科技资料

Molecular & Cellular Biology of Prostate Cancer

MOLECULAR AND CELLULAR BIOLOGY OF PROSTATE CANCER

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TISSUE SPECIFICITY AND CELL DEATH ARE ASSOCIATED WITH SPECIFIC ALTERATIONS IN NUCLEAR MATRIX PROTEINS*

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INTRODUCTION

The ability of identical signals to interact with specific tissues in the same animal and result in the expression of different genes, is a fundamental question in cell regulation. A suitable model system for investigating the tissue specific regulation of gene expression is found in comparing the dihydrotestosteone (DHT) response in ventral prostate with the seminal vesicle. The rat ventral prostate and the seminal vesicle both contain the same genome, posses nuclear DHT receptors, and respond to DHT with the production of markedly different secretory products (figure 1). Upon DHT stimulation, the ventral prostate produces several specific secretory proteins. Similarly, the seminal vesicle produces its own unique tissue specific secretory proteins (figure 2). These tissue specific secretory proteins are all under control of the androgen receptor. Evidently there is another form(s) of regulation which determines what genes are activated when the cells are stimulated with androgen. We hypothesize that this regulation is brought about by alterations in the three dimensional conformation of the genome within the nucleus. It is believed that the genomic configuration within the nucleus is determined in part by the binding of DNA loop domains to the nuclear matrix proteins. If the ventral prostate and seminal vesicle each possess unique conformations of their DNA, it is possible that the androgen receptor is able to bind and activate tissue specific transcription on different locations of the DNA depending on the tissue. Since the nuclear matrix is the organizing structure of the DNA in the nucleus, tissue specificity in three dimensional DNA organization may be caused by a unique nuclear matrix composition. We propose that the nuclear matrix is tissue specific and is involved in the regulation of gene expression.

MAJOR SECRETORY PROTEINS

A major secretory protein of the ventral prostate has been identified as prostatein or prostate binding protein by a number of investigators. This protein is a steroid binding protein with a molecular weight of approximately 40,000 kD and a pI of 4.8 (Lea, O.A. et al., 1979). Prostatein is a glycoprotein which consists of four subunits. The major subunit is known as C3 and has a molecular weight of 14 kD (Viskochil, D.H., et al., 1983). When the intact prostatein protein is run under denaturing SDS/PAGE, the protein separates into two bands of 22 kD and 20 kD which further dissociate to the 14 kD C3, the 10 kD C2, and the 6 Kd C1 subunits (Lea, O.A. et al., 1979). The seminal vesicle produces six major secretory proteins which are referred to as SVS I to VI (Ostrowski, M.C. et al., 1979). SVS IV, the most abundant protein secreted by the seminal vesicle, has a molecular weight of 17 kD (Wagner, C.L., and Kistler, W.S., 1987). SVS IV is the major protein that we will use as a marker of androgen regulated seminal vesicle secretory proteins and the C3 subunit of prostatein will be used as an example of a ventral prostate specific androgen regulated secretory protein (figure 2).

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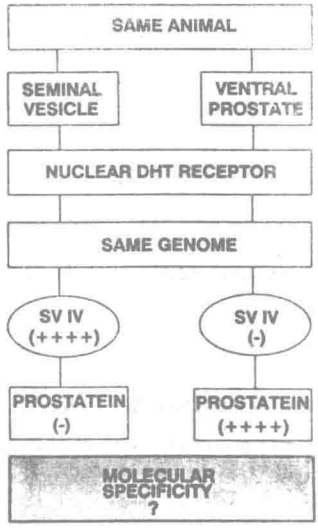


Figure 1: Statement of Problem. Why does the same signal interact with identical genomes in different tissues to produce different gene expressions? In the same animal, the ventral prostate and seminal vesicle possess nuclear DHT receptors and similar genomes. Upon DHT stimulation, each produces tissue specific secretory proteins. The major protein produced by the seminal vesicle is SV IV and the major protein produced by the ventral prostate is prostatein. This research attempts to determine what controls this tissue specificity on a molecular basis.

