Symposium: Genetic aspects of male (in)fertility

Genetic control of spermiogenesis: insights from the *CREM* gene and implications for human infertility

Dr Paolo Sassone-Corsi

Paolo Sassone-Corsi is Directeur de Recherche at the CNRS, France. Born in Naples, Italy, he received his PhD in Genetics in 1979. After a post-doctoral period with Pierre Chambon, he went to The Salk Institute, San Diego, California, as visiting scientist with Inder Verma. From 1989 he has been Group Leader at the Institut de Génétique et de Biologie Moléculaire et Cellulaire in Strasbourg, France. He has received various awards: Member of the European Molecular Biology Organization (1990); EMBO Gold Medal (1994); Rosen Prize (Fondation de la Recherche Médicale), France (1996); Grand Prix Liliane Bettencourt pour la Recherche, France (1997); Segerfalk Award Lecture, Lund University, Sweden (2001); Grand Prix Charles-Léopold Mayer de l'Académie des Sciences, Paris (2003); ISI Highly Cited (2003); Edwin B Astwood Award Lecture of The Endocrine Society, USA (2004); The Umesono Memorial Award Lecture, The Salk Institute, San Diego, USA (2004). Additional information is available on the web site [www.isihighlycited.com].

Csilla Krausz[1], Paolo Sassone-Corsi[2,3]
[1]Andrology Unit, Department of Clinical Physiopathology, University of Florence, Viale Pieraccini, 6, Florence 50139, Italy
[2]Institut de Génétique et de Biologie Moléculaire et Cellulaire, 1, rue Laurent Fries, 67404 Illkirch, Strasbourg, France
[3]Correspondence: Tel: +33 388 653410; Fax: +33 388 653246; e-mail: paolosc@igbmc.u-strasbg.fr

Abstract

Male germ cell differentiation requires a highly cell-specific gene expression programme that is achieved by unique chromatin remodelling, transcriptional control, and the expression of testis-specific genes or isoforms. The regulatory processes governing gene expression in spermatogenesis have fundamentally unique requirements, including meiosis, ongoing cellular differentiation and a peculiar chromatin organization. The signalling cascades and the downstream effectors contributing to the programme of spermatogenesis are currently being unravelled, revealing the unique features of germ cell regulatory circuits. This paper reports on the unique role that CREM exerts as a master regulator. Targeted inactivation of the genes encoding CREM and ACT has been achieved. ACT selectively associates with KIF17b, a kinesin motor protein highly expressed in germ cells. It has been found that KIF17b directly determines the intracellular localization of ACT. Thus, the activity of a transcriptional co-activator is intimately coupled to the function of a kinesin via tight regulation of its intracellular localization. The conservation of these elements and of their regulatory functions in human spermatogenesis indicates that they are likely to provide important insights into understanding the molecular mechanisms of human infertility.

Keywords: *ACT, CREM, genetics, infertility, kinesin, spermiogenesis*

Introduction

A spectacular array of extensive biochemical and morphological changes is required for a male germ cell to undergo the remarkable transition during which spermatogonia, round mitotically dividing cells, develop into spermatozoa, elongated meiotic cells. These changes occur within a very short time-frame and are under tight control. An intrinsic genetically defined programme, manifested in the expression of unique gene products at specific times in response to various signal transduction processes, is essential for spermatogenesis. This programme is conserved among species, as reflected in the fact that rat spermatogonial stem cells give rise to normal rat spermatozoa when transplanted into the mouse testis (Clouthier *et al*., 1996). In addition, there is a highly specialized epigenetic programme that involves the dramatic histone-to-protamine transition as well as the use of a number of unique histone variants.

The genetic programme of spermatogenesis is based on highly specific transcriptional regulatory pathways. Indeed, increasing evidence reveals that transcriptional mechanisms are quite different in haploid germ cells with respect to somatic cells (Sassone-Corsi, 2002). These include the use of distinct promoter elements and specific transcription factors as well as mechanistically different routes for activation. Many generally expressed genes use alternative promoters in male germ cells, and several genes have a homologue whose expression is specific for the male germ line. Transgenesis experiments have revealed that various *cis*-acting regulatory elements direct expression exclusively to the testis, demonstrating the presence of germ cell-specific factors (Sassone-Corsi, 1997;

Eddy and O'Brien, 1998; Hecht, 1998; Kleene, 2001). In spermatogenic cells, several unique transcription factors have been identified (e.g. SPRM1, TAK-1, OCT-2), but their target genes have yet to be determined (Winer and Wolgemuth, 1993; Eddy, 1998; Eddy and O'Brien, 1998). One of the best characterized transcription factors in testis is CREM (cAMP response element modulator), which encodes a number of different isoforms by various alternative regulatory processes (Sassone-Corsi, 1995).

