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PROSPECTS

Nuclear Structure and the Three-Dimensional Organization of DNA

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Abstract The organization of DNA within the nucleus has been demonstrated to be both cell and tissue specific and is arranged in a non-random fashion in both sperm and somatic cells. Nuclear structure has a pivotal role in this three-dimensional organization of DNA and RNA and contributes as well to forming fixed organizing sites for nuclear functions, such as DNA replication, transcription, and RNA processing. In sperm, DNA is also organized in a specific fashion by the nuclear matrix and DNA-protamine interactions. Within somatic cells, the nuclear matrix provides a three-dimensional framework for the tissue specific regulation of genes by directed interaction with transcriptional activators. This differential organization of the DNA by the nuclear matrix, in a tissue specific manner, contributes to tissue specific gene expression. The nuclear matrix is the first link from the DNA to the entire tissue matrix system and provides a direct structural linkage to the cytomatrix and extracellular matrix. In summary, the tissue matrix serves as a dynamic structural framework for the cell which interacts to organize and process spatial and temporal information to coordinate cellular functions and gene expression. The tissue matrix provides a structural system for integrating form and function.

Key words: nuclear matrix, nuclear scaffold, tissue matrix, extracellular matrix, cytoskeleton, gene expression

The structural components of the nucleus are known to have a central role in the specific topological organization of DNA. DNA in the nucleus is not randomly organized and although only approximately ten percent of the DNA actually encodes genes, only specific genes are positioned in a manner that permits the expression of both housekeeping and cell type specific genes. The average mammalian somatic cell nucleus contains a linear equivalent of two meters of DNA packed by a 200,000-fold linear condensation into a 10 micron nucleus. The DNA has many forms of higher order structure which are organized in a particular pattern that results in the expression only of appropriate tissue specific genes. With the use of in situ hybridization, there is now direct evidence for specific three-dimensional organization of the DNA within the nucleus [1–5]. Manuelidis and coworkers demonstrated the specific and reproducible compartmentalization of unique chromosomal domains within the nuclei of human central nervous system cell lines and established that functionally distinct cell types have specific patterns of interphase chromosome three-dimensional organization. Comparison of the chromosomal topography of human lymphocytes, amniotic fluid cells, fibroblasts, and human cerebral and cerebellar samples have further demonstrated that the topological DNA organization is cell type specific [6,7]. The use of confocal microscopy has further enabled the visualization of multiple probes simultaneously and has confirmed the dynamic and specific cellular localization of specific genes within the nucleus [8,9]. In summary, many studies have now demonstrated the specific three-dimensional organization of DNA within the nucleus. The difference in this organization can occur with the same genomic sequence and is dictated in part by DNA interactions with a tissue specific nuclear matrix. Nuclear structure is therefore involved in both this topological organization of DNA and the functional aspects which coincide with this organization.

ORGANIZATION OF DNA

The DNA in both sperm and somatic cells is organized in a specific three-dimensional fash-

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ion. The mammalian sperm nucleus contains the most highly condensed eukaryotic DNA known [see review, 10]. This DNA is approximately sixfold more highly condensed than the DNA in the mitotic chromosome. The sperm nucleus does not contain sufficient volume to permit DNA organization even into a nucleosome structure [11,12] and therefore, the sperm is known to posses a unique type of DNA packaging [13]. The first order structure of DNA is the 2 nm right-handed double helix. The second order structure of DNA within mammalian sperm nuclei is dictated by protamines, which are highly positively charged molecules that neutralize the negative charge of the phosphate groups in the DNA backbone [13]. These positively charged protamines permit one strand of DNA to fit into the major groove of another DNA strand resulting in a stacked linear array of DNA. This linear array is further stabilized by inter-protamine disulfide bonds [14,15] and several experiments support the principle that the DNA in the mammalian sperm nucleus is organized into these tightly packed linear arrays [16–24]. Mammalian sperm DNA is specifically organized by the nuclear matrix into DNA loop domains, the third order of DNA packaging. The nuclear matrix was originally identified in somatic cells as the residual framework of the nucleus that consists of the peripheral pore complexes, an internal ribonucleic protein network, and the residual nucleoli [25]. The linear arrays of DNA in the sperm nucleus are arranged into loop domains by this nuclear matrix in a manner reminiscent of somatic DNA loop organization [17]. A structural component of the hamster sperm nucleus, the nuclear annulus, was recently identified [26] that anchors the DNA at the implantation fossa of the sperm nucleus and may serve as the fourth higher order organizational structure of sperm DNA. The DNA remains attached to this structure after the sperm nuclei were completely decondensed and the apparently large size of the DNA that remains associated with the nuclear annulus suggests that every chromosome has at least one attachment site (Fig. 1) [for review, see 10,17].