DNA COMPOSITION

Our hypothesis is based on the assumption that the genomes of the ventral prostate and seminal vesicle are identical. One possible exception involves the use of DNA methylation as a method of gene regulation. At present, DNA methylation does not seem to be a steroid regulatory mechanism used with these two genes (Kandala, J.C. et al., 1985; White, R. and Parker, M., 1983). Methylation of genes may be developmentally controlled (White, R. and Parker, M., 1983) but, SV IV is hypomethylated in both the ventral prostate and seminal vesicle (Kandala, J.C. et al., 1985). Rearrangements, deletions, and mutations have not been established as factors.

NUCLEAR MATRIX

The nuclear matrix is the residual element of the nucleus including the peripheral lamins and nuclear pore complexes (see Table 1). The nuclear matrix is the insoluble skeletal framework of the nucleus (Berezney, R. and Coffey, D.S., 1974). This matrix serves as the protein framework on which the DNA is organized into loop domains of approximately 60 kilobases (Pardoll, D.M. et al., 1980 ; Vogelstein, B. et al., 1980). The base of the loops are attached to the matrix and have been identified as the location of actively transcribed genes (Zehnbauer, B.A. and Vogelstein, B., 1985; Robinson, S.I. et al., 1983; Small, D. et al., 1985; Small, D. and Vogelstein, B., 1985; Mirkovitch, J. et al., 1984; Buttyan, R. and Olsson, C.A., 1986). The nuclear matrix has also been identified as a site of attachment of over 80% of the heteronuclear RNA (hnRNA) (Miller, T.E. et al., 1978; Peters, K.E., and Commings, D.E., 1980; van Eekelen, C.A. and van Venrooij, W.J., 1981; Long, B.H. and Schrier, W.H., 1983; Fey, E.G. et al., 1988). Transforming proteins and oncogene products such as the *myc* protein (Eisenman, R.N., et al., 1985), the large T antigen of the SV40 virus (Staufenbiel, M. and Deppert, W., 1983; Covey, L. et al., 1984) and the transcription factor EIA from adenovirus (Sarnow, P. et al., 1982) have been found to interact with the nuclear matrix. Recent evidence also indicates that the steroid receptor is associated with the nuclear matrix (Barrack, E.R. and Coffey, D.S., 1980; Donnelly, B.J. et al., 1984; Alexander, R.B. et al., 1987; Mowszowicz, I. et al., 1988).

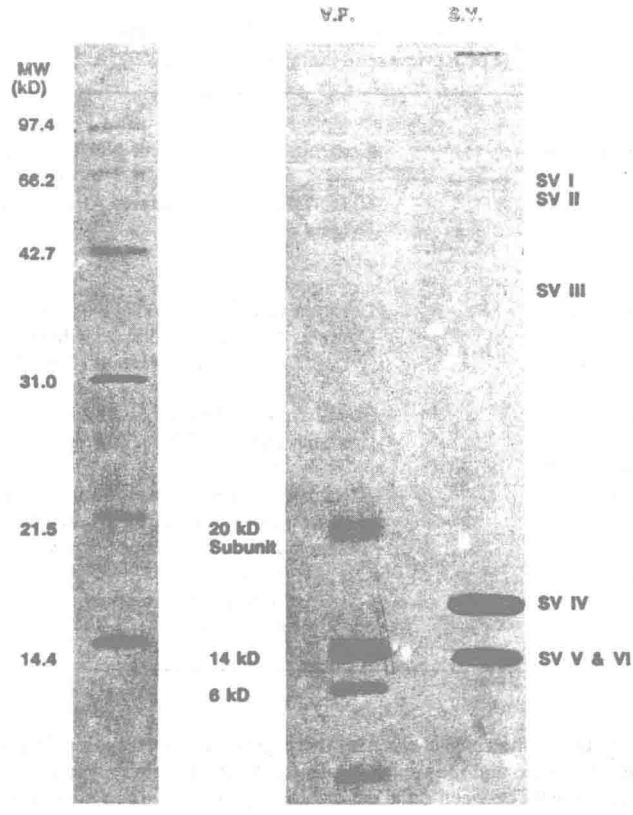


Figure 2: Major Secretory Proteins of the Seminal Vesicle(S.V.) and Ventril Prostate(V.P.) Rat seminal vesicle and ventral prostate secretory proteins reduced and separated on 15% SDS/PAGE. Major secretory proteins of the ventral prostate are similar to those identified by Lea, O.A. et al., 1979, as subunits of denatured prostatein. The 14 kD protein is the C-3 subunit. Major secretory proteins (SVS I-VI) of the seminal vesicle were identified as those described by Wagner, C.L. and Kistler, W.S., 1987.

