Proteomics of Spermatogenesis

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PREFACE

Although morphological events in mammalian spermatogenesis have been known for many years, it is only through recent development of experimental techniques in cellular and molecular biology that made it possible to understand molecular biology of male gametogenesis in sufficient detail. However, despite considerable research over past several decades, there has been no systematic attempt to organize protein sequences/structures involved in spermatogenesis. The **PROTEOMICS OF SPERMATOGENESIS,** first of its kind, is the first ever effort to describe proteomics of an organ system such as male reproduction and deals with germ cell specific proteins from the point of view of their structures and functions as well as their clinical applications. However, the subject focuses mainly on the description of protein isoforms, which have been either considered specific to- or dominantly expressed in germ cells and finally localized in spermatozoa. The book has been written keeping in mind that the subject may be beneficial not only to students of reproductive biology in understanding spermatogenesis, but may be useful in understanding the causes of genetic infertility in human males and in other mammalian species, although few examples on proteins during spermatogenesis of non-mammalian species also have been cited. The salient feature of proteomics of spermatogenesis is the compilation of up to date information, based on the available data in literature, which has been interpreted and described in the words of original researchers. More importantly, each chapter in relation to a group of proteins has been properly introduced, although the classification of these proteins is arbitrary and based on their cellular localization or their functions. The knowledge of germ cell specific protein isoforms and understanding of sperm specific proteins and polypeptides acquired during maturation in epididymis offers potential application for targeted intervention in testis without generalized effects on stages of spermatogenesis, and in the development of a contraceptive vaccine in males and females. Although each chapter is unique, but under a broader base the book can be classified into sections such as (i) Spermatogenesis (Chapters 1-5), (ii) Cytoskeleton proteins (Chapters 6-10, and Chapter 25), (iii) Proteins involved in the regulation of gene expression, and in the transcriptionl and translational activity (Chapters 11-17), (iv) Informational macromolecules and their relevance in cell communication during spermatogenesis and sperm-oocyte interactions (Chapters 18-22), (v) Proteins participating in cell adhesion and fertilization (Chapters 23-27, and Chapter 34), (vi) Is-proteins which regulate sperm motility (Chapters 28-29) and participate in quality control of sperm functions (Chapters 30 and 31). In addition, the discovery of association of germ cell specific isoforms with non-germ cell/ somatic cell tumors has opened new challenges for their application in the diagnosis and prognosis of oncogenesis and immunotherapy of variety of malignancies (Chapter 32). Therefore, the main objectives of proteomics of spermatogenesis were to acquaint the reproductive biologists and andrologists with the current status of basic and applied research on specialized proteins of mammalian germ cells, their role in spermatogenesis, and to help in identifying research strategies that might yield information useful in the design of male anti-fertility agent, and antigenic peptides as future perspectives for development of contraceptive-cum-cancer vaccines in males and females. Since each topic has been properly introduced, proteomics of spermatogenesis may be referred to as a text book for students undergoing advanced training in reproductive biology and as a guide for Research and Development by pharmaceutical industries.

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G.S.Gupta

CONTENTS

Chapter 1

SPERMATOGENESIS

1.1. TESTIS COMPARTMENTS

The main function of testis is to produce the male gametes and steroid hormones. Spermatogenesis and steroidogenesis take place in two different compartments: seminiferous tubules and interstitium respectively that are morphologically and functionally distinguished from each other. Although anatomically divided, both compartments are functionally connected to each other, and their integrity is essential for normal germ cell production. The functions of the testis and thereby also the functions of its compartments are primarily regulated by the hypothalamus and the pituitary gland, whereas at the testicular level various local regulatory molecules modulate the endocrine hormone actions in somatic and germ cells directly. In mammalian species, the testicular tubular compartment consists of a variety of cell types. The Sertoli cells comprise the main structural component of the seminiferous epithelium. They are responsible for the physical support of the germ cells, in addition to providing nutrients and growth factors. The germ cells are sequentially organized into several layers signifying the respective mitotic or meiotic processes and spermatid development. The presence of distinct germ cell associations allowed stages of the spermatogenic cycle of the seminiferous epithelium to be described on the basis of morphological changes in spermatid morphology. Although the staging is arbitrary, it is of great help in describing structural and physiological changes in the seminiferous epithelium. Each seminiferous tubule is surrounded by mesenchymal cells, which comprise the peritubular myoid cells whose contractile elements generate peristaltic waves along the tubules, but do not present a tight diffusion barrier. The interstitium, the other compartment is populated by androgen producing Leydig cells, which are heterogeneous in respect to their physiological and structural features. Vascular smooth muscle cells, macrophages and endothelial cells are also located in the interstitial space of the testis. The physiological function of macrophages has not been well studied. However, their presence is crucial for (re)population of Leydig cells during development and after experimental depletion. Immune cells, known to secrete a number of growth factors and cytokines, are part of the intra-testicular communication pathways. In addition, neuronal connections also influence cellular interaction in the testis.

