

Proteomics of Spermatogenesis

Proteomics of Spermatogenesis

Dr. G.S. Gupta, Ph.D.

*Former Professor and Chairman
Department of Biophysics
Panjab University
Chandigarh-160014
India*

 Springer

A C.I.P. Catalogue record for this book is available from the Library of Congress.

ISBN: 0-387-25398-X

©2005 Springer Science+Business Media, Inc.

All rights reserved. This work may not be translated or copied in whole or in part without the written permission of the publisher (Springer Science+Business Media, Inc., 233 Spring Street, New York, NY 10013, USA), except for brief excerpts in connection with reviews or scholarly analysis. Use in connection with any form of information storage and retrieval, electronic adaptation, computer software, or by similar or dissimilar methodology now known or hereafter developed is forbidden. The use in this publication of trade names, trademarks, service marks and similar terms, even if they are not identified as such, is not to be taken as an expression of opinion as to whether or not they are subject to proprietary rights.

Printed in the United States of America.

9 8 7 6 5 4 3 2 1

springeronline.com

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 transcriptional 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.

The author acknowledges the financial grant from Department of Science and Technology under USERS scheme and Emeritus Fellowship from University Grants Commission during the tenure of this project. The author also appreciates the cooperation of various research

investigators for providing scientific literature and various copyright owners such as publishing agencies and scientific societies, and authors for granting copyright permissions to reproduce figures and other illustrations from original journals and books.

The author wishes to express his personal gratitude to Professor K.N.Pathak, the Vice-Chancellor and Dr. S.N. Sanyal, the Chairman, Department of Biophysics, Panjab University, Chandigarh for providing space and office facilities of the department during the entire tenure of writing of the manuscript. I am also thankful to my colleagues and friends, and office staff for their support and co-operation during this task. My special appreciation goes to Kishori (wife), Rajesh (son), and other family members who had been supporting this venture with great patience and their personal inconveniences.

G.S.Gupta

CONTENTS

| | |
|--|-----------|
| 1. SPERMATOGENESIS | 1 |
| 1.1. TESTIS COMPARTMENTS | 1 |
| 1.1.1. Intra-Testicular Communication | |
| 1.1.2. Seminiferous Tubules | |
| 1.2. SPERMATOGENESIS | 3 |
| 1.2.1. Mitotic Phase | |
| 1.2.2. Meiotic Phase | |
| 1.2.3. Post-meiotic Phase (Spermiogenesis) | |
| 1.3. ENDOCRINE HORMONES AND SPERMATOGENESIS | 8 |
| 1.3.1. Action of LH | |
| 1.3.3. FSH Action | |
| 1.3.4. FSH Receptor | |
| 1.3.5. Local Factors in Trophic Hormone Action | |
| 1.4. TESTOSTERONE AND SPERMATOGENESIS | 13 |
| 1.4.1. Androgen Receptors | |
| 1.4.2. Androgen Binding Protein | |
| 1.4.3. Estradiol | |
| 1.5. PROLACTIN | 17 |
| 1.6. SEROTONIN RECEPTOR IN SERTOLI CELLS | 17 |
| 1.7. REFERENCES | 18 |
| 2. PARACRINE ROLE OF SERTOLI CELL | 21 |
| 2.1. SERTOLI CELL | 21 |
| 2.1.1. Co-culture Experiments | |
| 2.1.2. Dependence of Sertoli Cell Products on Stage of Spermatogenesis | |
| 2.2. BASEMENT MEMBRANE COMPONENTS OF SEMINIFEROUS TUBULES | 23 |
| 2.2.1. Collagen Type IV and Type II | |
| 2.2.2. Laminin | |
| 2.2.3. Fibulins and Other Components | |
| 2.3. SERTOLI CELL PRODUCTS IN GERM CELLS DEVELOPMENT | 25 |
| 2.3.1. Cytokines | |
| 2.3.2. Growth Factors | |
| 2.3.3. Stem Cell Factor | |
| 2.3.4. Sertoli Cell's Cystatins | |
| 2.3.5. Mannose-6-phosphate Receptor | |
| 2.3.6. Other Sertoli Cell Products | |

| | |
|---|-----------|
| 2.4. SERTOLI CELL JUNCTION ADHESIONS | 32 |
| 2.4.1. Neural Cell Adhesion Molecule (NCAM) at Sertoli Cell-Gonocyte Junction | |
| 2.4.2. $\alpha 6 \beta 1$ Integrin | |
| 2.4.3. Connexin 43 and Zonaoccludin-1 | |
| 2.4.4. Tpx-1 | |
| 2.4.5. Clusterin | |
| 2.4.6. Osteopontin | |
| 2.4.7. Other Junction Proteins | |
| 2.5. INTRACELLULAR BRIDGES/ ECTOPLASMIC SPECIALIZATIONS | 37 |
| 2.6. SERTOLI CELL TIGHT JUNCTIONS DYNAMICS | 38 |
| 2.7. SPERMATION | 38 |
| 2.8. GERM CELL-SERTOLI CELL INTERACTIONS | 40 |
| 2.9. P-MOD-S FROM PERITUBULAR CELLS | 42 |
| 2.10. REFERENCES | 42 |
| 3. NON-STEROIDAL SIGNAL MOLECULES IN SPERMATOGENESIS | 47 |
| 3.1. INHIBIN FAMILY | 47 |
| 3.1.1. Inhibin | |
| 3.1.2. Activins | |
| 3.1.3. Follistatin | |
| 3.2. GROWTH FACTORS | 52 |
| 3.2.1. Insulin Like Growth Factors | |
| 3.2.2. Fibroblast Growth Factors | |
| 3.2.3. Epidermal Growth Factor | |
| 3.2.4. Transforming Growth Factor- α | |
| 3.2.5. Transforming Growth Factor- β | |
| 3.2.6. Anti-Mullerian Hormone | |
| 3.2.7. Platelet Derived Growth Factor | |
| 3.2.8. Nerve Growth Factor | |
| 3.2.9. Neurotrophic Factors | |
| 3.2.10. Vascular Endothelial Growth Factor | |
| 3.2.11. Hepatocyte Growth Factor/Activator-Inhibitor | |
| 3.3. PLASMINOGEN ACTIVATORS | 62 |
| 3.3.1. Ly-6/Urokinase-Type Plasminogen Activator Receptor | |
| 3.3.2. Plasminogen Activator Inhibitor Proteins | |
| 3.4. PEPTIDE HORMONES | 64 |
| 3.4.1. Rennin-Angiotensin System | |
| 3.4.2. Angiotensin Converting Enzyme | |
| 3.4.3. Kallikrein-Kinin System | |
| 3.4.4. Proopiomelanocortin Peptides | |
| 3.4.5. Proenkephalin and Prodynorphin peptides | |
| 3.4.6. Action of Other Peptide Hormones | |
| 3.5. REFERENCES | 72 |
| 4. SPECIFICITY OF RETINOL, ESTROGEN AND STEROID LINKED PROTEINS | 77 |
| 4.1. ACTION OF RETINOIDS | 77 |