Further evidence for the role of the nuclear matrix in the regulation of gene expression comes from studies of specific protein components of the nuclear matrix. Several studies have indicated that mitogenic stimulation (Bladon, T. et al., 1988) and induction of differentiation (Stuurman, N., et al., 1989) cause changes in the protein composition and structure of the nuclear matrix. Studies of the ovalbumin gene have provided insight into the role of the nuclear matrix in transcriptional control. Using DNA probes for different regions of the ovalbumin gene, sequences which are adjacent to and within the ovalbumin gene are associated with the nuclear matrix only in the hen oviducts and not in the nuclear matrices of other tissues in the same animal where the ovalbumin gene is not expressed (Robinson, S.I. et al., 1982; Robinson, S.I. et al., 1983; Ciejek, E.M. et al., 1983). Androgens have previously been shown to cause changes in nuclear proteins (Chung, L.W.K. and Coffey, D.S., 1971; Carmo-Fornesca, M., 1988). Using one dimensional electrophoresis, a 20 kD androgen dependent nuclear peptide was found after castration and subsequent androgen treatment (Kishimoto, R. et al., 1982; Venkatraman, J.Y. et al., 1984). The role of this 20 kD protein in regulation of gene expression is unknown. It is evident from these studies that steroid receptors may interact with the matrix and that the matrix may be important in controlling gene expression.

Table 1: Functions of the Nuclear Matrix
(For Review see Pienta, K.J., et al., 1989)

Nuclear Morphology	-Controls 3-D Structure of Nucleus and DNA
DNA Organization	-60 kD DNA Loop Domains Attached at Bases -Origins of Replication
DNA Replication	-Location of Fixed Sites of DNA Replication -Contains Replisome Complex which Includes DNA Polymerase and Newly Replicated DNA
RNA Synthesis	-Site of Actively Transcribed Genes -Contains hnRNA, RNA Splicing
Cell Regulation	-Associates with Steroid Hormone Receptors -Interaction with *myc*, large T antigen, EIA -Changes in Protein Composition with: Differentiation Mitogenic Stimulation Androgens

Alterations in nuclear matrix proteins may be responsible for the differential gene expression of the seminal vesicle and the ventral prostate. Nuclear matrix proteins have recently been shown to vary significantly between different cell lines (Fey, E.G. and Penman, S., 1988). This study also found differences in nuclear matrix proteins when comparing transformed and non-transformed cell lines of similar origins. The nuclear matrix proteins in the transformed cells are currently under investigation as possible probes for the diagnosis of cancer and in determination of the tissue of origin of some cancers.

MODIFICATIONS OF NUCLEAR MATRIX PROTEINS

The components of the nuclear matrix have been found to undergo a number of protein modifications including, phosphorylation, methylation and ADP-ribosylation. The major modification studied is that of phosphorylation. Several groups have demonstrated the phosphorylation of the nuclear matrix proteins (Allen, S.L. et al., 1977; Sevaljevic, L. et al., 1982; Henry, S.M. and Hodge, L.D., 1983; Moy, B.C., and Tew, K.D., 1986). Phosphorylation of the nuclear matrix proteins has been found to be under androgenic control (Goueli, S.A. and Ahmed, K., 1984) The nuclear components themselves were demonstrated to be involved in protein phosphorylation. Recently, the nuclear matrix of rat liver was found to possess tyrosine kinase activity (Teraoka, H. et al., 1989). The role of phosphorylation and other protein modifications in the matrix has not been resolved although, it is probable that these changes, especially phosphorylation, are somehow involved in the regulation of the activity of the nuclear matrix.

NUCLEAR MATRIX ISOLATION PROCEDURES AND ANALYSIS

There are several different procedures currently used for nuclear matrix isolation. The procedure utilized in these experiments involves the use of mild salt extraction with ammonium sulfate which causes minimal disruption of the nuclear matrix proteins and structure (figure 3). The protein components of the matrix were analyzed utilizing a high resolution two dimensional electrophoresis system developed in our laboratory.

