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Stem cell factor/c-kit system in spermatogenesis

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One of the major unresolved questions with male infertility is the identification of the molecular origin of a great majority of the spermatogenetic arrests currently diagnosed as idiopathic male infertility. During the past years, several families of regulating factors have been implicated in spermatogenesis defects observed essentially in animal models. Among these factors are signalling molecules, and particularly the stem cell factor (SCF)/c-kit system. The SCF and its receptor c-kit are an appropriate example to illustrate the role of signalling molecules in the physiology and pathology of spermatogenesis. The SCF/c-kit regulates primordial germ cell migration, proliferation and apoptosis during fetal gonadal development. The SCF/c-kit also regulates spermatogonia proliferation in the adult animal. In mutant mice, abnormalities of the SCF/c-*kit* gene expression, such as gene deletion, point mutation, alternative splicing defect, lead to different types of spermatogenesis alterations (e.g. decrease in primordial germ cell migration, decrease in spermatogonia proliferation). More recently, defects in SCF/c-*kit* gene expression have also been shown in human testicular dysfunctions. Indeed, a reduction in SCF/c-*kit* expression has been evidenced in oligozoospermia/azoospermia associated with an increase in the germ cell apoptosis process. In addition, c-kit seems to be a good marker of seminoma testicular tumours. This review reports a large number of data—obtained essentially in animal models—that suggest an important role for the SCF/c-kit system in spermatogenesis and, as a corollary, its potential involvement in spermatogenic defects.

Key words: c-*kit*/germ cells/stem cell factor/testicular development/testis

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Introduction

Infertility affects an estimated 10% of couples, and in roughly half of these cases the defect can be traced to the male. A large portion of these men are infertile either because of insufficient spermatozoa (oligoazoospermia) or lack of spermatozoa (azoospermia). The cause of these defects is still unclear. Paradoxically, the use of different assisted reproduction techniques such as intracytoplasmic sperm injection (ICSI) has increased the need of identifying and understanding the molecular basis of different forms of spermatogenic arrests of unknown origin. The explosive growth in the use of such assisted reproduction techniques focuses our attention on at least two points: (i) the genetic components of spermatogenic defects may be transmitted by ICSI more readily than by in-vivo fertilization (for reviews see Lamb, 1999; Schlegel, 1999); and (ii) the requirement of a haploid gamete for ICSI demands the understanding of the cellular and molecular mechanisms involved in the process of spermatogenesis, whereby undifferentiated spermatogonia divide and differentiate into mature fertile spermatozoa.

Recently, male infertility has received the attention of investigators involved in different fields such as toxicology, genetics, molecular and cellular biology. Their work shed light on the pathophysiological mechanisms underlying spermatogenetic defects. In toxicology, several observations suggest that the reproductive health of both humans and wildlife have suffered adverse effects as a result of environmental exposure to chemicals that interact with the endocrine system. These substances, termed endocrine disruptors, are supposed to exert their effects during the embryological development of the gonad and the genital tract and/or on adult testicular functions, namely on steroidogenesis

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and spermatogenesis (for reviews see Ashby *et al*., 1997; Cooper and Kavlock, 1997; Cheek and McLachlan, 1998). The genetic causes of male infertility have been demonstrated in different gene defects, particularly those on the Y chromosome, and also on other chromosomes such as 1, 3, 5, 6, 9 and 10. The use of animal (mice) models with spontaneous or induced gene mutations, as well as knock-out models, have shown that a growing number of genes are now clearly implicated in the molecular process of spermatogenesis (for review see Okabe *et al*., 1998). Finally, a large number of data, mainly obtained in in-vitro models, indicate that germ cells are sources and targets of signalling molecules such as growth factors, cytokines and peptides (Gnessi *et al*., 1997; Mauduit *et al*., 1999). These observations, which were partly confirmed in in-vivo models using gene knock-out or overexpression [for example for transforming growth factor βs (TGFβ) and related peptides (see Gnessi *et al*., 1997)], suggest that some key steps in spermatogenesis are under the control of regulatory factors. It is therefore possible that defects in the expression and/or action of these molecules are a possible cause of male infertility. These signalling molecules may represent the intratesticular relay of the endocrine system which controls spermatogenesis, mainly through Sertoli cells (Benahmed, 1996).

