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DNA Loop Domain Organization: The Three-Dimensional Genomic Code

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Abstract It is well known that aberrations in the nuclear matrix contribute to the development of cancer, but many aspects of this process remain unknown. The mammalian sperm nuclear matrix serves as a distinctive model of DNA loop domain organization by the nuclear matrix since the integrity of the DNA structure can be measured by the ability of the paternal chromosomes to participate in embryogenesis. The structure of the nuclear matrix is known to be important for normal cellular functions such as transcriptional regulation and DNA replication. Even small aberrations in DNA structural organization in the sperm cell could have disastrous consequences for the embryo if they were essential for function. Recent work from our laboratory suggests that sperm nuclei with disrupted nuclear matrix structures but intact DNA cannot participate fully in embryogenesis, suggesting that the structural organization of DNA may provide important, heritable information that is necessary for development. We term the DNA sequence together with its three-dimensional organization the ''genomic code.'' We suggest that the sperm nucleus is an ideal model for understanding the principles of the involvement of the three-dimensional structure of DNA in normal cellular function. Finally, the implications for cancer about what we can learn using sperm DNA as a model about the ''genomic code'' are discussed. J. Cell. Biochem. Suppl. 35:23–26, 2000.

Key words: DNA loop; sperm; nuclear matrix

Evidence for a structural component of the eukaryotic nucleus was first described in 1948 [Zbarskii and Debov, 1948], but it was not until 1974 that the first connection was made between the physical aberrations in cancer cells that pathologists had long used to diagnose the disease and the structural proteins of the nucleus [Berezney and Coffey, 1974]. Since that time, the nuclear matrix or nuclear scaffold, as the normal structural component of the nucleus has been termed, has been shown to be the site of DNA replication [Berezney and Coffey, 1975; Vogelstein et al., 1980; Dijkwel and Hamlin, 1995] and involved in RNA transcription in an as yet undefined way [Ciejek et al., 1983; Nelson et al., 1986; Mirkovitch et al., 1987; Zhao et al., 1993; Gerdes et al., 1994]. Furthermore, Coffey's prediction that proteins in the nuclear matrix are altered in cancer has been verified experimentally [Getzenberg et al., 1991; Partin et al., 1997]. More recently, a series of elegant experiments from Stein and colleagues [McNeil et al., 1999; Tang et al., 1999] have demonstrated that gene translocations that are known to occur in cancer of nuclear matrix proteins can alter their sublocalization within the nucleus. Thus, the data to date (reviewed much more extensively in other articles in this volume) suggest that the nuclear matrix plays crucial roles in the normal functions of DNA, and that alterations in this structure play an important role in carcinogenesis.

However, many things have yet to be learned about the extent of the functions of the nuclear matrix. We still do not understand how crucial the integrity of the nuclear matrix is for DNA replication and RNA transcription, nor do we fully understand the underlying structure of the nuclear matrix, itself. Our laboratory has been investigating the structural organization

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of DNA in the nuclear matrix of the sperm cell as the simplest model of biologically active DNA that is available [Ward, 1994]. The sperm cell has no active transcription, nor does it replicate its DNA, yet it is still organized into DNA loop domains by a nuclear matrix [Ward et al., 1999; Kalandadze et al., 1990; Choudhary et al., 1995]. Recently, we have exploited another aspect of this model system, its function in embryogenesis. The sperm cell is pluripotent in that it provides half the genome to the one-celled embryo that differentiates into every other type of tissue during embryogenesis. This makes it a unique model to test the integrity of the DNA's three-dimensional organization because any aberration in structure that is required for function will likely disrupt embryogenesis. Using this model, we have obtained evidence that suggests that the structural integrity of the three-dimensional organization of the DNA within the sperm nucleus may be a necessary component for embryogenesis to occur. That is, the organization of DNA into loop domains by the nuclear matrix may be a heritable component of the chromosome that contributes regulatory information to the developing embryo. We call the combination of this heritable structural information with the genetic information encoded within the DNA sequence the "genomic code," and discuss the potential implications of this for normal development and cancer.

DNA LOOP DOMAIN ORGANIZATION BY THE SPERM NUCLEAR MATRIX

The structural organization of sperm DNA and somatic DNA are very different from each other, but share one interesting element. Sperm DNA is mostly bound by protamines and a few histones, whereas somatic cells are bound solely by the histone octamer forming the familiar "beads on a string" nucleosome. Protamines are (arginine-rich) basic proteins that bind in the major groove to the sperm DNA [Prieto et al., 1997]. Rather than the relatively open configuration the nucleosome structure gives somatic cell chromatin, protamines condense the DNA into a tightly packaged toroid that contains roughly 50 kb of DNA [Hud et al., 1993, 1995]. With this marked difference in DNA packaging, sperm and somatic cell chromatin do share one major commonality. Both have a nuclear matrix which organizes the DNA into loop domains. Several laboratories have demonstrated that sperm DNA is organized by specific sequences to the nuclear matrix, as in somatic cells [Kalandadze et al., 1989; Ward and Coffey, 1990; Nadel et al., 1995; Kramer and Krawetz, 1996]. When the loop domain organization of individual genes in sperm cells were compared with that of somatic cells, sperm DNA was consistently found to have a unique type of organization. Recent work from our laboratory (submitted for publication) has demonstrated that DNA loop domain structure in sperm cells is independent of protamine expression or protamine binding to DNA. The differences between sperm and somatic cell loop domain organization may be attributed to the difference in the nuclear matrix regulation since the nuclear matrix proteins for different cell types vary [Getzenberg and Coffey, 1990]. This suggests that the specific loop domain organization in spermatozoa is not due to the more condensed DNA packaging in these cells, but instead related to functional aspects of the chromatin, that will be discussed in the next section.