This procedure employed 10% SDS/PAGE in the second dimension and required several modifications of the original O'Farrell technique for two dimensional electrophoresis (O'Farrell, 1975). The gels were silver stained (Accurate Scientific) after enhancement techniques.

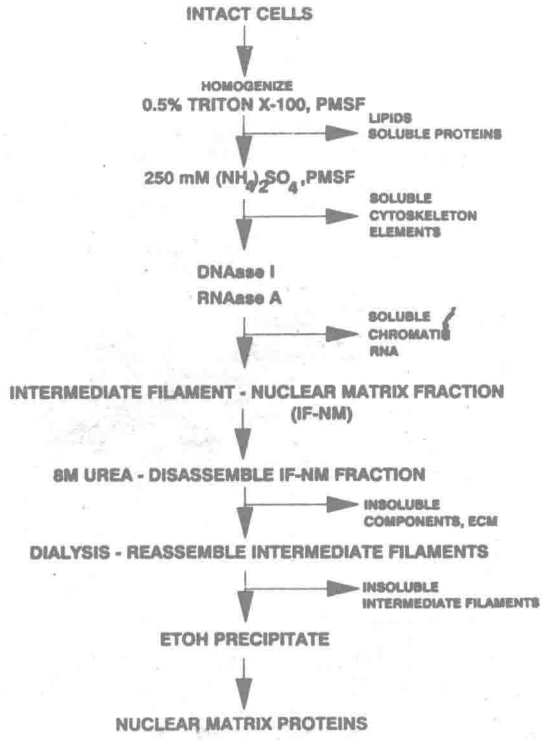


Figure 3: Nuclear Matrix Purification Procedure. Sequential isolation procedure from fresh tissue. Lipids and soluble proteins are removed with triton X-100. The soluble cytoskeletal elements are then removed with 0.25 M ammonium sulfate. DNAase I and RNAase A are then used to remove the soluble chromatin elements. The remaining intermediate filament-nuclear matrix fraction is then disassembled by solubilization in 8 M urea and 1% -mercaptoethanol at room temperature as described in Fey, E.G. et al.,1984. Removal of the urea with dialysis reassembles the intermediate filaments and the nuclear matrix proteins are removed from the supernatant by ethanol precipitation.

RESULTS

The nuclear matrix proteins of both the rat ventral prostate and seminal vesicle were compared using the two dimensional electrophoresis technique described. This technique provides high resolution of the protein components. When the gels from both the ventral prostate and seminal vesicle nuclear matrices are compared, the protein patterns of the two tissues are different. Thorough analysis reveals major proteins unique to each of the tissues which are indicated with arrows in the figures (figures 4 A & B). Numerous quantitative changes as well as minor qualitative protein changes were also noted in the gels. The major protein changes are unique for each tissue and any of these proteins can be used as a tool for tissue identification (figures 4 C & D).

To determine the effects of androgens, the protein patterns of both the intact rat ventral prostate and seminal vesicle nuclear matrices were compared with that of the 23 hour castrate ventral prostate and seminal vesicle nuclear matrices (figures 5 A & B). On visual examination these protein patterns from castrates are not markedly different from their intact counterparts. However, careful analysis of the protein patterns reveals several major proteins and numerous minor proteins which both disappear and