1.1.1. Intra-Testicular Communication

At the organ and cell levels, a number of signaling factors operate for rapid communication and responsiveness. Presently, cellular communication is categorized into endocrine, paracrine and autocrine signaling. A signaling molecule can functionally cover more than one category.

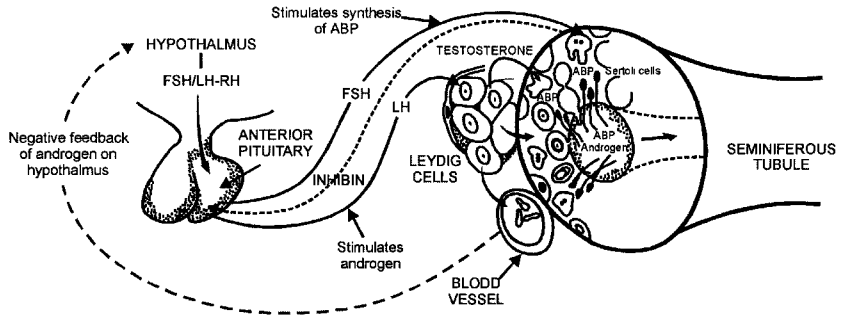

Fig.1.1: Hypothalmus-pituitary-testis axis in mammalian male reproduction.

To ensure coordinate organ function, response and activation, cells in one organ synthesize and release signaling molecules that act on distant organs. This signaling mechanism, termed endocrine signaling is mediated by hormones. Hormones are transported via the bloodstream from the site of production, and reach their cellular target through diffusion or mediated by receptor. Hence, endocrine communication, albeit indispensable and highly effective, is relatively slow **(Fig 1.1)**.

In addition to the regulation of testis function by hypothalamus and the pituitary gland, another level of interaction exists between the neighbouring cellular elements within each of two testicular compartments. While paracrine factors secreted from cells act through diffusion on neighbouring cells, the secreted molecules that act back on the cells from which they originate are referred to autocrine factors. Intracrine signaling has occasionally been used to describe factors that are produced and active within the same cell. In juxtacrine signaling, exoplasmic components of plasma membrane bind and act on adjacent cells through direct cell contact. However, same molecule can work for endocrine, paracrine and autocrine functions. Within testis, paracrine communication comprises not only signaling between neighbouring cells but also between the testicular compartments and among cells far from being in close proximity to each other. In testis, the paracrine mechanisms occur between immune cells, fibroblasts and Leydig cells in the interstitium, between interstitial cells and peritubular cells, between peritubular cells and Sertoli cells, between Sertoli cells and germ cells and among germ cells themselves. Sertoli cells are closely linked by tight and gap junctions from puberty onwards. This structure is known as the 'blood-testis barrier', which represents a tight diffusion barrier dividing the testis into two functional compartments (basal and adluminal) within each seminiferous tubules. Sertoli cells, the only cell type extending into two compartments have the important role of coordinating the secretion of signaling factors into tubular compartments **(Fig. 1.1)**. Sertoli cells also are endowed with a variety of structural features, which enable them to establish and maintain contact with the adjacent germ cells. It is evident that communication between the compartments is essential for functioning of the testis although the precise mechanisms of these interactions are less evident. Presently, more than 100 local factors have been identified and considered to be important for the testis function, but little is known concerning the relevance of these factors in human male infertility. Hence it would seem necessary to distinguish between local factors that are essential for spermatogenesis and those that show redundancies.

1.1.2. Seminiferous Tubules

Seminiferous tubules are enclosed by one or more layers of adventitial cells derived from primitive connective tissue elements of the interstitium. In rodents, a single layer of polygonal cells form a continuous epitheloid sheet surrounding the tubule. Because of their atypical shape and epitheloid organization, they are referred to as myoid cells or peritubular cells. In larger species, ram, bull, boar, man and monkey the adventitial cells form multiple layers. The properties of these cells differ from species to species. In adult mammals, the seminiferous tubules are lined by a complex stratified epithelium composed of two major categories of cells, supporting cells and spermatogenic cells. Supporting cells of single kind, called Sertoli cells were earlier believed to form a syncytium. But later they were shown to be individual cells uniformly spaced on the basal lamina with germ cells occupying expanded intercellular spaces between them. Sertoli cells cease to divide at the time of puberty but persist for whole life of an individual. The three dimensional configuration of Sertoli cell is extraordinarily complex.