While the first two levels of DNA organization in the sperm are not sequence specific, sperm DNA loop domains are specifically organized with respect to the nuclear matrix. Recent work has demonstrated the specific organization of several genes with the sperm nuclear matrix [28,29]. It is evident that although the DNA in the sperm nucleus is highly compacted, it is organized into loop domains which enable the association of specific genes with the sperm nuclear matrix. These sperm DNA loop domains are also common to the somatic cell, but are approximately half the length (46 ± 7 kbp in sperm versus 76 ± 11 kbp in brain) and contain no superhelical density [17].

The DNA in the eukaryotic somatic nucleus while not as tightly compacted as that of the sperm nucleus, is still highly organized by the structural components of the nucleus and a common feature of all eukaryotes are the DNA loop domains [17]. The nuclear matrix is the dynamic structural subcomponent of the nucleus that directs the three-dimensional organization of DNA into loop domains of approximately 60 kilobases (kb) [28–32]. The concept of a loop domain was originally proposed by Cook et al. and is now thought to be the basic unit of higher order DNA structure which is present throughout the cell cycle in eukaryotic cells from sperm to interphase nucleus to chromosome [33,34].

The organization of the DNA within the DNA loop domain has revealed that the DNA in the mammalian chromosome is organized at many different levels [recently reviewed in 35] (Fig. 2). The first order structure of DNA is the 2 nm right-handed double helix. The second level of DNA organization is that of the nucleosome that consists of histone octamers with approximately 200 base pairs of DNA wound around each histone octamer core and results in a packing ratio of approximately six. The third level of DNA structure is that of the 30 nm filament which is the form of DNA filament that comprises the DNA loop domain. The 30 nm structure was first described as being formed from wrapping the DNA helix around cores of histones to form a 10 nm fiber of nucleosomes and six of these nucleosomes then form a solenoid to produce the 30 nm diameter filament [40]. There have been many models proposed to describe the 30 nm filament all of which predict a packing ratio of approximately 35–50 for the DNA within the filament. These models all attempt to explain the arrangement of the linker DNA located between the nucleosome cores. Two current models, the solid solenoid in which the core of the solenoid is filled with the loops of the linker DNA [41] and the double crossed linker, in which

