-427.
18. Southern EM. (1975) J. Mol. Biol., 98, 503-517.
19. Hart,R.P. and Folk,W.R. (1982) J. Biol. Chem., 257, 11706-11711.
20. Kalandadze,A.G., Bushara,S.A., Vassetzky,Y.S.,Jr., and Razin,S.V. (1990) Biochem. Biophys. Res. Comm., 168, 9-15.
21. Ballachey, B. E., Hohenboken, W. D., Evenson, D. P. (1987) Biol. Reprod., 36, 915-925.
22. Blow, J. J., and Laskey, R. A. (1986) Cell, 47, 577-587.