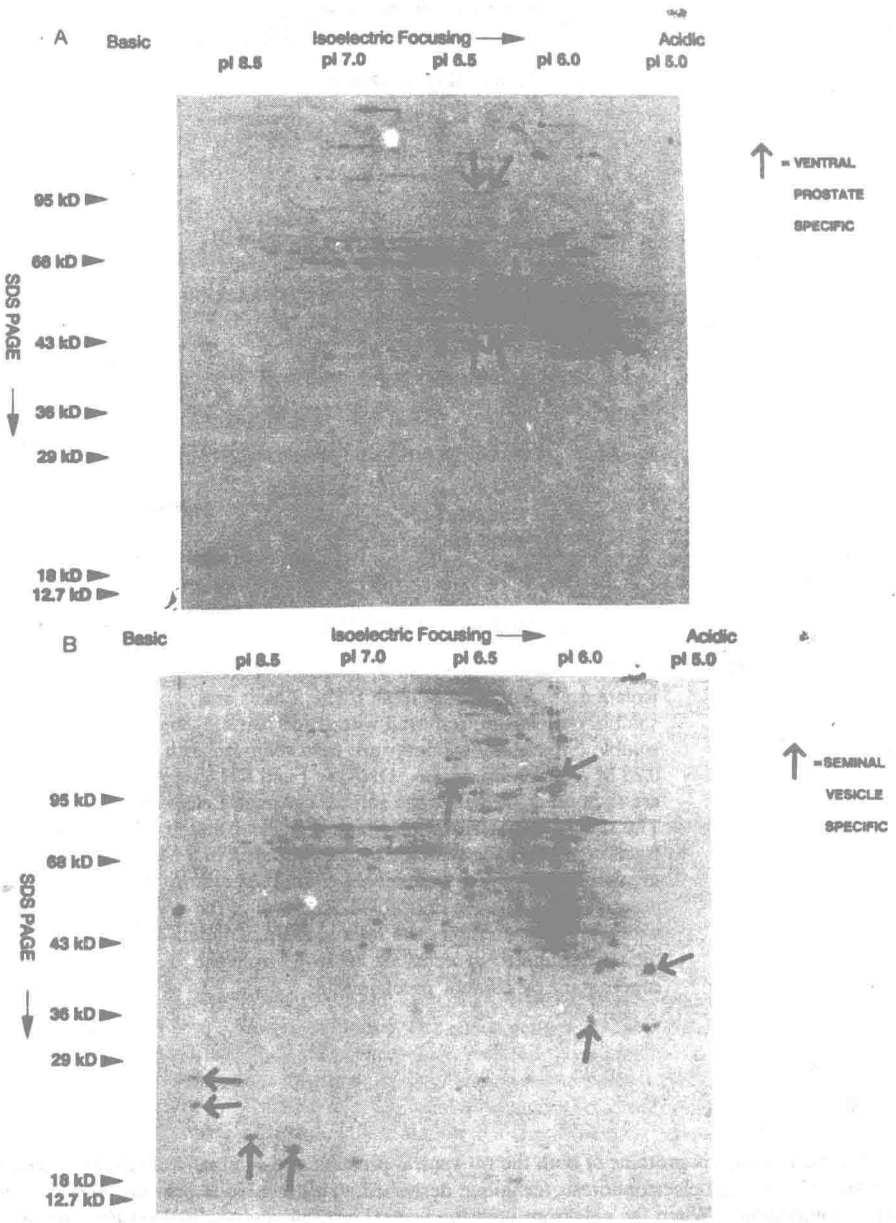
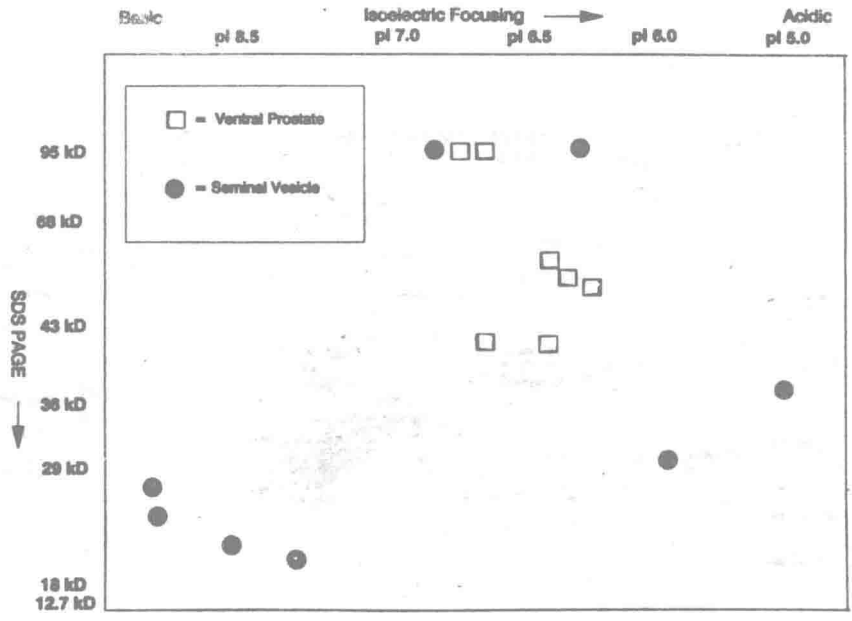