This review focuses on a signalling system, the stem cell factor (SCF) and its receptor c-kit, which is an appropriate example to illustrate the new insights in the pathophysiology of male infertility resulting from work in the various fields mentioned earlier.

Male germ cell proliferation and maturation

Stem cells (from haematopoietic, nervous and gonadal systems) are a subject of increasing interest because of their biological and medical importance (for review see Morrison *et al*., 1997). Gonadal stem cells undergo migration, proliferation apoptosis and/or differentiation (DeRooj and Grootegoed, 1998). Indeed, primordial germ cells (PGC) originate from embryonal ectoderm. In the mouse embryo (8 days post-coitum; dpc), 15 to 100 PGC are recognizable in the yolk sac endoderm (Eddy *et al*., 1981; Wassarman, 1996). At about 8/9 dpc for mouse (24–33 dpc for human), PGC translocate by passive transfer to the hindgut, while they begin to proliferate (170–350 PGC). Then, by a migratory process that probably involves chemoattractant factors (Kierszenbaum, 1994), PGC join the gonadal ridge (at 10.5/11 dpc; 33–37 dpc for human), their number increases to a maximum of 20 000–25 000 (Tam and Snow, 1981). PGC then interact with epithelial and mesenchymal cells of the gonad to form the testicular cords (sexual differentiation occurs at 12/13 dpc; 44–48 dpc for human); PGC then change morphologically and are called gonocytes. At 13/14 dpc, in the male mouse, gonocytes enter a mitotic arrest (Monk and McLaren, 1981) which will be maintained until 3/4 days post-partum (dpp).

After birth (about 6 dpp), gonocytes divide to form type A1 spermatogonia. At maturity, these spermatogonia divide to produce another type A1 spermatogonium and a type A2 spermatogonium. Thus, the A1 spermatogonium appears as a stem cell capable of regenerating itself and producing a new cell type. The A2 spermatogonium divides to produce the A3 spermatogonia, which will differentiate into A4 spermatogonia. After the type A4 spermatogonia, the intermediate spermatogonia divide to form the type B spermatogonia which are the precursors of the spermatocytes—the cells which will enter meiosis (Gilbert, 1994; DeRooj and Grootegoed, 1998). Spermatocytes will then further differentiate into mature spermatozoa. In mice, the first haploid germ cells appear at about 20/21 dpp (Bellvé *et al*., 1977) (Table I).

Table I. Age-dependent development and appearance of germ cells in the mouse testis

Days post-coitum (pc) or post-partum (pp)	Appearance of germ cells
7.5 pc	10–100 PGC in the yolk sac (Eddy *et al*., 1981)
8.5 to 9.5 pc	Passive translocation of the PGC from the yolk sac to the hindgut
9.5 to 11.5 pc	Active migration of PGC from the dorsal mesentery to the genital ridge. PGC are proliferating (no: 2500–5000)
10.5 to 12.5 pc	SRY expression, testicular differentiation
12 to 14 pc	PGC (no. 20 000–25 000) stopped their multiplication
12/14 pc to 3/4 pp	PGC are quiescent and are called gonocytes (Monk and McLaren, 1981)
6 pp	Germ cells have differentiated to type A spermatogonia (Bellvé *et al*., 1977)
8 pp	Type B spermatogonia are present (Bellvé *et al*., 1977)
10 pp	Spermatocytes at the preleptotene and leptotene stages are present (Bellvé *et al*., 1977)
12 pp	Spermatocytes at the zygotene stage are present (Bellvé *et al*., 1977)
14 pp	Spermatocytes at the early pachytene stage are present (Bellvé *et al*., 1977)
18/20 pp	Spermatocytes at the late pachytene stage are present (Bellvé *et al*., 1977)
20 pp	Secondary spermatocytes and few round spermatids are present (Bellvé *et al*., 1977)
35 pp	Few spermatozoa are present (Bellvé *et al*., 1977)

Another key process that controls spermatogenesis is apoptosis. Indeed, testicular germ cells enter apoptosis, with such a process occurring at two periods in the mouse (Wang *et al*., 1998): at about 13 dpc, when PGC have reached the gonadal ridge; and at about 10/13 days after birth, when meiotic prophase begins.