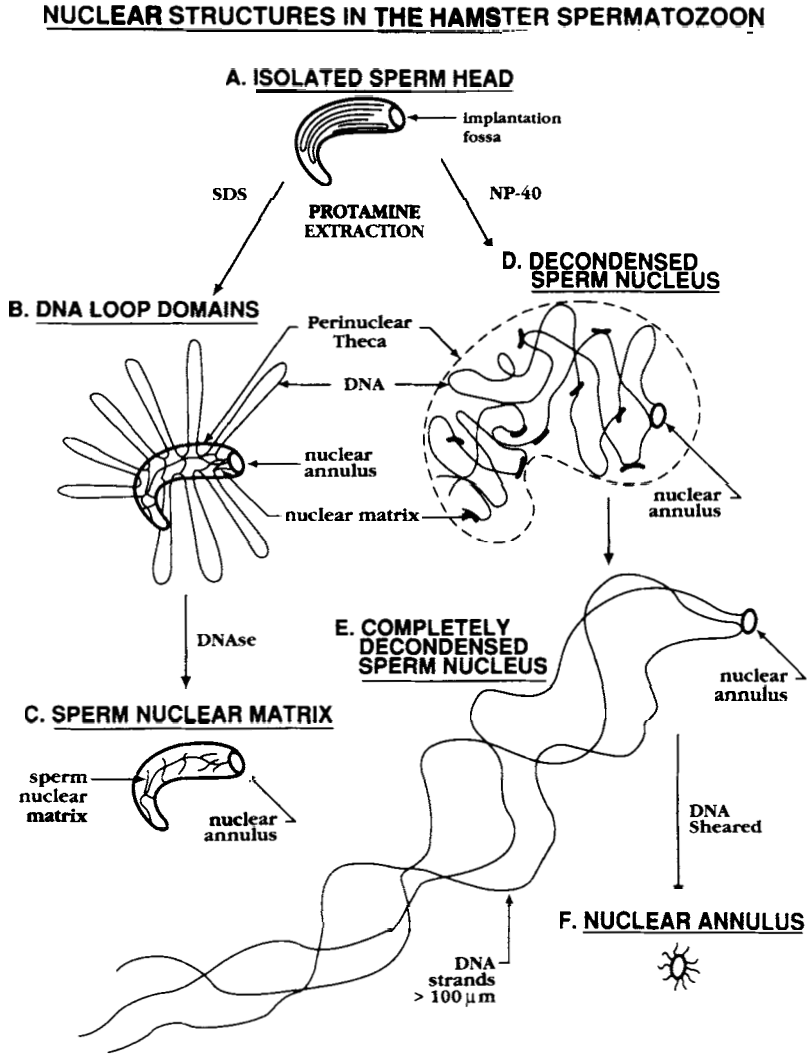
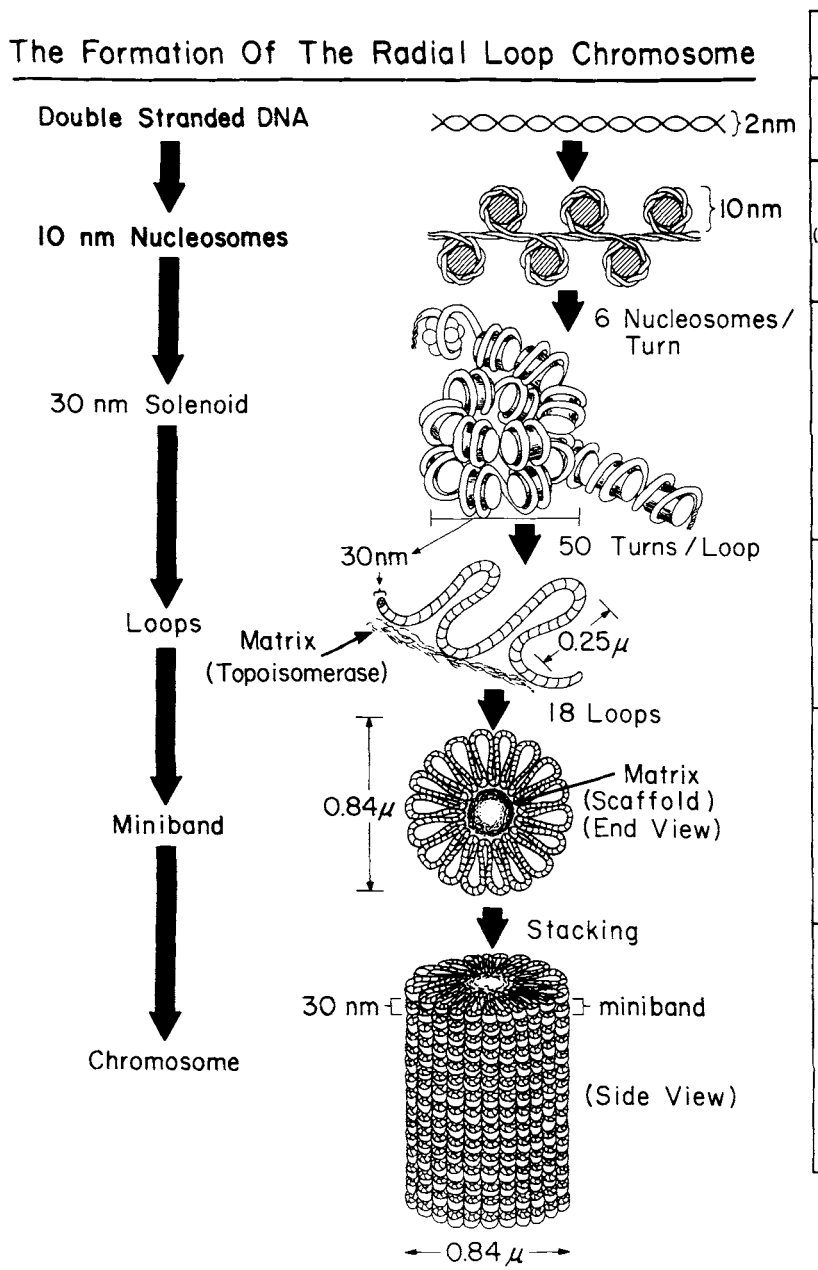