The spermatogenic cells include severed morphologically defined cell types: spermatogonia, primary spermatocytes, secondary spermatocytes, spermatids and spermatozoa. Ontogenetically these spermatogenic cell types are not distinct but are successive stages of a process which after proliferation and differentiation lead to formation of mature differential spermatozoon. The proliferative activity in epithelium is confined to spermatogonia and spermatocytes near the base. The earlier cells, speramatogonium rests on the basal lamina propria or boundary tissue. Thus seminiferous epithelium in adults consists of a fixed population of non-proliferating supporting cells and a highly proliferating and differentiating population of germ cells with their stem cells at the base of the epithelium. As they develop, the germ cells are displaced upward along the sides of supporting cells. The topographical relations between germ cells and Sertoli cells change as germ cells move upwards from the base to the lumen, and have important implications in cell adhesion and communications. In seminiferous tubules, typical gap junctions or desmosomes are not found between Sertoli cells and germ cells in the upper two thirds of epithelium, due to free movement of germ cells to move upward as seen in other somatic tissues. However, specialized junctions (occluding junctions), described between adjacent Sertoli cells near the base of the epithelium, form the morphological basis of blood testis permeability barriers. These junctions divide the epithelium into basal compartment containing stem cells of spermatogenesis and adluminal compartment consisting of more advanced stages of spermatogenesis. In addition, these junctions regulate the permeability selective molecules necessary for spermatogenesis, without interruption of the permeability barrier. A number of studies have suggested that the basement membrane (BM) around seminiferous tubules has an important role in supporting testis differentiation, influencing in particular the differentiation of peritubular cells and the proliferation and differentiation of Sertoli cells, and their interaction with germ cells. In addition to compartmentalization, BM of seminiferous tubules acts as substrate for cells in contact and also provides important signals for differentiation, maintenance, and remodeling of tissues.

1.2. SPERMATOGENESIS

Spermatogenesis is a process by which spermatozoa are formed from spermatogonial stem cells during adult's reproductive phase. The process of sperm formation is initiated in the mouse embryo at around day 11.5 postcoitum (pc), when primordial germ cells (PGC) colonise the genital ridge. Under the influence of Y chromosome bearing Sertoli cells, the PGCs proliferate, some of which undergo apoptosis, while the remainder convert to gonocytes. The gonocytes

proliferate for a few days and then arrest in G_0/G_1 phase of cell cycle. After remaining quiescent until after birth, gonocytes are reactivated, and differentiate into spermatogonia to initiate the process of spermatogenesis. In rat and mouse the gonocytes resume proliferation (first wave of proliferation) within a few days after birth to form adult type spermatogonia. In mice the first wave of spermatogenesis occurs on day 5 after birth and at 6 months of age after birth in men. While some spermatogonia become self-renewing spermatogonial stem cells, most of them differentiate into spermatocytes, and meiosis begins at approximately day 10 pp in mice and at puberty in man. In the mouse, haploid spermatids are generated by day 20, and spermatozoa first appear in seminiferous tubules by approximately day 35. The onset of puberty and associated increase in gonadotrophin and androgen levels result in the progression of spermatocytes and the appearance of haploid spermatids. Thus, the entire process of spermatogenesis occurs in three sequential phases of cell proliferation and differentiation called: i) mitotic phase, ii) meiotic phase, and iii) post-meiotic phase, which involves stepwise progression of morphologically undifferentiated spermatids to highly differentiated spermatozoa. In mouse spermatogenesis, the mitotic phase lasts for 10 days, meiotic phase for 11 days, while post-meiotic phase lasts for 14 days. The final division produces preleptotene spermatocytes, which begin meiotic phase and undergo last cell cycle S-phase of spermatogenesis..