Specifically, in the testis, the transformation of spermatogonial stem cells into differentiated haploid spermatozoa is a complex process which is highly dependent upon the somatic Sertoli

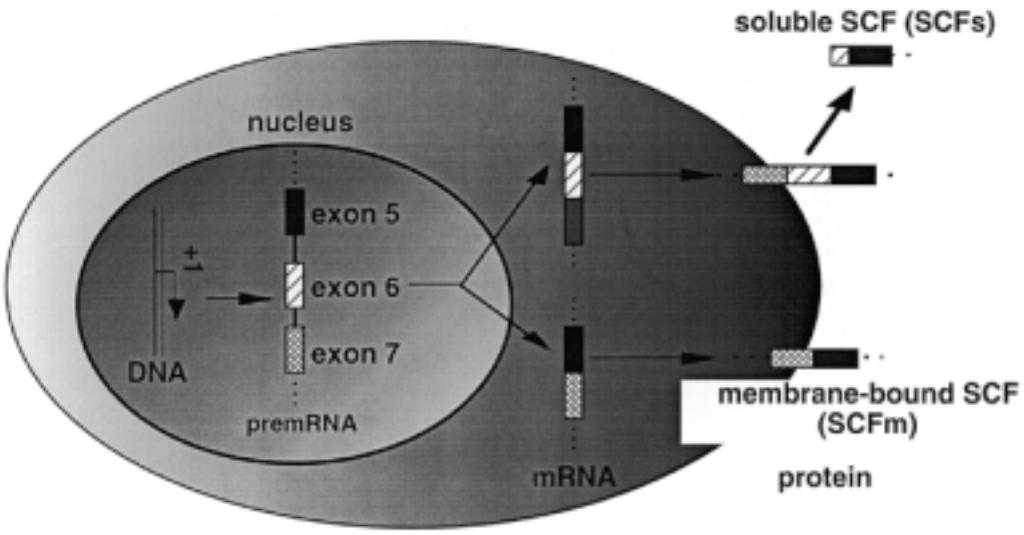

Figure 1. Alternative splicing of stem cell factor (SCF) pre-mRNA. Exon 6 (white box) which encodes the proteolysis sites is either maintained or skipped from the cytoplasmic SCF mRNAs, generating SCFs (soluble) or SCFm (membrane-bound) forms respectively. Exon 7 (gridded box) contains the transmembrane domain that anchors the SCF protein to the membrane.

cells. One of the major questions today is the identification of the Sertoli cell-derived factors driving quiescent stem cells into proliferation. Although these factors are largely unknown, some growth factors and cytokines have been suggested to be at play (for reviews see Roberston *et al*., 1993; Benahmed, 1996; Gnessi *et al*., 1997). The SCF/c-kit system illustrates the Sertoli cell–germ cell interaction because SCF is expressed specifically in Sertoli cells while its receptors, c-kit, are expressed in stem germ cells.