Fig. 1. DNA organization in the hamster sperm nucleus. The sperm nuclear matrix: (**A**) An isolated hamster sperm nucleus; (**B**) A sperm nucleus that has been isolated from spermatozoa washed in 0.5% of the ionic detergent, SDS, immediately upon extrusion and then treated with high salt and reducing agent to extract the protamines. The DNA loop domains extrude from the nucleus and can be visualized as a fluorescent halo surrounding the sperm nucleus when stained with ethidium bromide; (**C**) When treated with DNAse I, the sperm matrix retains its characteristic shape. The nuclear annulus: (**D**) A sperm nucleus that has been prepared from spermatozoa washed in the nonionic detergent NP-40 and then extracted as in (B) for 10 minutes. Under these conditions, the sperm nucleus decondenses [17]; (**E**) A fully decondensed sperm nucleus, prepared as in (D) and extracted for an additional 30 minutes. All nuclear and perinuclear structures have dissipated except for the nuclear annulus, a structure that was originally located at the implantation fossa. All the DNA remains anchored to the nuclear annulus [26]; (**F**) A nuclear annulus from which most of the DNA has been mechanically sheared away. A small amount of DNA remains firmly anchored to the annulus [26]. The perinuclear theca is a cytoplasmic structure that surrounds the sperm nucleus [27]. It dissipates when nuclei decondense (D,E) and remains intact in sperm nuclear matrix preparations (B,C). (Reproduced from W.S. Ward and D.S. Coffey [10] with permission of the Society for the Study of Reproduction.)

the DNA linker domains are straight while crossing the core of the solenoid, are best supported by the in vivo data.

These loop domains also exist in the metaphase chromosome. There are a number of models currently available which describe the organization of these loops in the metaphase chromosome. The two models which most closely fit the data are those of the radial loop [42,43] and the radial coil [reviewed in 44]. The fundamental difference between the radial loop and the radial coil models is that in the radial loop model, the cross-section of the loops are half the size of the loops in the radial coil model. The radial loop



Base Pairs per Turn	Packing Ratio
10 b.p.	1
80 b.p (160 b.p. per 2 turns)	6 - 7
1,200 b.p. (per turn)	40
60,000 b.p. (per loop)	680
1.1×10^6 b.p. (per miniband)	1.2×10^4
18 loops/ Miniband	1.2×10^4

Fig. 2. Schematic of the levels of organization within a chromatid of a chromosome. Approximately 160 bp of 2 nm DNA helix is wound twice around the histone octamers to form the 10 nm nucleosomes. These nucleosomes form a "beads on a string" fiber, which winds in a solenoid fashion with 6 nucleosomes per turn to form the 30 nm chromatin filament. The 30 nm filament forms the 60 kbp DNA loops that are attached at their bases to the nuclear matrix structure. The loops are then wound into the 18 radial loops that form a miniband unit or 1 turn on the chromatid. The minibands are continuously wound and stacked along a central axis to form each chromatid. Variations in intrachromosomal length are achieved by the winding, unwinding, and compacting of the minibands. Topoisomerase II is located at the base of the interphase loop [36], on the nuclear matrix [37], and in the central protein scaffold of the metaphase chromosome [38]. (Reproduced from K.J. Pienta et al. [39] with permission of the American Association for Cancer Research, Inc.)

model accurately predicts the number of loops per turn observed in cross-sections of chromatids as measured from scanning electron micrographs [42], whereas the radial coil model is able to explain the 240 nm chromatid coils demonstrated by several authors as the higher order structure within chromosomes [reviewed in 46; 44,45]. Recent evidence indicates that the radial loop model may best explain normal chromosome structure (Fig. 2) [for review, see 47].

FUNCTIONAL ASPECTS OF DNA ORGANIZATION

In the interphase nucleus, the 30 nm filaments form approximately 50,000 loop domains each of approximately 60 kbp that are attached at their base to the inner portions of the nuclear matrix [32,48–51]. The nuclear matrix is the nuclear scaffolding which has previously been demonstrated to play a central role in the regulation of important cellular processes, such as DNA replication and transcription (Fig. 3). DNA replication has been shown to occur at the base of the loops in a complex termed the replisome, that consists of DNA polymerase, topoisomerases, DNA methylase, dihydrofolate reductase, thymidylate synthetase, and ribonucleoside diphosphate reductase [53]. The replication of DNA in the nucleus occurs in small units that have been termed, replicons, which are synthesized in a precise order and temporal sequence, and these replicons have now been shown to be equivalent to the DNA loop domains [32]. These replicon loops are passed down through the fixed sites of the multienzyme complex termed the replisome units [31,32,54–56]. During replication, each of the 60 kilobase replicons must pass through these fixed sites of replication within a 30-minute period. Thus, the DNA double helix must be unwound at a speed of over 100 rpm at each replicating site [35]. The requirement for DNA unwinding emphasizes the need for the DNA topoisomerases or gyrases which play central roles in the regulation of DNA topology. DNA topoisomerase II has been shown to be a component of both the metaphase chromosome scaffold [38] and the interphase nuclear matrix [36,37]. The topoisomerase II enzyme has been localized at the base of the DNA loops and shown to exist in the wake of the DNA replicating fork [57].