| | | |
|-----------|--|------------|
| 4.1.1. | Retinol Binding Proteins | |
| 4.1.2. | Retinol to Retinoic Acids | |
| 4.1.3. | <i>Stra 8-A</i> : A Retinoic Acid Inducible Gene | |
| 4.2. | NUCLEAR RECEPTORS | 79 |
| 4.3. | RETINOID RECEPTORS AND THEIR HOMOLOGUES | 80 |
| 4.3.1. | Retinoid Receptors | |
| 4.3.2. | Germ Cell Nuclear Factor (GCNF) | |
| 4.3.3. | Retinoid Testis Receptor (RTR) | |
| 4.3.4. | Tr 2-11 Homologue | |
| 4.3.5. | hTAKI | |
| 4.3.6. | Nuclear Receptor Co-activator | |
| 4.4. | ACTION OF ESTROGENS | 84 |
| 4.4.1. | Estrogen Receptors | |
| 4.4.2. | P450 Aromatase | |
| 4.4.3. | P450 Arom Gene (<i>CYP19</i>) | |
| 4.4.4. | P450arom Deficiency | |
| 4.5. | OTHER STEROIDS LINKED ENZYMES IN GERM CELLS | 88 |
| 4.5.1. | Testis-Specific Lanosterol 14 α -Demethylase (CYP51) | |
| 4.5.2. | Steroidogenic Acute Regulatory (StAR) Protein and Homologues | |
| 4.5.3. | T-StAR/ETOILE | |
| 4.6. | REFERENCES | 93 |
| 5. | HOMEOSTASIS OF GERM CELLS AND APOPTOSIS | 97 |
| 5.1. | APOPTOSIS | 97 |
| 5.1.1. | Death Signals | |
| 5.1.2. | Caspases | |
| 5.2. | THE MECHANISMS OF APOPTOSIS | 98 |
| 5.2.1. | The Intrinsic or Mitochondrial Pathway | |
| 5.2.2. | The Extrinsic or Death Receptor Pathway | |
| 5.2.3. | Apoptosis-Inducing Factor (AIF) Release Pathway | |
| 5.3. | INHIBITORS OF APOPTOSIS PROTEINS (IAPS) | 102 |
| 5.4. | APOPTOSIS DURING SPERMATOGENESIS | 102 |
| 5.4.1. | FAS System in Testis | |
| 5.4.2. | Apaf-1 and Cytochrome C in Germ Cell Apoptosis | |
| 5.4.3. | p53 Induced Pathway | |
| 5.4.4. | Bcl-2 Protein Family | |
| 5.5. | FACTORS CONTROLLING APOPTOSIS IN SPERMATOGENESIS | 107 |
| 5.5.1. | Paracrine Control of Apoptosis | |
| 5.5.2. | Endocrine Control of Apoptosis | |
| 5.5.3. | Selective Apoptosis of Damaged Germ Cells | |
| 5.5.4. | Sertoli Cell-Germ Cell Contact | |
| 5.5.5. | Phagocytosis of Apoptotic Cells | |
| 5.6. | REFERENCES | 109 |
| 6. | NUCLEAR SKELETON PROTEINS: NON-HISTONES | 111 |
| 6.1. | CHROMOSOMAL ORGANIZATION | 111 |
| 6.2. | CELL DIVISION | 111 |

| | | |
|-----------|---|------------|
| 6.2.1. | Mitosis | |
| 6.2.2. | Meiosis | |
| 6.3. | THE SYNAPTONEMAL COMPLEX | 115 |
| 6.4. | SYNAPTONEMAL COMPLEX PROTEINS | 117 |
| 6.4.1. | Synaptonemal Complex Protein-1 | |
| 6.4.2. | Synaptonemal Complex Protein-2 | |
| 6.4.3. | Synaptonemal Complex Protein-3 | |
| 6.4.4. | Other SC Proteins | |
| 6.5. | STRUCTURAL MAINTENANCE CHROMOSOME PROTEINS | 122 |
| 6.6. | t-COMPLEX POLYPEPTIDES | 124 |
| 6.6.1. | TCP-1 | |
| 6.6.2. | Tcte 2 | |
| 6.7. | CENTROMERE PROTEIN-B | 126 |
| 6.8. | NUCLEAR LAMINS | 126 |
| 6.9. | NUCLEAR ASSOCIATED PROTEIN (NASP) | 128 |
| 6.10. | SPERM CYLICIN | 129 |
| 6.11. | NUCLEAR PORE ASSOCIATED PROTEINS | 130 |
| 6.12. | HIGH MOBILITY GROUP (HMG) PROTEINS | 130 |
| 6.13. | OTHER NUCLEAR ANTIGENS | 131 |
| 6.14. | REFERENCES | 134 |
| 7. | NUCLEAR SKELETON PROTEINS: CHROMOSOMAL | 137 |
| | BASIC PROTEINS | |
| 7.1. | MAMMALIAN TESTIS HISTONES | 137 |
| 7.2. | HISTONE H2A/TH2A | 138 |
| 7.2.1. | Somatic Variants | |
| 7.2.2. | Testis Specific TH2A Variant | |
| 7.3. | HISTONE H2B/TH2B | 139 |
| 7.3.1. | Somatic H2B Variants | |
| 7.3.2. | Testis TH2B | |
| 7.4. | HISTONE H3 /TH3 VARIANTS | 141 |
| 7.5. | HISTONE H4t | 142 |
| 7.6. | HISTONE H1 AND TESTIS VARIANT H1t | 143 |
| 7.6.1. | Histone H1 | |
| 7.6.2. | Testis Specific Histone-1 (H1t) | |
| 7.7. | HISTONES IN CHROMOSOME ASSEMBLY | 150 |
| 7.8. | UBIQUITINATION OF HISTONES | 152 |
| 7.9. | TRANSITION PROTEINS | 152 |
| 7.10. | PROTAMINES | 155 |
| 7.10.1. | Characterization and Functions | |
| 7.10.2. | Protamine Genes | |
| 7.11. | NATURE OF BASIC PROTEINS IN OTHER SPECIES | 160 |
| 7.11.1. | Basic Proteins of Winter Flounder | |
| 7.12. | REFERENCES | 162 |
| 8. | MICROTUBULES | 167 |
| 8.1. | INTRODUCTION | 167 |
| 8.2. | FLAGELLAR STRUCTURE AND DOUBLET SLIDING | 168 |

| | |
|---|-----|
| 8.3. TUBULINS | 169 |
| 8.4. MICROTUBULES IN SPERMATOGENIC CELLS | 170 |
| 8.4.1. Testicular- α Tubulin | |
| 8.4.2. Cell Specific Expression of β -Tubulin Isoforms | |
| 8.4.3. Glutamylated and Glycosylated Tubulins (Post-Translational Modifications) | |
| 8.4.4. δ -Tubulin | |
| 8.5. MICROTUBULE ASSOCIATED PROTEINS | 175 |
| 8.5.1. Tektins | |
| 8.5.2. Microtubule Associated Protein-2 like Proteins | |
| 8.5.3. E-MAP-115/MTEST 60 | |
| 8.5.4. TBP-1-Like Subfamily with ATPase and Protease Domains | |
| 8.5.5. CLIP50 | |
| 8.5.6. CAS | |
| 8.6. RNA BINDING PROTEINS IN MICROTUBULES | 181 |
| 8.6.1. Spermatid Perinuclear RNA Binding Protein (Spnr) | |
| 8.6.2. Testis-Brain RNA Binding Protein | |
| 8.6.3. Fragile X Mental Retardation-1 Protein | |
| 8.7. OTHER MICROTUBULE ASSOCIATED PROTEINS | 182 |
| 8.8. CENTROSOME | 183 |
| 8.9. CENTROSOME PROTEINS | 185 |
| 8.9.1. γ -Tubulin in MT Nucleation | |
| 8.9.2. Centrin and Other Proteins in Centrosome | |
| 8.10. REFERENCES | 187 |
| 9. MICROTUBULE BASED MOTOR PROTEINS | 191 |
| 9.1. KINESIN MOTOR | 191 |
| 9.1.1. Kinesin Related Proteins | |
| 9.1.2. Kinesin Family C-Terminal 5A Gene (KIFC5A) | |
| 9.1.3. Kinesin Motor in <i>Drosophila</i> | |
| 9.1.4. Structure-Function Relation | |
| 9.2. DYNEIN MOTORS | 196 |
| 9.3. AXONEMAL DYNEINS | 197 |
| 9.3.1. Doublet Tubules of Sperm Flagella | |
| 9.3.2. Outer Row Dyneins | |
| 9.3.3. Outer Arm Dynein Structure | |
| 9.3.4. Tctex-1: A Cytoplasmic LC Dynein in Inner Dynein Arm | |
| 9.3.5. C and A Heavy Chain Dynein (C/A Dynein) | |
| 9.4. RADIAL SPOKE PROTEINS | 203 |
| 9.5. FORCE GENERATION BY DYNEIN ARMS AND BEAT RHYTHMICITY | 203 |
| 9.6. CYTOPLASMIC DYNEINS | 205 |
| 9.6.1. Tctex-2: An Analogue of Outer Dynein Arm LC2 | |
| 9.6.2. Cytoplasmic Dynein in Sertoli Cells and Germ Cells | |
| 9.7. STRUCTURE-FUNCTION RELATIONS IN DYNEINS | 207 |
| 9.7.1. AAA Domains and Organization of the Dynein Motor Unit | |
| 9.8. REFERENCES | 209 |