1.2.1. Mitotic Phase

In mitotic phase, also called spermatocytogenesis, primitive spermatogonia proliferate by mitosis to give rise to several successive generations of spermatogonia, each generation being more differentiating than the preceding one. Traditionally spermatogonia have been divided into two types of spermatogonia (A and B type). These can be distinguished with little difficulty. The type A spermatogonia do not have heterochromatin, whereas type B spermatogonia possess abundant heterochromatin in their nuclei. In human testis, the type A spermatogonium has a spherical or ellipsoid nucleus and one or two nucleoli attached to inner part of nuclear envelope. The type B spermatogonium has spherical nucleus containing a single nucleolus and the chromatin of varying size (heterochromatin), many of which are distributed along the nuclear envelope. In rats and mice intermediate type spermatogonia can also be observed. The A type spermatogonia undergo a series of divisions that result into other type A spermatogonia. During spermatogonial division, A single (A_s), a paired (A_p) and A aligned (A_{al}) spermatogonia can be seen according to their arrangement on the basal side of seminiferous tubules. Single spermatogonium (A_s) is the stem cell for spermatogenesis. On division, A_s produces two new stem cells, where as A_p spermatogonia are connected through intercellular cytoplasmic bridges, the functional significance of which is not clear. The paired spermatogonia (A_p) further divide to form chains of 4, 8 and 16 A_{al} spermatogonia **(Fig.1.2)**. The A_{al} spermatogonia undergo five successive divisions giving rise to A2, A3, A4, intermediate and finally B spermatogonia. B spermatogonia further divide to give primary spermatocytes, which are produced by last mitotic division during spermatogenesis (de Rooij and Grootegoed, 1998). Type A spermatogonia express a very high level of telomerase (Ravindranath et al., 1997). The expression of telomerase decreases with further stages of spermatogenesis and disappears in late spermatids.

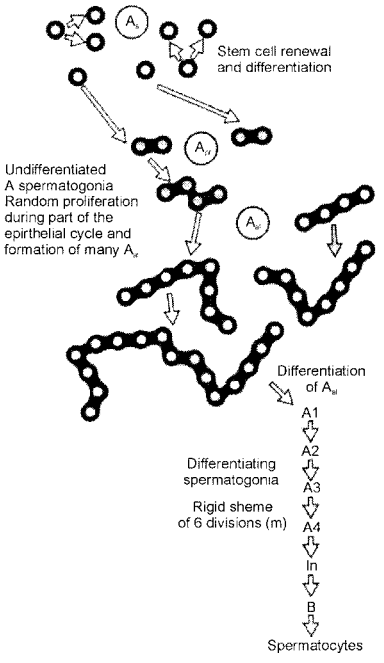

Fig.1.2. Scheme of spermatogonial multiplication and stem cell renewal, which probably applies to all mammals except humans. Stem cells (A_s) proliferate, renewing the stem cell pool and also producing undifferentiated A type paired spermatogonia (A_{pr}), joined together by intercellular cytoplasmic bridges. Further division of A_{pr} produce chains of aligned spermatogonia (A_{al}), which differentiate through six mitotic divisions into A1, A2, A3, A4, intermediate (In), and B spermatogonia to become primary spermatocytes. Reproduced with permission from de Rooij DG, Grootgoed JA.Curr Opin Cell Biol 10; 694-701: 1998 © Elsevier

1.2.2. Meiotic Phase

Most of the somatic cells contain chromosomes in pairs and hence called diploid, while gametes (sperm and ovum) possess only one of each pair. Such cells are called haploid. Haploidy of mammalian gametes is essential, since after fertilization, the zygote establishes the diploid character of chromosome number. The special type of nuclear division, which forms haploid gametes, is termed 'Meiosis'. The meiotic phase terminates at the primary spermatocytes, which at first resembles the cytological characteristics of spermatogonia from which they arise. Primary spermatocytes enter into prophase I of maturation or meiotic division. Their chromatin reorganizes into thread like chromosomes, characteristic of leptotene stage of meiosis. During meiotic phase (leptotene, zygotene, pachytene, diplotene and diakinesis) chromosomes condense. Two important events in meiosis are: linear pairing of chromosomes and interchange of genetic segments between homolgous chromatids during zygotene stage through formation of synaptonemal complex. This is followed by two meiotic divisions that occur in rapid succession without DNA replication to produce spermatids, which are re-modeled into spermatozoa. The process of meiosis and formation of synaptonemal complex has been discussed in more details in Chapter 6. During meiosis a wide variety of genes are up-regulated in spermatocytes. Some of these genes are transcribed only in spermatogenic cells, whereas others produce transcripts specific or unique to spermatocytes. The expression and regulation of several of these genes during meiosis has been recorded during last decade (McCarrey, 1998; Eddy and O'Brien, 1998) (see Chapter 14). The RNA synthesis is low at preleptotene, leptotene, zygotene and early pachytene spermatocytes. However, RNA synthesis increases rapidly in pachytene spermatocytes of mouse, rat, hamster, and human testes. Nuclear RNA synthesis is highest at zygotene stage in both mouse and human spermatocytes suggesting that RNA synthesis occurs during meiosis (Eddy and O'Brien, 1998).