These data illustrate that the evolutionary forces which led to the condensation of sperm DNA, probably to protect it during the process of fertilization, forfeited almost every aspect of chromatin structure except for the organization of DNA into loop domains attached to a nuclear matrix. The data also suggest that the DNA loop domain organization is specifically regulated. Our conclusion from this work is that sperm DNA organization plays an important role in the function of the paternal DNA. These ideas have been reviewed more extensively in a previous Prospect article in this journal [Ward, 1994]. Our laboratory is now examining the possibility that the sperm nucleus provides the embryo not only with the DNA sequence, but with a structural organization of that DNA into loop domains that serve as functional units during development.

ROLE OF THE NUCLEAR MATRIX IN EMBRYOGENESIS

Mature spermatozoon DNA is inactive because there is no replication or transcription occurring, therefore what could be the role of the sperm nuclear matrix organization? One very important role that our laboratory is now studying is its possible involvement in embryogen-

esis. We took advantage of the pluripotency of sperm DNA to study the importance of the organization of that DNA into loop domains for embryogenesis. Mouse sperm nuclei were extracted in isotonic buffer with an ionic detergent, ATAB, with and without dithiothreitol (DTT) and then injected into oocytes using intracytoplasmic sperm injection (ICSI). The embryos were then transferred to foster mothers, and their development to live births were followed [Ward et al., 1999]. The results are illustrated in Figure 1. Nuclear matrix stability was measured by the ability of the sperm nuclei to form nuclear halos when extracted with 2 M NaCl. Nuclear halos are extracted nuclei that are surrounded by a halo of DNA, comprised of DNA loop domains that are attached at their bases to the nuclear matrix. We found that spermatozoa treated with ATAB in the presence of DTT decondensed completely when they were extracted with 2 M NaCl, while those that were extracted with ATAB formed nuclear halos. Interestingly, when oocytes were injected with sperm nuclei that had stable nuclear matrices, 30% of the embryos developed to live births. However, none of the oocytes that were injected with sperm nuclei that had unstable nuclear matrices developed (Fig. 1).

These experiments are important because in both cases, the sperm nuclei were injected when the DNA was still highly condensed, making it unlikely that the sperm DNA, itself, was damaged. The only difference between the two types of nuclei that were injected was the stability of the nuclear matrix. This is the first evidence that the organization of the DNA within the sperm nucleus plays an important role in embryogenesis. The data support our previous suggestion [Ward, 1994] that the sperm nucleus provides the embryo with heritable information that is "encoded" within the way that the DNA is organized into loop domains.

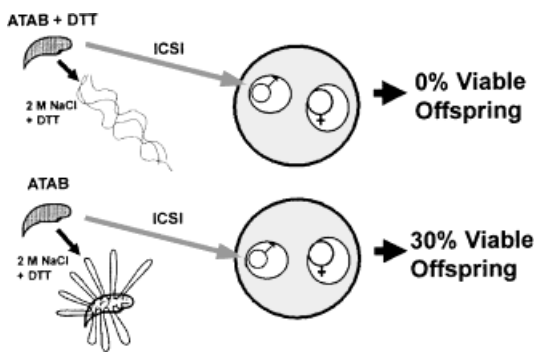

Fig. 1. Evidence that an intact sperm nuclear matrix is required for embryogenesis. This figure summarizes the results recently described using ICSI to study the role of sperm DNA organization in embryogenesis [Ward et al., 1999]. Mouse sperm nuclei that had the ability to form nuclear halos in vitro, were also able to participate in embryogenesis when injected into oocytes. However, sperm nuclei that had unstable nuclear matrices, were not able to participate in embryogenesis. In both types of sperm nuclei, the DNA is expected to be intact.

CONCLUSIONS: THE STRUCTURAL COMPONENT OF THE "GENOMIC CODE"

The experiments described above demonstrate one way in which we hope to exploit the pluripotency of the sperm cell to determine how the nuclear matrix functions in embryogenesis. Our hypothesis is that the organization of DNA into loop domains provides a level of heritable instructions, in addition to the genetic information encoded within the DNA sequence, that are passed down from one generation to the next. This idea is diagramed in Figure 2. The idea is that the linear genetic information in the DNA molecule can be "annotated" by its structural organization on the nuclear matrix. Stated another way, the three-dimensional organization of the DNA provides heritable information, itself. The attachment sites of DNA to the sperm nuclear matrix may provide the embryo with cues for origins of DNA replication, start sites for mRNA transcription, and other functions as yet undetermined. We term this combined genetic and structural information the "genomic code."

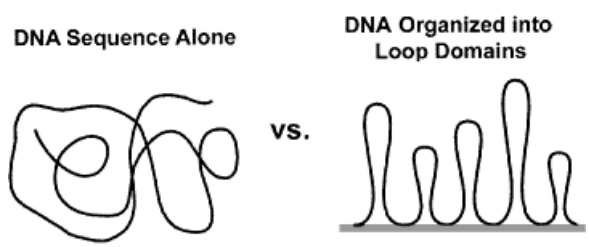

Fig. 2. The three dimensional structure of DNA contains information. The DNA contains genetic information encoded in the sequence of the base pairs. When this linear molecule is organized into loop domains by the nuclear matrix, additional, heritable information is added by marking certain positions along the DNA by attaching these points to the nuclear matrix. We call the combination of the DNA sequence and the three-dimensional organization of this DNA the ''Genomic Code.''

The sperm nucleus, therefore, provides us with a unique opportunity to study the function of the nuclear matrix that cannot be studied by other means. By determining what roles the nuclear matrix plays in embryogenesis, we will be able to predict how aberrations in this structure affect the development of cancer.

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