| | |
|---|------------|
| 10. SEX CHROMOSOMAL PROTEINS AND AUTOSOMAL HOMOLOGUES | 211 |
| 10.1. X-CHROMOSOME ABERRATIONS AND SPERMATOGENESIS | 212 |
| 10.1.1. Xp22 Contiguous Gene Syndrome | |
| 10.1.2. Translocation of X-Chromosome Genes to Autosomes and Y-Chromosome | |
| 10.2. X-CHROMOSOME LINKED PROTEINS | 213 |
| 10.2.1. Pro-mAKAP82 | |
| 10.2.2. SPAN-X | |
| 10.2.3. Cleavage Stimulation Factor Like Protein | |
| 10.3. Y-CHROMOSOME ABERRATIONS | 214 |
| 10.3.1. Y-Chromosome and Sex Reversal | |
| 10.3.2. The SRY: a Sex-Determining Region on Y Gene in Mammals | |
| 10.4. Y CHROMOSOME AND SPERMATOGENESIS | 217 |
| 10.4.1. AZFa Region | |
| 10.4.2. ZFb Region | |
| 10.4.3. AZFc Region | |
| 10.4.4. RBM Gene Family | |
| 10.5. THE DAZ GENE FAMILY AS AZF CANDIDATE | 222 |
| 10.6. THE CDY GENE FAMILY | 223 |
| 10.7. OTHER SPERMATOGENESIS-RELATED GENES ON Y-CHROMOSOME | 223 |
| 10.8. AUTOSOMAL GENES PRODUCTS | 224 |
| 10.8.1. SOX9 and Other SOX Proteins | |
| 10.8.2. Autosomal DAZ like (DAZL) Proteins | |
| 10.8.3. Murine DazL1 Binding mRNAs | |
| 10.8.4. Boule and DAZ | |
| 10.8.5. MORC Gene | |
| 10.8.6. Other Gene Products in Infertility | |
| 10.9. XY BODY | 230 |
| 10.10. REFERENCES | 231 |
| 11. CELL CYCLE COMPONENTS | 235 |
| 11.1. CELL CYCLE | 235 |
| 11.2. CELL CYCLE GENES IN YEAST | 235 |
| 11.2.1. Cdc2, Cdc28 and Cdc13 Genes | |
| 11.2.2. Cdc25 and Wee1 Genes | |
| 11.2.3. Cyclin and Other Genes in Budding Yeast | |
| 11.3. DEPENDENCE OF MITOSIS ON DNA SYNTHESIS | 237 |
| 11.4. BIOCHEMISTRY OF CELL DIVISION: AN OVERVIEW | 238 |
| 11.4.1. MPF and Kinase Activity | |
| 11.4.2. Phosphorylation / Dephosphorylation of Cdc2 | |
| 11.5. MPF ACTIVITY IN SPERMATOGENESIS | 240 |
| 11.5.1. Cdk activity in Spermatogenic Cells | |
| 11.5.2. Multiple Forms of Cdks in Spermatogenesis | |
| 11.5.3. Human Wee1 | |
| 11.5.4. Other Protein Kinases in Cell Cycle | |
| 11.6. CELL CYCLINS IN SPERMATOGENIC CELLS | 243 |
| 11.6.1. Cyclin B: A Component of MPF | |
| 11.6.2. Cyclin A1 in Male Germ Cells | |

| | |
|--|------------|
| 11.6.3. Cyclin A2 | |
| 11.6.4. D-Type Cyclins | |
| 11.6.5. Cyclin G Associated Kinase (GAK)-Cdk5 | |
| 11.6.6. Cyclin H / Cdk7 Complex | |
| 11.6.7. Sperm Cyclin I | |
| 11.6.8. Cyclin K | |
| 11.7. CDC25 PHOSPHATASES IN MALE GERM CELLS | 253 |
| 11.8. CYCLIN DEPENDENT KINASE INHIBITORS | 254 |
| 11.8.1. INK4 Family | |
| 11.8.2. Cip/Kip Family of Cdk Inhibitors | |
| 11.9. ACTIVATION OF MAPK PATHWAY DURING MEIOSIS | 257 |
| 11.10. REFERENCES | 258 |
| 12. ISOPROTEINS IN DNA SYNTHESIS | 261 |
| 12.1. EUKARYOTIC DNA POLYMERASES | 261 |
| 12.1.1. DNA Polymerases in Spermatogenesis | |
| 12.1.2. DNA Polymerase β | |
| 12.1.3. Pol λ | |
| 12.1.4. DNA Polymerase ξ | |
| 12.2. DNA LIGASES | 266 |
| 12.3. DNA HELICASES | 267 |
| 12.4. DNA TOPOISOMERASES | 270 |
| 12.5. REVERSE TRANSCRIPTASE | 272 |
| 12.6. TELOMERE PROTEINS | 273 |
| 12.6.1. Telomere Binding Proteins | |
| 12.6.2. Telomerase and Telomere Length | |
| 12.6.3. Tankyrase | |
| 12.7. REFERENCES | 277 |
| 13. DNA REPAIR AND RECOMBINATION | 279 |
| 13.1. MISMATCH REPAIR | 279 |
| 13.1.1. Mismatch Repair Genes | |
| 13.1.2. Proteins with Nuclease Activity | |
| 13.2. PROTEIN SPECIFICITY IN RECOMBINATION REPAIR | 285 |
| 13.2.1. RAD52 | |
| 13.2.2. RAD51 | |
| 13.2.3. RAD51 Paralogs | |
| 13.2.4. Excision Repair Cross Complimenting Genes | |
| 13.2.5. BLM Helicase | |
| 13.3. POST-REPLICATION REPAIR GENE PRODUCTS | 292 |
| 13.3.1. RAD6 and its Mammalian Homologues: HR6A and HR6B | |
| 13.3.2. Mammalian Homologues of Rad 18 | |
| 13.3.3. RAD30 Gene | |
| 13.4. CELL CYCLE CHECK POINT CONTROL | 296 |
| 13.4.1. RAD1 | |
| 13.4.2. ATR/ATM Gene Products | |
| 13.5. CRE RECOMBINASE | 298 |
| 13.6. REFERENCES | 299 |