The nuclear matrix also plays a central role in RNA processing. Newly synthesized heteronuclear RNA and small nuclear RNA are enriched on the nuclear matrix [57–64]. The nuclear matrix has also been shown to be the site of attachment for products from RNA cleavage and for RNA processing intermediates [65]. Spliceosome complexes involved in the regulation of

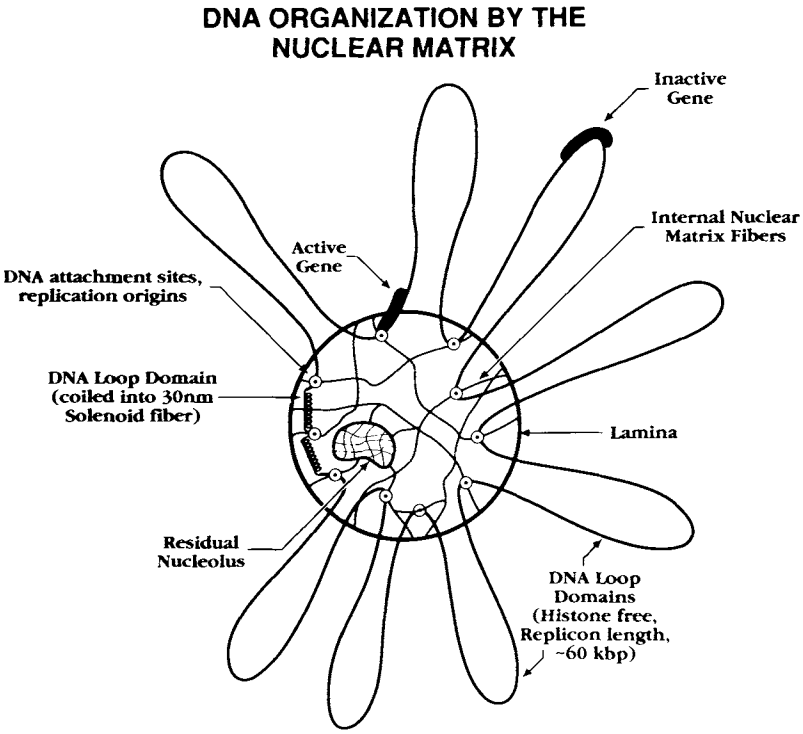


Fig. 3. DNA organization by the nuclear matrix in somatic cells. In somatic cells, the DNA that is coiled into 30 nm solenoid filaments is organized into loop domains attached at their bases to the proteinaceous nuclear matrix. When the histones are extracted by salt, the DNA loop domains extend out of the nucleus and can be visualized as a fluorescent halo surrounding the extracted nucleus when stained with ethidium bromide [16,31]. Active genes are close to the site of attachment to the nuclear matrix (see Table 1), and DNA replication origins are permanently associated with the matrix [31,32,52]. (Reproduced from W.S. Ward and D.S. Coffey [10] with permission of Society for the Study of Reproduction.)

RNA splicing have been localized to the nuclear matrix [66]. RNA [63,67–70] and ribonucleoprotein particles and fibers [59,69,71] may themselves have an important role in the structure of the nuclear matrix.