Figure 4 A & B: A. Intact - Ventral Prostate. B. Intact - Seminal Vesicle. High resolution two dimensional gel electrophoresis of rat nuclear matrix proteins was performed with modifications of O'Farrell, 1975. Second dimension electrophoresis was 10% SDS/PAGE under reducing conditions. 15ug. of protein was loaded on each gel. Gels were stained with silver stain (Accurate Chemical) after enhancement.



D **There are quantitative differences in proteins between gels, however, proteins which consistently appear as unique are described below:**

Seminal Vesicle		Ventral Prostate	
M.W.	pI	M.W.	pI
96 kD	6.27	95 kD	6.76
95 kD	6.84	95 kD	6.71
38 kD	5.10	52 kD	6.51
31 kD	6.01	50 kD	6.44
27 kD	*9.75	48 kD	6.37
24 kD	*9.50	41 kD	6.71
22 kD	8.50	41 kD	6.51
21 kD	8.00		

* = estimated pI

Figure 4 C & D: Tissue Specific Nuclear Matrix Proteins. Schematic of major tissue specific nuclear matrix proteins of ventral prostate and seminal vesicle.

appear when the rat is castrated (figures 5C & D). Quantitative alterations in the proteins were also noted between the intact and the castrate.

The phosphorylation patterns of the nuclear matrix proteins were also studied and these patterns were compared between ventral prostate and seminal vesicle. There are a large number of phosphorylated proteins in the nuclear matrix. The role of these phosphorylated proteins in nuclear matrix function and gene regulation is currently under investigation.

The results from these experiments can be summarized as follows. 1. There is a group of nuclear matrix proteins which are tissue specific for both the rat ventral prostate and seminal vesicle. 2. There is a series of nuclear matrix proteins that can vary depending on the hormonal state of the organ (castration, hormonal withdrawal). 3. Additionally, there also is a set of nuclear matrix proteins which are common between the seminal vesicle and ventral prostate (for example the lamins).