| | |
|--|------------|
| 14 TRANSCRIPTIONAL CONTROL | 303 |
| 14.1. TRANSCRIPTION IN EUKARYOTES | 303 |
| 14.2. CHAUVINIST GENES | 304 |
| 14.3. REGULATORY FACTORS IN GENE EXPRESSION OF GERM CELLS | 305 |
| 14.3.1. Intrinsic and Extrinsic Factors | |
| 14.3.2. Chromatin Structure | |
| 14.3.3. Role of Nuclear Matrix in Replication | |
| 14.3.4. Role of Chromosomal Proteins in Transcription | |
| 14.3.5. In-situ Modification of Nucleoproteins | |
| 14.4. GENE METHYLATION AND GENE EXPRESSION IN SPERMATOGENESIS | 307 |
| 14.4.1. DNA Methylation of Testis Specific Genes | |
| 14.4.2. CpG Islands and Spermatogenesis | |
| 14.4.3. Methyl-CpG-Binding Proteins | |
| 14.4.4. DNA Methyltransferase | |
| 14.5. POLY-(ADP)-RIBOSYLATION | 311 |
| 14.5.1. Poly-(ADPR)-Polymerase | |
| 14.5.2. Poly(ADP-ribosyl)transferase (pADPRT) | |
| 14.6. ACETYLATION AND REGULATION OF PROTEIN FUNCTIONS | 312 |
| 14.6.1. Histone Acetylation | |
| 14.6.2. ESET Histone Methyltransferase | |
| 14.6.3. Functional Significance of Acetylation | |
| 14.7. PHOSPHORYLATION / DEPHOSPHORYLATION | 314 |
| 14.8. PROTEIN BINDING SITES IN DNA | 314 |
| 14.9. FAMILIES OF DNA BINDING PROTEINS | 315 |
| 14.9.1. Helix-Turn-Helix (HTH) and Homeodomain | |
| 14.9.2. Zinc Finger Proteins | |
| 14.9.3. Leucine Zipper | |
| 14.9.4. The Helix-Loop-Helix (HLH) | |
| 14.9.5. β -Sheet Motifs | |
| 14.9.6. Other Families | |
| 14.10. REFERENCES | 321 |
| 15. PROTEINS IN TRANSCRIPTIONAL ACTIVITY OF SPERMATOGENIC CELLS | 323 |
| 15.1. TRANSCRIPTIONAL ACTIVITY IN GERM CELLS | 323 |
| 15.1.1. RNA Polymerases in Eukaryotes | |
| 15.1.2. Initiation of RNA Synthesis in Eukaryotes | |
| 15.1.3. Pre-Initiation Complex with RNA Polymerase II | |
| 15.2. RNA SYNTHETIC MACHINERY IN SPERMATOGENESIS | 324 |
| 15.2.1. RNA in Meiosis and Post-meiotic Stages | |
| 15.2.2. RNA Polymerases | |
| 15.2.3. TATA Binding Protein | |
| 15.2.4. TFIID Subunit TAF7 | |
| 15.2.5. Transcription Elongation Factor (S-II) | |
| 15.3. RNA PROCESSING IN GERM CELLS | 328 |

| | |
|--|------------|
| 15.3.1. Alternative RNA Splicing | |
| 15.3.2. Polyadenylation | |
| 15.3.3. Testis Specific Poly (A) Polymerase | |
| 15.3.4. Poly-A Binding Protein | |
| 15.3.5. Cleavage Stimulation Factor – 64 | |
| 15.4. TRANSLATIONALACTIVITY IN GERM CELLS | 331 |
| 15.4.1. Eukaryotic Translation Elongation Factor 1 (eEF1) | |
| 15.5. m-RNA BINDING PROTEINS | 332 |
| 15.5.1. Eukaryotic Translation Factor (eIF-4E) | |
| 15.5.2. RNA Helicases | |
| 15.5.3. Protamine m-RNA Binding Protein | |
| 15.5.4. Testis Nuclear RNA Binding Protein (Tenr) | |
| 15.5.5. Testis-Brain RNA Binding Protein (Translin) | |
| 15.5.6. Y- Box Proteins | |
| 15.5.7. Other mRNA-Binding Proteins in Germ Cells | |
| 15.6. mRNA AND RNP IN SPERMATOZOA | 344 |
| 15.7. REFERENCES | 345 |
| 16. TRANSCRIPTION FACTORS ASSOCIATED WITH SPERMATOGENESIS | 347 |
| 16.1. CRE-TRANSCRIPTION FACTORS (b-ZIP CLASS PROTEINS) | 347 |
| 16.2. CRE-MODULATOR (CREM) PROTEIN | 347 |
| 16.2.1. Signal Transduction | |
| 16.2.2. Germ Cell Specific CREM Isoform | |
| 16.2.3. CREM Activator Protein | |
| 16.2.4. CREM and Spermatogenic Genes | |
| 16.2.5. Location of CRE in Promoters of Germ-Cell Specific Genes | |
| 16.3. CRE-BINDING (CREB) PROTEIN | 354 |
| 16.3.1. CREB mRNA Isoforms | |
| 16.3.2. CREB Promoter | |
| 16.3.3. Hormonal Control of CREB | |
| 16.3.4. CREB Regulation by NF- κ B and Other Factors | |
| 16.3.5. Activating Transcription Factor 4 (ATF4/CREB2) | |
| 16.4. OTHER LEUCINE ZIPPER PROTEINS | 357 |
| 16.4.1. RT7 : A Germ Cell Specific Protein | |
| 16.4.2. Nurit Protein | |
| 16.5. HOMEBOX PROTEINS IN GERM CELLS | 358 |
| 16.6. ZINC FINGER PROTEINS | 359 |
| 16.6.1. Zinc Fingers of Class 1 and 2 | |
| 16.6.2. GATA Binding Proteins | |
| 16.6.3. Basonuclin | |
| 16.6.4. Ret Finger Protein | |
| 16.6.5. RING Finger Proteins | |
| 16.7. RNA BINDING PROTEINS AS TRANSCRIPTION FACTORS | 370 |
| 16.8. TCFL5 –A BASIC HELIX-LOOP-HELIX PROTEIN | 370 |
| 16.9. OTHER TRANSCRIPTION FACTORS IN TESTIS | 371 |
| 16.10. REFERENCES | 372 |

| | |
|--|-----|
| 17. PROTO-ONCOPROTEINS | 377 |
| 17.1. INTRODUCTION | 377 |
| 17.2. C-KIT AND STEM CELL FACTOR (SCF) | 377 |
| 17.2.1. Truncated Form of c-Kit | |
| 17.2.2. Activation of Phosphatidylinositol 3'-Kinase Pathway | |
| 17.2.3. Stem Cell Factor (SCF) or c-Kit Ligand (KL) | |
| 17.3. THE MYC FAMILY | 383 |
| 17.3.1. The C-Myc | |
| 17.3.2. Other Myc Proteins in Testis | |
| 17.4. THE MYB FAMILY | 386 |
| 17.4.1. A-Myb and B-Myb in Germ Cell | |
| 17.5. THE JUN FAMILY | 387 |
| 17.5.1. Jun-B, C-Jun, Jun-D | |
| 17.5.2. C-FOS AND C-FOS RELATED ANTIGENS | |
| 17.6. C-ROS TYROSINE KINASE | 389 |
| 17.6.1. Epididymis c-Ros and Infertility | |
| 17.6.2. Regulation of c-Ros Receptor by Protein Tyrosine Phosphatase | |
| 17.7. PIM-1 AND PIM-2 | 390 |
| 17.8. C-MOS FACTOR | 392 |
| 17.9. INT-1 AND INT-2 ONCOPROTEINS | 395 |
| 17.10. CELLULAR-ABELSON PROTO-ONCOGENE (C-ABL) PROTEIN | 396 |
| 17.11. Bcl-2 FAMILY | 397 |
| 17.12. OTHER PROTOONCOGENE PRODUCTS IN TESTIS | 399 |
| 17.13. REFERENCES | 400 |
| 18. G PROTEINS AND ASSOCIATED SIGNAL TRANSDUCTION MOLECULES | 405 |
| 18.1. CYCLIC AMP AND Ca^{2+} MEDIATED SIGNAL TRANSDUCTION | 405 |
| 18.2. G PROTEINS | 405 |
| 18.2.1. Heterotrimeric G Proteins | |
| 18.3. G Proteins in Testis Germ Cells | 408 |
| 18.3.1. $G\alpha$ | |
| 18.3.2. $G\gamma$ -Subunit | |
| 18.3.3. G Proteins in Sperm | |
| 18.4. SMALL G PROTEINS | 411 |
| 18.4.1. Ras Proteins | |
| 18.4.2. Ran-GTPase and Germ Cell Specificity | |
| 18.4.3. Rap1: A Ras-like GTPase | |
| 18.4.4. Rab Proteins | |
| 18.4.5. Rho family | |
| 18.4.6. Rho targets (Rhopilin and Ropporin) | |
| 18.5. REGULATORS OF RHO GTPases | 418 |
| 18.5.1. GTPase-Activating Proteins | |
| 18.5.2. Rho GDP Dissociation Inhibitor (Rho GDI) | |
| 18.6. ADP-RIBOSYLATION FACTORS | 421 |
| 18.7. ADENYLYL CYCLASES | 422 |
| 18.7.1. Sperm Membrane Bound Adenylyl Cyclase | |