The nuclear matrix has also been demonstrated to be the site of mRNA transcription. Active genes have been found to be associated with the nuclear matrix only in cell types in which they are expressed (Table I). Genes that are not expressed in these cell types are not found to be associated with the nuclear matrix. In addition, transcription factors including the myc protein, the large T antigen of the SV40 virus, and E1A from adenovirus have all been found to be associated with the nuclear matrix [90–93]. The association of nuclear oncogene proteins with the matrix was found to be sensitive to the method of isolation [94]. Further investigation into the association of active genes with the nuclear matrix has revealed a DNA loop anchorage site next to the enhancer region of several genes [86,87,95–101]). These sequences have been termed matrix associated regions (MARs) or scaffold attached regions (SARs) and are usually approximately 200 base pairs in length, are A-T rich, and contain topoisomerase cleavage sequences along with other sequences, such as poly-adenylation signals [89]. Although these sequences often have a high degree of homology with topoisomerase II cleavage sequences, only one has been found to actually bind topoisomerase II [102]. The MARs have also been shown to functionally confer transcriptional activity in genes in which they are inserted. Classic experiments by Stief and colleagues involved utilizing the matrix associated DNA sequences of the chicken lysozyme gene and inserting this sequence into a transfectable expression vector. When this reporter system is flanked by the 5′ MAR, its expression is markedly elevated and is independent of chromosome position [103]. A similar experiment was carried out using the 5′ MAR of the chicken lysozyme gene and a transient transfection system, again demonstrating increased and position independent transcription of the gene construct [88]. To further determine the role of these MARs in gene expression, deletion experiments were carried out to remove the MARs from genes which normally contained these sequences and noting the effect of this deletion on the transcriptional activity of the gene. In the immunoglobulin kappa gene, deletion of the intronic MAR led to a fourfold decrease in expression. When both the intronic MAR and a MAR in the enhancer region were removed from this gene, the expression dropped elevenfold [104]. These experiments were then conducted in vivo, when transgenic animals were produced from these constructs, both of the genes with the intact and deleted MARs were expressed in a tissue specific manner, although those genes with a deleted MAR(s) exhibited only two- to threefold lower activity [105]. This data demonstrates the importance of precise DNA organization which is necessary to result in appropriate gene expression.

TABLE I. Examples of Active Genes Reported to Be Associated With the Nuclear Matrix From Tissues Expressing These Genes and Absent From Non-Expressing Tissues

Tissue/cell type	Gene	Reference
Rat liver	Ribosomal RNA	32
NIH 3T3 cells	SV40	72
HeLa cells	Newly transcribed sequences	73
Chicken oviduct	Ovalbumin	74
Nine cell types	Polyoma and avian sarcoma viruses	75
Chicken oviduct	Ovalbumin	76
HeLa cells	Newly transcribed sequences	77
Human lymphocytes	Immunoglobulin	78
Mouse lymphocytes	α-globin	79
Chicken liver	Vitellogenin II	80
Chicken erythrocytes	β-globin	81
Drosophila	Heat shock	82
Rat ventral prostate	Prostatein C-3	83
Rat liver	α_2-macroglobulin	84
Human skin	Proα2(I) collagen	85
Human erythrocytes	Globin	86
Chicken erythrocytes	α-globin	87
Chicken	Lysozyme	88
Human HL-60	*c-myc*	89

The nuclear matrix has a central role in hormone action and this has been well demonstrated for steroid hormones. All of the steroid hormone receptors with the exception of the glucocorticoid receptor are found constitutively in the nucleus [106]. The glucocorticoid receptor has been shown to be a cytoplasmic protein that upon binding of ligand to receptor becomes localized in the nucleus [107]. Extensive work has identified the high affinity binding of the steroid receptors to the nuclear matrix in many estrogen and androgen responsive tissues [108–113].

The binding of steroid receptors to the nuclear matrices of individual tissue is both steroid and tissue specific and requires the presence of an activated steroid receptor complex with bound steroid [114–116]. For example, androgens were found to bind to the nuclear matrices of androgen responsive tissues and estrogens to the nuclear matrices of estrogen responsive tissues. Tissues that do not demonstrate hormone responsiveness are not found to have hormone receptors on their nuclear matrices. In addition, the level of steroid receptor binding in these nuclear matrices was demonstrated to vary with the hormonal state of the animal. Receptors were not found bound to the nuclear matrix in the hormone withdrawn state, and with subsequent administration of specific hormones the receptor complexes were then again shown to bind to the nuclear matrix.

The binding of steroid hormones to DNA and the subsequent activation of gene transcription is an example of the tissue specific expression of genes that have been shown to be under the control of a single transcriptional activator. The rat ventral prostate and seminal vesicle, both androgen induced sex accessory tissues, contain the same genome, possess nuclear dihydrotestosterone (DHT) receptors, and respond to DHT, but with the production of markedly different secretory proteins. The ventral prostate produces a major secretory protein with a molecular weight of approximately 40,000 daltons identified as prostatein [117]. Prostatein is a glycoprotein and consists of four subunits, the most abundant of which, C3, has a molecular weight of 14,000 daltons [118]. The seminal vesicle produces six major secretory proteins which have the nomenclature SVS I–VI. The most abundant secretory protein in the seminal vesicle is known as SVS-IV and has a molecular weight of 17,000 daltons [119]. Both the C3 in the prostate and the SVS-IV in the seminal vesicle are controlled by androgens and respond to DHT addition. Upon castration, in the absence of androgens, both the ventral prostate and seminal vesicle cease production of these major secretory proteins and begin the synthesis of alternative protein products. These two proteins serve as an illustration for one of the major questions in cell regulation; the determination of tissue specificity. Although DNA methylation may be involved in some aspects of DNA conformation, DNA methylation does not appear to account for the tissue specific expression of the prostatein C-3 and the SVS-IV genes [120,121]. For example, in this system, in the same animal the ventral prostate and seminal vesicle each respond to androgens with the production of vastly different secretory proteins when stimulated with the same signal.