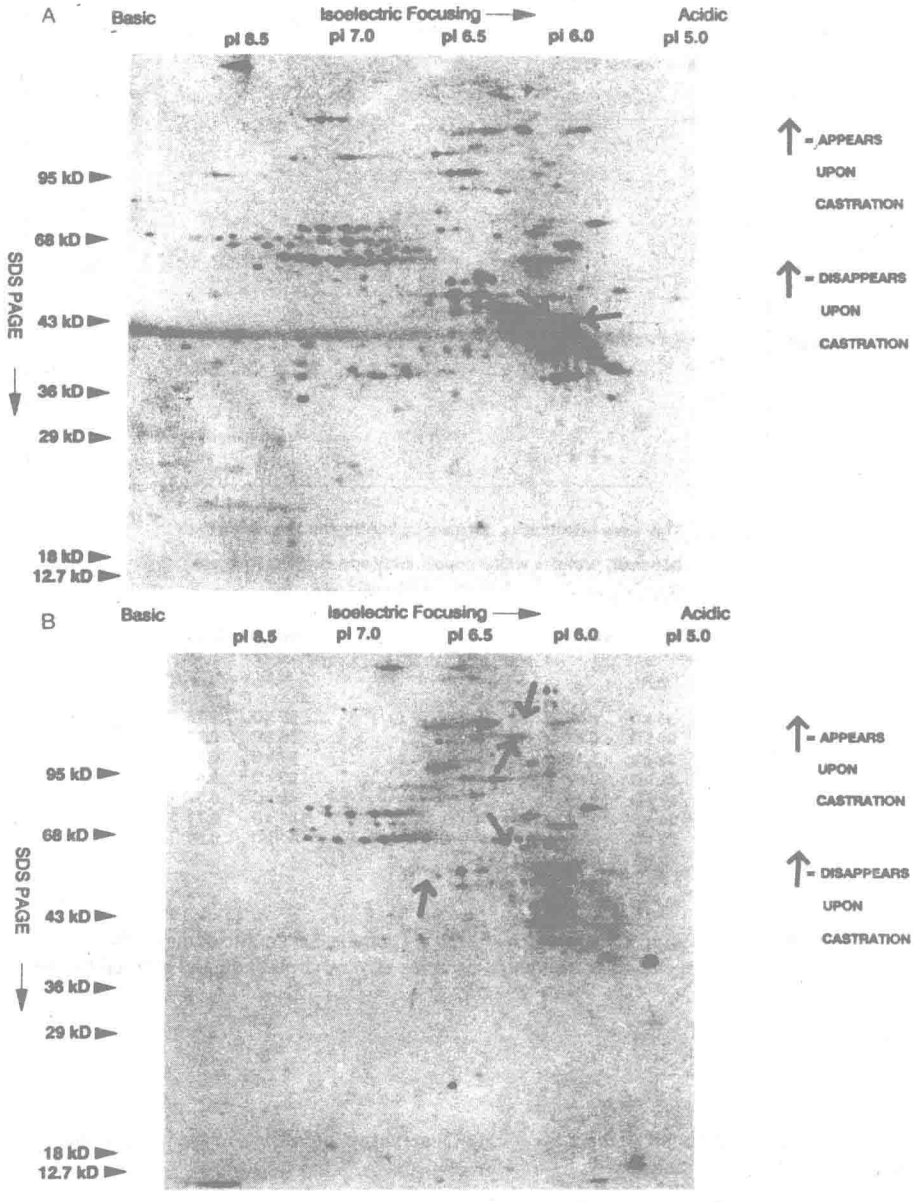
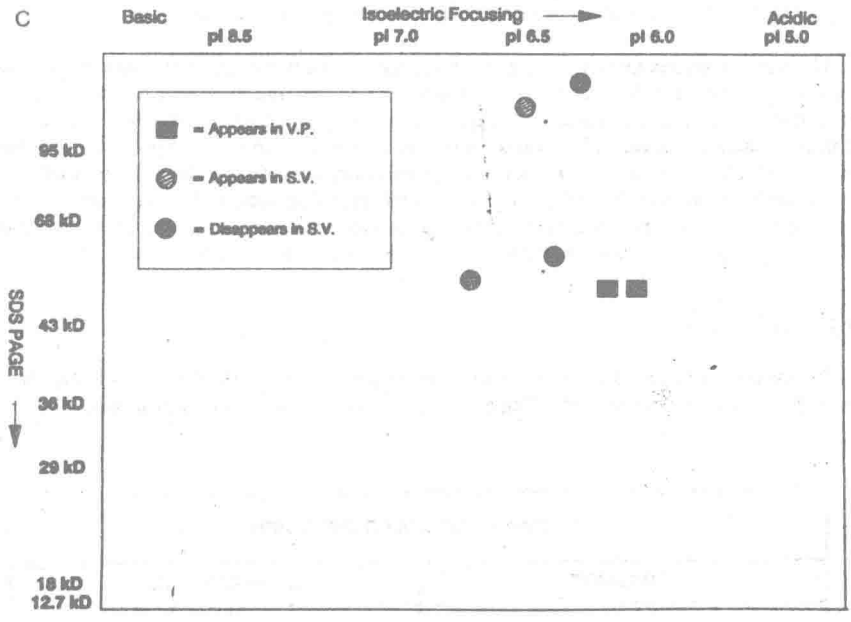


Figure 5 A & B: A. Castrate - Ventral Prostate. B. Castrate - Seminal Vesicle. High resolution two dimensional gel electrophoresis of rat nuclear matrix proteins was performed with modifications of O'Farrell, 1975. Second dimension electrophoresis was 10% SDS/PAGE under reducing conditions. 15ug. of protein was loaded on each gel. Gels were stained with silver stain (Accurate Chemical) after enhancement.