| | |
|--|------------|
| 18.7.2. Soluble Form of Adenylyl Cyclase in Germ Cells | |
| 18.7.3. Olfactory Adenylyl Cyclase Type 3 | |
| 18.7.4. Pituitary Adenylate Cyclase Activating Polypeptide (PACAP) | |
| 18.8. G-PROTEIN RECEPTORS | 426 |
| 18.8.1. G-Protein Odorant Receptors in Germ Cells | |
| 18.9. ADENOSINE RECEPTORS AND LIGANDS | 427 |
| 18.9.1. Adenosine Receptors | |
| 18.9.2. Fertilization Promoting Peptide (FPP) and Adenosine Receptors | |
| 18.10. RECEPTOR GUANYLYL CYCLASE | 429 |
| 18.10.1. Natriuretic Peptides of Egg as Ligand of Guanylyl-Cyclase | |
| 18.11. PHOSPHODIESTERASES IN TESTIS | 431 |
| 18.11.1. PDE1 and PDE2 Genes | |
| 18.11.2. PDE3 Isoforms | |
| 18.11.3. PDE4 Isoforms | |
| 18.11.4. Other PDE Isoforms | |
| 18.12. c-AMP-GEF PATHWAY | 434 |
| 18.13. REFERENCES | 435 |
| 19. PROTEIN KINASES | 439 |
| I SERINE/THREONINE KINASES | 439 |
| 19.1. C-AMP DEPENDENT PROTEIN KINASE-A | 439 |
| 19.1.1. Catalytic Sub-units of PKA | |
| 19.1.2. Regulatory Subunits of PKA | |
| 19.1.3. Functions of Protein Kinase A | |
| 19.1.4. PKA Inhibitor Proteins | |
| 19.2. PROTEIN KINASE-C | 445 |
| 19.2.1. PKC δ and PKC θ Isoforms in Testis | |
| 19.2.2. Functions of Protein Kinase-C | |
| 19.2.3. PKC Substrates in Sperm | |
| 19.3. Ca ²⁺ /CALMODULIN DEPENDENT PROTEIN KINASES | 449 |
| 19.3.1. CaMKI and CaMKII | |
| 19.3.2. CaMK IV in Testis | |
| 19.3.3. Calspermin : A Germ Cell Homologue of CaMKIV with no Kinase Activity | |
| 19.4. LIM KINASES | 453 |
| 19.5. TESTIS SPECIFIC SERINE PROTEIN KINASES | 454 |
| 19.5.1. TESK-1 | |
| 19.5.2. TESK 2 | |
| 19.5.3. Testis Specific Serine Kinase(s) | |
| 19.6. CASEIN KINASES | 458 |
| 19.6.1. Casein Kinase 1 | |
| 19.6.2. Casein Kinase II | |
| 19.7. AURORA LIKE KINASES | 461 |
| 19.7.1. Aurora Like Kinase 3 (ALK3) | |
| 19.7.2. AIE 1 and AIE/2 | |

| | |
|---|------------|
| 19.8. MICROTUBULE ASSOCIATED PROTEIN KINASES | 462 |
| 19.8.1. Polo Like Kinase | |
| 19.8.2. p56 KKIAMRE | |
| 19.8.3. MAST205 with Kinase Activity | |
| 19.9. CELL CYCLE CHECK POINT KINASES | 464 |
| 19.10. MITOGEN ACTIVATED PROTEIN KINASES | 465 |
| 19.10.1. ERK 1/ ERK2/ERK7 and Other MAP-Kinases | |
| 19.10.2. p21-Activated Kinases (PAKs) | |
| 19.11. RAC-PROTEIN KINASES | 467 |
| 19.12. HASPIN | 469 |
| 19.13. MALE GERM CELL ASSOCIATED KINASE (MAK) | 469 |
| 19.14. OTHER SERINE / THREONINE KINASES | 470 |
| II PROTEIN TYROSINE KINASES | 471 |
| 19.15. TYROSINE KINASES IN TESTIS | 472 |
| 19.15.1. Src Kinase | |
| 19.15.2. Testis Specific Tyrosine Kinase FER (FerT) | |
| 19.15.3. G protein-Coupled Receptor Kinase 4 (GRK4) | |
| 19.15.4. Tyro-Receptors | |
| 19.16. PHOSPHORYLATION OF SPERM TYROSINE | 475 |
| 19.17. TYROSINE KINASES IN SPERM | 476 |
| 19.17.1. Zona Receptor Kinase | |
| 19.17.2. Sp 42 | |
| III DUAL SPECIFICITY PROTEIN KINASES | 478 |
| 19.18. CDC2-LIKE KINASE-3 (CLK3): A LAMMER KINASE | 478 |
| 19.19. NIMA LIKE KINASES | 478 |
| 19.19.1. Nek1 and Nek2 | |
| 19.19.2. Murine Nek3 and Nek4 | |
| 19.19.3. Human Nek6 and Nek7 | |
| 19.20. DYRK-1B | 481 |
| 19.21. C-GMP DEPENDENT PROTEIN KINASE | 481 |
| 19.22. PHOSPHOLIPID RELATED KINASES | 482 |
| 19.22.1. Phosphoinositide-Dependent Protein Kinase-1 (PDK1) | |
| 19.22.2. Phosphatidylinositol 3-Kinase | |
| 19.22.3. Phosphatidylinositol 4-Kinase | |
| 19.22.4. Diacylglycerol Kinase | |
| 19.23 OTHER PROTEIN KINASES | 484 |
| 19.23.1 5'-AMP-Activated Protein Kinase (AMPK) | |
| 19.23.2. Glycogen Synthase Kinase-3 | |
| 19.23.3. Other Non-specific Protein Kinases in Germ Cells | |
| 19.24. REFERENCES | 485 |
| 20. PROTEIN PHOSPHATASES | 493 |
| 20.1. INTRODUCTION | 493 |
| I SERINE-THREONINE PHOSPHATASES | 493 |
| 20.2. CLASSIFICATION | 493 |
| 20.3. PROTEIN PHOSPHATASE TYPE-1 | 494 |