There are two major transcriptional waves during spermatogenesis, one throughout spermatogonial proliferation, the other during early spermiogenesis (Sassone-Corsi, 2002). In the mouse, there is a considerable elevation in the activity of the basal transcriptional machinery shortly after meiosis (Schmidt and Schibler, 1995), which marks the beginning of spermiogenesis and continues until the transition from round to elongating spermatid. At this point, that is several days before the completion of spermiogenesis, the main wave of transcription ceases (Monesi, 1964; Kierszenbaum and Tres, 1975) due to extensive chromatin condensation through replacement of somatic-type and testis-specific histones by transition proteins first and then by protamines. Lacking the possibility of direct transcriptional control, the elongating and elongated spermatids hence necessitate various sophisticated post-transcriptional mechanisms for control of gene expression (Eddy, 1998). The post-meiotic transcription phase and its exact regulation and timing is thus of central importance to the successful completion of spermatogenesis, as the spermatid has to organize the production of all factors required for its final differentiation before cessation of transcription.

Pathways of chromatin remodelling in spermatogenesis

The seminiferous epithelium constitutes a remarkable anatomical structure in which the differentiation of germ cells occurs. Various steps of this developmental process rely on a complex paracrine dialogue with Sertoli cells. Testosterone secreted by Leydig cells under the influence of pituitary-secreted LH, and FSH acting on Sertoli cells, stimulates gene transcription and the secretion of peptides that promote germ cell differentiation. Biochemical stimulation of germ cells is thought to occur via the secretion of regulatory molecules from Sertoli cells such as growth factors and proteases (Griswold, 1998).

Spermatogonia yield cells with three possible fates. One spermatogonial subpopulation undergoes self-renewal through mitotic divisions, some cells undergo apoptosis, while about half of all the cells are committed to differentiate into mature spermatozoa by entering the programme of meiosis (de Rooij, 2001). This period of spermatogonial proliferation and differentiation is followed by meiotic prophase I, when the most mature type B spermatogonia undergo division to the preleptotene spermatocyte stage. These early spermatocytes undergo the S-phase of the cell cycle, giving rise to diploid leptotene spermatocytes, and mark the beginning of meiotic prophase. In zygotene cells, aligned homologous chromosomes pair and the synaptonemal complex forms (Parra *et al*., 2003). As synapsis is completed by the pachytene stage, genetic crossover is possible at sites along the synaptonemal complex known as recombination nodules (Carpenter, 1987). In the largest spermatocyte, at diplotene, meiosis I is completed; the synaptonemal complex disintegrates, bivalent chromosomes align on the metaphase plate and sister chromatids dissociate into two daughter cells. The second meiotic division of secondary spermatocytes results in the production of haploid spermatids and coincides with a wave of gene transcription. Some genes that are essential for mammalian meiosis include *SCP3* (synaptonemal complex protein 3), *CDK-2* (cyclin dependent kinase), *β-Trcp1* (β-transducin repeat containing protein) and v-*Mos* oncogene (Rosenberg *et al*., 1995; Yuan *et al*., 2000; Ortega *et al*., 2003). The chromatin remodelling events at meiosis are as yet largely unknown, but recent evidence indicates that histone modifications are associated with chromosomal association and segregation (Prieto *et al*., 2004). This suggests that intracellular signalling pathways specific to germ cells may operate to direct chromatin remodelling, similarly to what happens at mitosis in somatic cells (Cheung *et al*., 2000).

The post-meiotic developmental phase, so-called spermiogenesis, involves the differentiation of spermatids into spermatozoa. Spermiogenesis constitutes a remarkable process, as germ cells undergo an enormous morphogenetic transformation involving DNA compaction, cytoplasmic ejection and acrosome and flagellar formation. Male germ-cell-specific nuclear proteins, the transition proteins (TP) and protamines, sequentially replace histones, to allow for DNA compaction, and to permit reshaping of the round spermatid nucleus (Heidaeran and Kistler, 1987; Sassone-Corsi, 2002). The highly ordered process required for production of the male gamete involves precise steps of transcriptional control to govern the changing patterns of gene expression. Thereby, a link exists between genetic and epigenetic control of male germ cells: the transcriptional programme that controls the expression of TP and protamines is logically essential to the reprogramming of the nuclear organization.

CREM: a master-switch regulator in testis

CREM (cyclic AMP-response element modulator) is a transcription factor that belongs to the basic domain-leucine zipper (bZip) class of proteins (Foulkes *et al*., 1991). In somatic cells, transcriptional activation by CREM requires a specific phosphorylation event that turns this protein into a powerful activator. In CREM, the phosphorylation site (Ser117) is within the so-called P-Box region, which is highly conserved among the other members of the family, CREB (cyclic AMP-response element binding protein) and ATF-1 (activating transcription factor-1) (Sassone-Corsi, 1995; De Cesare *et al*., 1999). Several lines of evidence now support the notion that CREM and CREB can be phosphorylated in the P-Box by multiple kinases, which are activated by a variety of signals elicited by growth factors and stress (De Cesare *et al*., 1999). Phosphorylation is the prerequisite for the consequent recruiting of CBP (CREB-binding protein), a co-activator that also carries HAT (histone acetyltransferase) activity, which implies events of chromatin remodelling for transcriptional activation. Importantly, CREM is not phosphorylated in testis (Fimia *et al*., 1999), indicating a distinct control pathway in male germ cells.

CREM plays an essential role in the control of post-meiotic