D There are quantitative differences in proteins between gels, however, proteins which consistently appear as unique are described below:

Appear Upon Castration

Ventral Prostate		Seminal Vesicle	
M.W.	pI	M.W.	pI
50 kD	6.27	105 kD	6.58
50 kD	6.14		

Disappear Upon Castration

Ventral Prostate		Seminal Vesicle	
M.W.	pI	M.W.	pI
NO MAJOR PROTEINS		110 kD	6.37
		59 kD	6.44
		52 kD	6.86

Figure 5 C & D: Casrate Induced Changes in Nuclear Matrix Proteins. Schematic of major nuclear matrix proteins which both appear and disappear in ventral prostate and seminal vesicle.

DISCUSSION

Our results demonstrate that the nuclear matrix is tissue specific. The nuclear matrix proteins which are specific for the seminal vesicle and ventral prostate may be involved in organizing the DNA into a specific three dimensional conformation which is unique for each tissue. This characteristic DNA structure can allow for the differential gene expression seen in the seminal vesicle and ventral prostate. These unique nuclear matrix proteins may be involved in binding DNA and/or interacting with the androgen receptor to permit transcription of the specific secretory products(figure 6).

The nuclear matrix undergoes characteristic alterations in protein composition upon castration. We have demonstrated that there are proteins which both appear and disappear in the ventral prostate and seminal vesicle nuclear matrices upon androgen withdrawal. These proteins may be indicative of alterations in nuclear matrix structure which occur in the seminal vesicle and ventral prostate from castrate animals. Specific alterations may be responsible for initiating the early events in programmed cell death which is known to occur in both the seminal vesicle and ventral prostate following androgen withdrawal. The exact function of those proteins which are tissue specific or appear/disappear upon castration is unknown. It is possible that these proteins are important determinants of gene expression.

FUTURE DIRECTION

The evidence presented in this chapter is the beginning of an extensive investigation into the function of the nuclear matrix in controlling gene expression. Future research will concentrate on learning

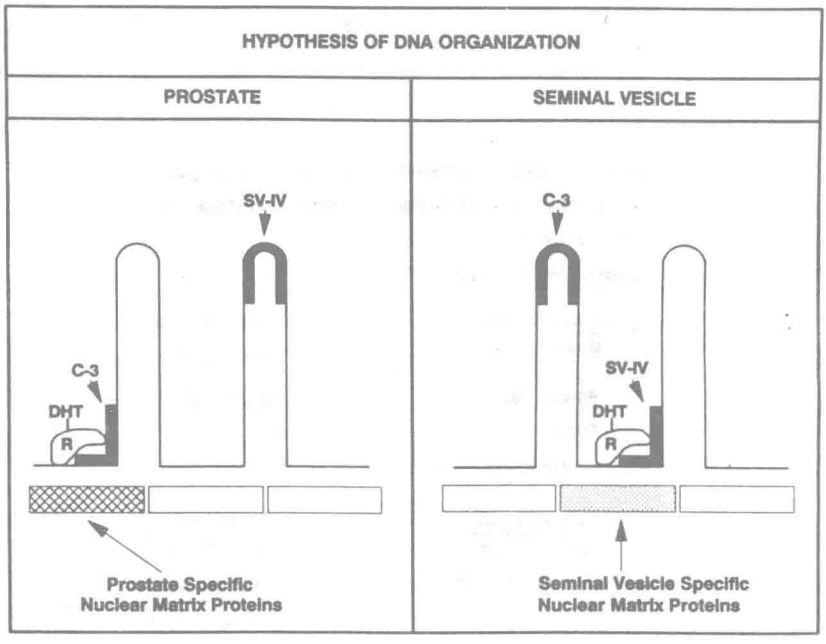


Figure 6: Model of tissue specific DNA organization. It has been reported that active genes are associated with the nuclear matrix (see text). This schematic is our hypothesis of tissue specific DNA loop organization in the ventral prostate and the seminal vesicle. Tissue specific nuclear matrix proteins are involved in the localization of specific DNA sequences and genes in proper configuration for DHT receptor to interact and allow activation of gene expression. By controlling the three dimensional conformation of the DNA, the tissue specific nuclear matrix proteins confer specificity to the protein products. The genes are on different DNA loops but not necessarily adjacent loops as is shown here for simplicity. R is the DHT receptor. C-3 and SV-IV signify gene sequences.