| | |
|--|-----|
| 20.3.1. Protein Phosphatase-1 γ 2 | |
| 20.4. PROTEIN PHOSPHATASE TYPE-2 | 495 |
| 20.4.1. Protein Phosphatase-2A | |
| 20.4.2. Ca ²⁺ /Calmodulin Dependent Protein Phosphatase 2B | |
| 20.4.3. Protein Phosphatase 2C | |
| 20.5. PROTEIN PHOSPHATASE-4 or PPx | 500 |
| 20.6. Cdc25 PROTEIN PHOSPHATASES | 502 |
| 20.7. INHIBITORS OF PROTEIN PHOSPHATASES | 502 |
| II. PROTEIN TYROSINE PHOSPHATASES | 503 |
| 20.8. PROTEIN TYROSINE PHOSPHATASE-1 | 503 |
| 20.8.1. Tesis Specific Cytoplasmic PTPase | |
| 20.8.2. Transmembrane PTPase with Tensin Homology (TPTE) | |
| 20.8.3. Osteotesticular Protein Tyrosine Phosphatase (OST-PTP) | |
| III. DUAL SPECIFICITY PROTEIN PHOSPHATASES | 506 |
| 20.9. DSP FAMILY | 506 |
| 20.9.1. Testis-and Skeletal-Muscle-Specific DSP (TMDS) | |
| 20.9.2. Low Molecular Weight Dual Specificity Phosphatases | |
| 20.10. PTPase IN GERM CELLS-SERTOLI CELLS INTERACTIONS | 507 |
| 20.11. REFERENCES | 509 |
| 21. ION CHANNELS AND AQUAPORINS | 513 |
| 21.1. ION CHANNELS IN SPERM PHYSIOLOGY | 513 |
| 21.2. POTASSIUM CHANNELS | 513 |
| 21.2.1. Ca ²⁺ Activated K ⁺ Channel | |
| 21.2.2. Cyclic Nucleotides and K ⁺ Channels | |
| 21.2.3. SLO3 | |
| 21.2.4. Rectifier K ⁺ Channel | |
| 21.2.5. Volume Regulatory K ⁺ Channel | |
| 21.3. SODIUM CHANNELS | 518 |
| 21.4. CATION CHANNELS | 520 |
| 21.4.1. Voltage Gated Ion Channels | |
| 21.5. CALCIUM CHANNELS | 521 |
| 21.5.1. Presence of Two Ca ²⁺ Channels in Sperm | |
| 21.5.2. T-Type Low Voltage Activated VDCC | |
| 21.5.3. Neoglycoproteins and Ca ²⁺ Channels | |
| 21.5.4. L-Type High Voltage Dependent VDCC in Testis | |
| 21.6. AMINO ACID NEUROTRANSMITTER RECEPTOR / Cl ⁻ CHANNELS | 527 |
| 21.6.1. GABA _A Receptor/Cl ⁻ Channel | |
| 21.6.2. Glycine Receptor (GlyR)/ Cl ⁻ Channel | |
| 21.7. ANION CHANNELS | 528 |
| 21.7.1. Voltage-Dependent Anion Selective Channels (VDACs) | |
| 21.7.2. Close Cell-Cell Chloride Channel (CLC CL ⁻ Channel) | |
| 21.8. VDCC AND ACROSOME REACTION | 529 |
| 21.9. AQUAPORINS (WATER CHANNELS) | 531 |
| 21.9.1. Aquaporins in Male Reproductive Tract | |

| | |
|--|------------|
| 21.9.2. Aquaporin 7 and 8 | |
| 21.9.3. Aquaporin 9 | |
| 21.10. REFERENCES | 534 |
| 22. ACTION OF PHOSPHOLIPASES | 539 |
| 22.1. INOSITOL TRIPHOSPHATE MEDIATED SIGNAL TRANSDUCTION | 539 |
| 22.2. INOSITOL TRIPHOSPHATE RECEPTORS IN GERM CELLS | 540 |
| 22.3. PHOSPHOLIPASES | 541 |
| 22.3.1. Phospholipase A2 | |
| 22.3.2. LA2 in Sperm | |
| 22.3.3. Phosphatidic Acid Preferring Phospholipase A1 | |
| 22.3.4. Phospholipase B | |
| 22.3.5. Phospholipase – C | |
| 22.3.6. Sperm PLC in Egg Activation | |
| 21.3.7. Role of Egg PLC in Egg Activation | |
| 22.4. HORMONE-SENSITIVE LIPASE | 552 |
| 22.5. REFERENCES | 552 |
| 23. ACROSOMAL ENZYMES | 555 |
| 23.1. INTRODUCTION | 555 |
| 23.2. ACROSOME BIOGENESIS | 555 |
| 23.3. GLYCOSYLTRANSFERASES AND GLYCOSIDASES | 556 |
| 23.4. β 1,4-GALACTOSYLTRANSFERASE | 557 |
| 23.5. OTHER GLYCOSYLTRANSFERASES | 559 |
| 23.6. HYALURONIDASE AND PH-20 | 560 |
| 23.6.1. Characterization | |
| 23.6.2. Post-Testicular Modifications | |
| 22.6.3. Contraceptive Effects | |
| 23.7. GLYCOSIDASES | 568 |
| 23.7.1. N-Acetyl-b-D-Glucosaminidase | |
| 23.7.2. β -D-Glucuronidase | |
| 23.7.3. β -D-Galactosidase | |
| 23.7.4. Arylsulfatase A | |
| 23.7.5. Sperm α -L-Fucosidase | |
| 23.7.6. Mannosidase | |
| 23.8. PROTEINASES | 574 |
| 23.8.1. β Acrosin | |
| 23.8.2. Cathepsins | |
| 23.8.3. Testicular Serine Protease -1 and -2 (TESP1 and TESP2) | |
| 23.8.4. Testisin | |
| 23.9. OTHER ENZYMES IN ACROSOME | 580 |
| 23.10. REFERENCES | 581 |
| 24. ACROSOMAL PROTEINS (NON-ENZYMATIC) | 585 |
| 24.1. ACROSOMAL MATRIX | 585 |
| 24.2. ACROSOMAL MATRIX PROTEINS | 586 |
| 24.2.1. Mouse Sp 56 | |

| | |
|---|------------|
| 24.2.2. Guinea pig AM 67: An Orthologue of Sp56 | |
| 24.2.3. Acrins | |
| 24.2.4. SP17: A Zona-Binding Protein | |
| 24.3. ACROSOMAL VESICLE PROTEIN 1 (SP-10) | 590 |
| 24.4. HAMSTER P26H AND ITS HUMAN ORTHOLOGUE | 593 |
| 24.5. PERIACROSOMAL PLASMA MEMBRANE PROTEIN (PM52) | 595 |
| 24.6. ACTIN AND ACTIN BINDING PROTEINS IN ACROSOME | 595 |
| 24.7. FUSION PROTEINS IN SPERM | 596 |
| 24.8. BINDIN | 597 |
| 24.9. CYSTATIN-RELATED EPIDIDYMAL SPERMATOGENIC PROTEIN | 598 |
| 24.10. OTHER NON-ENZYMATIC ACROSOMAL PROTEINS | 598 |
| 24.11. REFERENCES | 600 |
| 25. ACTINS AND MYOSINS | 603 |
| 25.1. INTRODUCTION | 603 |
| 25.1.1. β - and γ -Actins in Spermatogenic Cells | |
| 25.1.2. F-Actin | |
| 25.1.3. Actin-Like Proteins (T-ACTINS) | |
| 25.2. ACTINS IN SPERMATOZOA | 606 |
| 25.3. ACTIN BINDING PROTEINS | 608 |
| 25.3.1. Basic Proteins of Cyclacin Group | |
| 25.3.2. Actin Capping Proteins | |
| 25.3.3. β 3 Capping Protein | |
| 25.3.4. Gelsolin | |
| 25.3.5. Thymosin β 10 | |
| 25.3.6. Other Actin Associating Proteins | |
| 25.4. ACTIN IN LIMULUS SPERM | 614 |
| 25.4.1. β -Scruin | |
| 25.5. MYOSINS | 616 |
| 25.5.1. Myosin X in Mouse Testis | |
| 25.5.2. Functions of Myosin in Reproduction | |
| 25.5.3. Myosins in Spermatogenic Cells of Non-Mammalian Species | |
| 25.6. REFERENCES | 620 |
| 26. CELL ADHESION PROTEINS | 623 |
| 26.1. CELL ADHESIONS | 623 |
| 26.2. INTEGRINS | 623 |
| 26.2.1. Integrins and Their Ligands in Testis and Sperm | |
| 26.3. CADHERINS | 627 |
| 26.3.1. Cadherins in Male Germ Cells | |
| 26.4. IMMUNOGLOBULIN SUPERFAMILY | 629 |
| 26.5. SELECTINS | 630 |
| 26.6. ADAM PROTEINS: THE FAMILY OF METALLO PROTEINASE DISINTEGRINS | 631 |
| 26.6.1. Fertilin α and β (ADAM1 and ADAM2) | |
| 26.6.2. Fertilin as a Co-receptor for Egg Integrins | |