One possibility to explain the tissue specific androgen regulated protein expression in the ventral prostate and seminal vesicle is that the DNA, which is organized by the nuclear matrix, is organized differently between the two tissues (Fig. 4). Previous work has demonstrated that the nuclear matrix components themselves are important regulators of gene expression. Mitogenic stimulation and the induction of differentia-

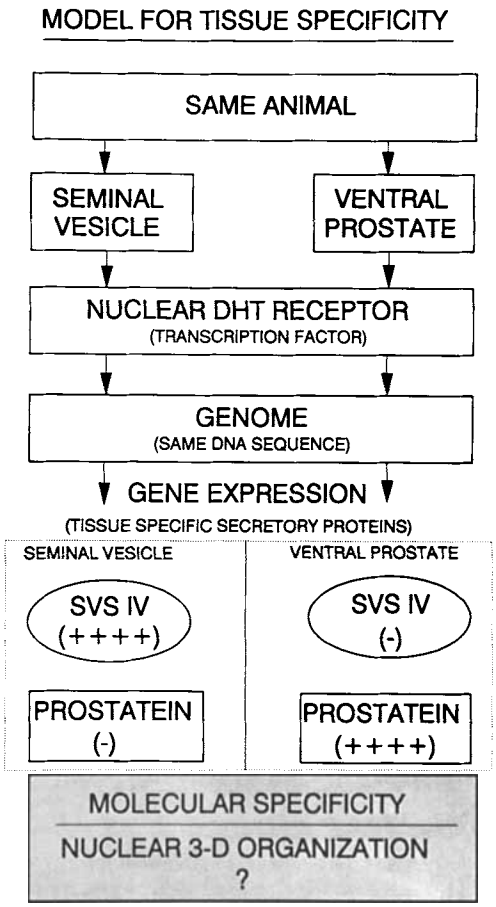


Fig. 4. Schematic of the Question of Tissue Specificity in Androgen Action. In the same animal, the DHT receptor interacts with identical genomes in different tissues to produce vastly different gene expressions of androgen-induced secretory proteins. For example, the major protein produced by the ventral prostate is prostatein, and the major secretory protein produced by the seminal vesicle is SVS-IV. (Reproduced from R.H. Getzenberg and D.S. Coffey [127] with permission of the Endocrine Society.)

tion have been demonstrated to cause alterations in nuclear matrix proteins and structure [122–125]. Differences in nuclear matrix protein patterns have recently been noted between different cell lines [126]. This same study also noted variations in the nuclear matrix proteins between transformed and non-transformed cell lines of similar origin. With these differences in nuclear matrix proteins found with differences in gene expression, the nuclear matrix proteins of the rat ventral prostate and seminal vesicle were investigated by Getzenberg and Coffey [127]. The protein components of the nuclear matrices from the rat ventral prostate and seminal vesicle were isolated based on the procedure of Fey and Penman, which uses a mild salt extraction to minimize destruction of the nuclear matrix proteins and structure [128]. The protein components of the nuclear matrices were analyzed using a high resolution two-dimensional gel electrophoresis system developed in our laboratory and based on a procedure by Cupo et al. [129]. Thorough analysis of the protein patterns revealed many qualitative and quantitative differences between the two tissues. The nuclear matrix patterns were also compared between normal tissues and those tissues isolated from rats that had been castrated for 23 hours. This comparison also uncovered many qualitative and quantitative differences between the nuclear matrices of the normal and castrate state. Overall, three sets of nuclear matrix proteins were found. The first set consists of those nuclear matrix proteins that are tissue specific in the rat ventral prostate and seminal vesicle. The second set consists of those proteins which both appear and disappear upon castration. The final set, which consisted of the majority of the nuclear matrix proteins, contains those proteins which did not vary between the tissues or with the hormonal state. The tissue specific protein composition of the nuclear matrix in the ventral prostate and seminal vesicle may play an important role in the regulation of gene expression. The nuclear matrix has been shown to be the organizing structure of the DNA in the nucleus, and therefore the different nuclear matrix components may allow for a different DNA organization in the two tissues. The difference in the three-dimensional organization of the DNA might provide for a tissue specific interaction of the DNA with the DHT receptor. The ability of the DHT receptor to bind to one site on the DNA in the ventral