| | |
|---|------------|
| 26.6.3. Role of CD9 in Sperm-Egg Interactions | |
| 26.7. CYRITESTIN (ADAM 3) | 641 |
| 26.8. OTHER ADAM PROTEINS | 642 |
| 26.9. ZONA ADHESINS | 646 |
| 26.9.1. Mouse | |
| 26.9.2. Pig | |
| 26.9.3. Tektorins and Zonaadhesins | |
| 26.10. ADHESION COMPONENTS OF IMMUNE SYSTEM | 647 |
| 26.10.1. Membrane Cofactor Protein (MCP) | |
| 26.10.2. Protectin (CD59) | |
| 26.10.3. Other Components of Immune System in Germ Cells | |
| 26.11. REFERENCES | 650 |
| 27. METALLOPROTEASES AND METALLOPROTEASE INHIBITORS | 655 |
| 27.1. MATRIX METALLOPROTEASES | 655 |
| 27.1.1. MMP-1, MMP-2, and MMP-9 | |
| 27.1.2. Epilysin (MMP-28) | |
| 27.1.3. Collagenase IV Metalloproteinases | |
| 27.1.4. Other Metalloproteinases | |
| 27.2. METALLOPEPTIDASES | 658 |
| 27.2.1. Neutral Endopeptidases (Neprilysins) | |
| 27.2.2. Endopeptidase 24.15 | |
| 27.2.3. Endothelin-1 Converting Enzyme | |
| 27.2.4. NRD Convertase (Nardilysin) | |
| 27.3. ENDOPROTEASES | 663 |
| 27.3.1. Proprotein Convertase | |
| 27.3.2. Calpain | |
| 27.4. TISSUE INHIBITORS OF METALLOPROTEINASES | 664 |
| 27.4.1. Testicular Inhibitors of Metalloproteinases (TIMPs) | |
| 27.4.2. Epididymal Protease Inhibitor (EPPIN) | |
| 27.4.3. Protein C Inhibitor | |
| 27.5. REFERENCES | 667 |
| 28. ISOENZYMES IN ENERGY PATHWAYS | 669 |
| 28.1. INTRODUCTION | 669 |
| 28.2. HEXOKINASE | 669 |
| 28.3. GLUCOSE-6-PHOSPHATASE | 671 |
| 28.4. GLUCOSE-PHOSPHATE ISOMERASE AND SPERMANTIGEN-36 | 672 |
| 28.5. FRUCTOSE-6-PHOSPHATE, 2 KINASE / FRUCTOSE-2, 6-BIS PHOSPHATASE | 672 |
| 28.6. PHOSPHOGLYCERATE KINASE-2 | 674 |
| 28.7. GLYCERALDEHYDE-3 PHOSPHATE DEHYDROGENASE | 679 |
| 28.8. PHOSPHOGLYCERATE MUTASE | 680 |
| 28.9. ENOLASE | 681 |
| 28.10. LACTATE DEHYDROGENASE-C | 682 |
| 28.11. PYRUVATE DEHYDROGENASE-E1 α | 685 |
| 28.12. GLUCOSE-6-PHOSPHATE DEHYDROGENASE-2 | 688 |
| 28.13. TESTICULAR CYTOCHROME C _t | 689 |

| | |
|--|------------|
| 28.14. ENZYMES WITH LIMITED SPECIFICITY | 691 |
| 28.15. REFERENCES | 692 |
| 29. FIBROUS SHEATH, DENSE FIBERS, AND PLASMA | 695 |
| MEMBRANE OF SPERM | |
| 29.1. OUTER DENSE FIBER PROTEINS | 695 |
| 29.1.1. ODF27/Odf1 | |
| 29.1.2. Odf1 Interacting Proteins | |
| 29.1.3. ODF84/Odf2 | |
| 29.1.4. Cysteine Rich Proteins in ODF | |
| 29.2. FIBROUS SHEATH PROTEINS | 701 |
| 29.2.1. Electrophoretic Studies | |
| 29.2.2. Thioredoxins | |
| 29.3. ANCHOR PROTEINS IN FIBROUS SHEATH | 704 |
| 29.3.1. AKAP220 | |
| 29.3.2. AKAP4 | |
| 29.3.3. Rat Testis AKAP80 | |
| 29.3.4. Sperm AKAP 82 | |
| 29.3.5. FSP95 | |
| 29.3.6. AKAP110 | |
| 29.3.7. Mitochondrial S-AKAP84 | |
| 29.3.8. Human Testis <i>hi</i> gene | |
| 29.3.9. Dual Specificity AKAPs | |
| 29.3.10. Fibrous Sheath Component 1 | |
| 29.3.11. c-GMP Dependent Protein Kinase Anchor Protein | |
| 29.4. OTHER PROTEINS OF FIBROUS SHEATH | 713 |
| 29.5. SPERM PLASMA MEMBRANE PROTEINS | 714 |
| 29.5.1. Na/K-ATPase | |
| 29.5.2. Periacrosomal Plasma Membrane Protein (PM52) | |
| 29.5.3. Calcium-Binding Tyrosine-Phosphorylation Regulated Protein (CABYR) | |
| 29.5.4. Human Sperm Membrane Protein-I | |
| 29.5.5. Other Protein Components of Sperm Membrane | |
| 29.6. REFERENCES | 717 |
| 30. PROTEINS IN ANTIPEROXIDATION | 721 |
| 30.1. REACTIVE OXYGEN SPECIES | 721 |
| 30.1.1. Types of ROS | |
| 30.1.2. ROS and Sperm Function | |
| 30.1.3. Oxidative Stress and DNA damage | |
| 30.2. PROTEINS IN ANTIPEROXIDATION | 723 |
| 30.2.1. Glutathione Peroxidase in Reproductive Tract | |
| 30.2.2. Classical Glutathione Peroxidase in Male accessory sex organs | |
| 30.2.3. Phospholipid Hydroperoxide Glutathione Peroxidase | |
| 30.2.4. Sperm Nucleus Glutathione Peroxidase (snGPx) | |
| 30.2.5. Glutathione-S-Transferases | |
| 30.2.6. Mu Class of Glutathione-S-Transferase in Testis | |
| 30.2.7. Superoxide Dismutase | |

| | |
|--|------------|
| 30.2.8. Search for Other Enzymes of Antiperoxidative Pathway | |
| 30.3. NITRIC OXIDE SYNTHASE | 739 |
| 30.4. HEME OXYGENASES | 740 |
| 30.5. SELENOPROTEINS (NON-ENZYMATIC) | 740 |
| 30.6. METALLOTHIONEINS | 742 |
| 30.6.1. Metallothioneins | |
| 30.6.2. Tesmin-60 | |
| 30.7. REFERENCES | 744 |
| 31. QUALITY CONTROL OF GERM CELL PROTEINS | 749 |
| I UBIQUITINATION AND PROTEOLYSIS | 749 |
| 31.1. UBIQUITIN SYSTEM | 749 |
| 31.2. UBIQUITIN SYSTEM IN VERTEBRATE GONADS | 752 |
| 31.2.1. Ubiquitin | |
| 31.2.2. E1-E2-E3 Enzymes | |
| 31.2.3. Deubiquitination Enzymes | |
| 31.2.4. Multiubiquitin Chain Binding Protein (Mcb1) | |
| 31.2.5. Proteasome | |
| 31.2.6. Tat Binding Protein 1 and Proteasome | |
| 31.2.7. Significance of Ubiquitination in Gametogenesis | |
| II MOLECULAR CHAPERONS | 759 |
| 31.3. Heat shock proteins in Spermatogenesis | 759 |
| 31.3.1. Properties of Heat Shock Proteins | |
| 31.3.2. 'Small' Heat Shock Proteins | |
| 31.3.3. Heat Shock Protein - 40 or DnaJ | |
| 31.3.4. Heat Shock Protein-60 | |
| 31.3.5. Heat Shock Protein-70 | |
| 31.3.6. Heat Shock protein-90 | |
| 31.3.7. Heat Shock Protein-110 | |
| 31.4. Heme Oxygenases as Chaperones | 766 |
| 31.5. Calreticulin And Calnexin | 766 |
| 31.5.1. Calreticulin | |
| 31.5.2. Calmegin (Calnexin-t) | |
| 31.6. IMMUNOPHILINS AND TETRATRICOPEPTIDE REPEATS | 769 |
| 31.7. SPECIALIZED CHAPERONES IN GERM CELLS | 771 |
| 31.8. REFERENCES | 773 |
| 32. CANCER ASSOCIATED TESTIS ANTIGENS | 777 |
| 32.1. TUMOR SUPPRESSOR ANTIGENS IN SPERMATOGENIC CELLS | 777 |
| 32.1.1. P53: Regulation of Spermatogenesis and Tumorigenesis | |
| 32.1.2. Retinoblastoma Family of Proteins | |
| 32.1.3. C9orf1 | |
| 32.1.4. hH-Rev107-3 Cdna | |
| 32.1.5. Testisin –A Serine Protease | |
| 32.1.6. Tumor Suppressor Gene: <i>PTEN</i> | |

| | |
|--|------------|
| 32.2. CANCER-TESTIS ANTIGENS | 781 |
| 32.2.1. Breast Cancer Antigens (BRCA-1 and BRCA-2) | |
| 32.2.2. PLU-1: A Nuclear Protein | |
| 32.2.3. MAGE Gene Products | |
| 32.2.4. LAGE-1 | |
| 32.2.5. CAGE-1 | |
| 32.2.6. Testis Specific Cyclin A1 in Testicular and Ovarian Tumors | |
| 32.2.7. Testis-Specific Protein Y-encoded (TSPY) | |
| 32.2.8. Markers of Seminomas | |
| 32.2.9. Other Testis Antigens Expressed in Cancer | |
| 32.3. DIAGNOSTIC AND THERAPEUTIC POTENTIAL OF C/T ANTIGENS | 790 |
| 32.3.1. MAGE in Squamous Cell Carcinoma and Childhood Astrocytoma | |
| 32.3.2. NY-ESO | |
| 32.3.3. Semenogelin 1 and HAGE in Leukemia | |
| 32.4. REFERENCES | 792 |
| 33. SELECTIVE GROUP OF GERM CELL SPECIFIC PROTEINS | 795 |
| 33.1. POLYAMINES AND ORNITHINE DECARBOXYLASE | 795 |
| 33.1.1. Polyamines | |
| 33.1.2. Ornithine Decarboxylase | |
| 33.1.3. Ornithine Decarboxylase Antizyme | |
| 33.2. SELECTIVE GROUP OF GERM CELL SPECIFIC PROTEINS | 799 |
| 33.2.1. Isoaspartyl Methyltransferase | |
| 33.2.2. Phosphoribosyl Pyrophosphate (PP-Rib-P) Synthetase | |
| 33.2.3. Glucosamine-6-Phosphate Deaminase | |
| 33.2.4. Carnitine Transferases | |
| 33.2.5. Organic Cation/Carnitine Transporters | |
| 33.2.6. NM23-H5 (Nucleoside Diphosphate Kinase) | |
| 33.2.7. Farnesyl Transferase | |
| 33.3. OTHER DOMINANT PROTEINS IN GERM CELLS | 803 |
| 33.4. REFERENCES | 809 |
| 34. SPERM MATURATION IN EPIDIDYMIS | 811 |
| 34.1. ROLE OF EPIDIDYMIS | 811 |
| 34.2. SPERM SURFACE ALTERATIONS | 812 |
| 34.3. PROCESSING OF SPERM PROTEINS IN EPIDIDYMIS | 813 |
| 34.4. PROTEINS ADSORBED BY SPERM DURING EPIDIDYMIS TRANSIT | 814 |
| 34.4.1. Mouse and Rat | |
| 34.4.2. Guinea pig/Rabbit/Hamster | |
| 34.4.3. Ram/Goat/Bull | |
| 34.4.4. Porcine Epididymal Proteins | |
| 34.4.5. Stallion | |
| 34.4.6. Primate Epididymis Secretory Proteins | |
| 34.5. HUMAN EPIDIDYMIS PROTEINS AND THEIR ANIMAL ORTHOLOGS | 821 |
| 34.5.1. Human Epididymal (HE1-HE4) Proteins | |

| | |
|---|------------|
| 34.5.2. CD52 (HE5) and Its Orthologs | |
| 34.5.3. P34H and its Orthologs | |
| 34.5.4. Cysteine Rich Secetary Protein (CRISP) Family | |
| 34.5.5. Cystatin-Related Epididymal Spermatogenic Protein | |
| 34.5.6. Clusterin | |
| 34.5.7. Other Epididymal Proteins in Sperm maturation | |
| 34.6. PROSTASOME | 832 |
| 34.7. REFERENCES | 833 |
| INDEX | 839 |

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.

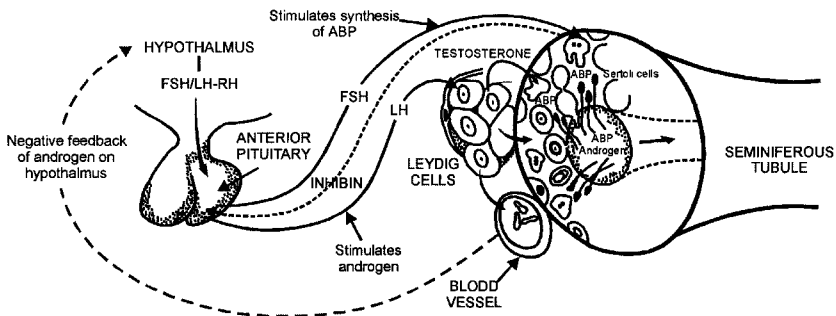


Fig.1.1: Hypothalamus-pituitary-testis axis in mammalian male reproduction.

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 several 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, spermatogonium 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

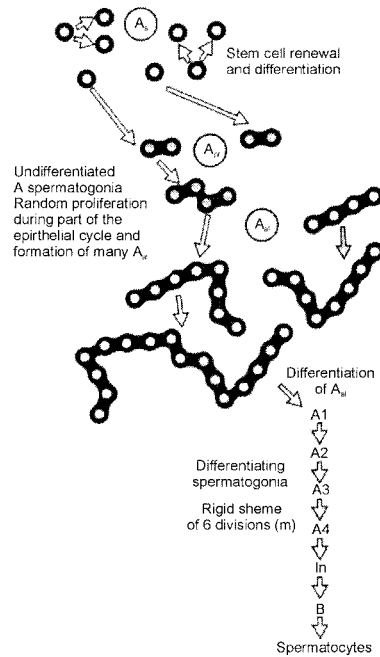
Spermatogenesis is a process by which spermatozoa are formed from spermatogonial stem cells during adult's reproductive phase. The process of sperm formation is initiated in the mouse embryo at around day 11.5 postcoitum (pc), when primordial germ cells (PGC) colonise the genital ridge. Under the influence 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, whereas 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_{in}), 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 homologous 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).