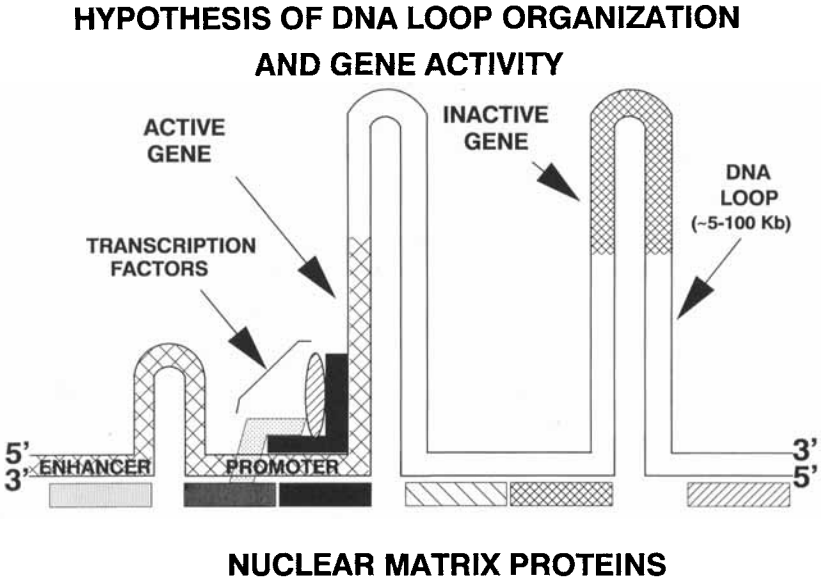


Fig. 5. Hypothesis of DNA organization and gene activity. Active genes have been shown to be associated with the nuclear matrix. This schematic is our hypothesis of tissue specific DNA loop organization. Tissue specific nuclear matrix proteins are involved in the binding and localization of specific DNA sequences and determining the position of genes in proper configuration for transcription factors to interact and allow activation of gene expression. By controlling the three-dimensional conformation of DNA, the tissue specific nuclear matrix proteins confer specificity to transcription factor/receptor binding. The genes are on different DNA loops, but not necessarily the adjacent loops shown here for simplicity. (Reproduced from K.J. Pienta et al. [35] with permission. Copyright CRC Press, Inc., Boca Raton, FL.)

prostate and another site on the DNA in the seminal vesicle could account for the differential gene expression seen between the two tissues (Fig. 5). Similarly, the differences seen upon castration may indicate changes in the three-dimensional organization of the DNA. These changes may determine the differences in gene expression seen in the castrate tissues. Current research in this area is exploring the properties of the tissue specific nuclear matrix proteins, their specific DNA binding capabilities, and what role, if any, they play in the regulation of gene expression.

THE TISSUE MATRIX SYSTEM

The nuclear matrix is the structural framework which organizes the DNA in the nucleus and has a direct role in the regulation of gene expression. The nuclear matrix is the component of the tissue matrix system which connects the DNA structurally and functionally to the cell periphery. The tissue matrix system is an integrated three-dimensional skeletal framework that organizes and coordinates cell structure and function. The concept of a tissue matrix was originally proposed by Bissell et al. [130] and Isaacs et al. [131], but the first experimental evidence came from the elegant studies of Penman and colleagues [128]. The skeletal networks of matrix systems consist of linkages and interactions of the nuclear matrix, the cytoskeleton, and the cell periphery. In addition, the tissue matrix includes the linkage of the cell matrix to the extracellular matrix. The interactive tissue matrix system may be defined as the dynamic structural subcomponent of the cell which interacts to organize and process spatial and temporal information to coordinate cell functions and gene expression [reviewed in 132]. This tissue matrix is capable of undergoing dynamic shifts in structure and conformation through polymerization, cross-linking, biochemical modifications, and contractile and vibratory movements that may allow coupling of extracellular signals directly to the nucleus and ultimately the DNA.

The tissue matrix system and principally the nuclear matrix has a central role in the organization of DNA within the nucleus. This structural organization is involved in the regulation of such processes as DNA replication, RNA splicing, transcription, and the tissue specific regulation of gene expression. It is this tissue matrix which communicates signals from the exterior and interior of the cell to the nucleus, where appropriate modifications in three-dimensional DNA organization are made to result in differences in gene expression. While soluble factors are clearly involved in many of these processes, it is the structural components and its dynamics which provide the overall organization and appear to play a key role in gene regulation.

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