SCF/c-*kit* expression

SCF/c-kit gene expression

The ligand SCF is encoded at the Steel (Sl) locus on chromosome 12 in the human, and on chromosome 10 in the mouse (Flanagan *et al*., 1991). In different species (mouse, rat and human), SCF is encoded by nine exons (Martin *et al*., 1990). SCF has been detected both as a membrane-bound (SCFm) and a soluble (SCFs) protein (Anderson *et al*., 1990; Toksoz *et al*., 1992). The soluble form is generated from an integral membrane protein precursor, by proteolytic cleavage at a site located in the proximal extracellular domain (Huang *et al*., 1992). Since the main proteolytic site involved in this process is encoded by a short 84 bp-long alternative exon (exon 6), the final localization of SCF is ultimately dictated by differential splicing (Flanagan *et al*., 1991) (Figure 1). Indeed, in-vitro experiments have shown that in a stromal cell line derived from SCF-deficient embryos transfected with human cDNA containing exon 6, SCF is expressed as a soluble protein (Toksoz *et al*., 1992). In stromal cells transfected with human cDNA lacking exon 6, SCF is expressed as a membrane-bound protein (Toksoz *et al*., 1992). Thus, detection of an mRNA containing exon 6 (SCFs) or lacking exon 6 (SCFm) determines whether the protein will be located in a soluble or membrane-bound form. Different studies have elucidated SCFs cleavage mechanisms. The main cleavage site (at 174–201 of the amino acid sequence) on exon 6 is represented by the Ala-Val dipeptide (Huang *et al*., 1992; Majumdar *et al*., 1994). Cleavage at this site is generated at high rate (Huang *et al*., 1992). When exon 6 is absent, another cleavage site (on exon 7) could be used, but at a low cleavage rate (Huang *et al*., 1992). Enzymatic cleavage on exon 6 is induced by protein kinase C inducers such as phorbol myristate acetate (phorbol ester; PMA) and the calcium ionophore A 23187 (Huang *et al*., 1992; Majumdar *et al*., 1994). Moreover, at least two cell-associated serine protease activities with distinct specificity participate in the membrane growth factor precursor cleavage (Pandiella *et al*., 1992) and more recently, a human mast cell chymase—a chymotrypsin-like protease—has been shown to cleave SCFs at the exon 6 site (Longley *et al*., 1997). In the testis, while no data have been reported on SCF cleavage, different proteases [tissue plasminogen activator (tPA), urinary plasminogen activator (uPA), metalloproteinases (MMP), cathepsin L] involved in maintenance of the structural integrity of the seminiferous tubules (Fritz *et al*., 1993) are expressed, suggesting that SCF cleavage could occur.

The c-*kit* gene is located at the W (White spotting) locus on chromosome 4 in the human, and chromosome 5 in the mouse. In human, c-*kit* is encoded by 21 exons (Giebel *et al*., 1992; Vandenbark *et al*., 1992). The proto-oncogene encodes a tyrosine kinase receptor for the SCF factor (Yarden *et al*., 1987). SCF binding induces a rapid and complete receptor

dimerization that involves activation (by autophosphorylation) of the catalytic tyrosine kinase and generates signal transduction (Blume *et al*., 1991; Reith *et al*., 1991; Lev *et al*., 1992).

C-kit expression in the male gonad

C-kit has been detected in the male gonad during embryogenesis and post-natal development. In the mouse embryo, c-*kit* mRNAs are detectable at 7.0 dpc in the PGC when they are located at the base of the allantois, and at 12.5 dpc when PGC have reached the gonadal ridge (Manova and Bachvarova, 1991).

In the post-natal testis, c-kit was detected from 1 dpp to 5 dpp on gonocytes (Orth *et al*., 1996). In the adult testis, c-kit is present in spermatogonia types A1 to A4 (Dym *et al*., 1995). Moreover, a truncated c-kit receptor was detected in spermatids and spermatozoa (Albanesi *et al*., 1996). These transcripts encoded a truncated version of the c-kit protein, lacking the extracellular, the transmembrane and part of the intracellular tyrosine-kinase domains (Albanesi *et al*., 1996). A stage-specific promoter (located in the 16th intron) has been shown to drive the expression of the truncated c-kit protein in spermatids (Albanesi *et al*., 1996). In somatic cells, c-*kit* is expressed in interstitial Leydig cells in the adult testis (Manova *et al*., 1990).

SCF expression in the male gonad

In the normal embryo, *SCF* mRNA has been detected at 9 dpc along the migratory pathway of PGC and in the genital ridge at 12.5 dpc (Matsui *et al*., 1990). Sertoli cells are the unique source of SCF in the testis (Rossi *et al*., 1991; Tajima *et al*., 1991).

Since there exist two mRNAs for SCF (SCFs and SCFm), their relative abundance in the adult mouse was evaluated. While SCFm and SCFs transcripts are equally abundant in spleen and heart (Flanagan *et al*., 1991; Huang *et al*., 1992; Marziali *et al*., 1993), SCFs is predominant in all other organs tested, including brain, bone marrow, kidney, lung, liver and thymus of adult mouse (Flanagan *et al*., 1991; Huang *et al*., 1992; Marziali *et al*., 1993). In contrast, in adult testis SCFm is predominant (Flanagan *et al*., 1991; Marziali *et al*., 1993). Thus, the alternative splicing pattern of SCF seems to be tissue-specific. The SCF alternative splicing pattern is also regulated during the male gonadal development. Indeed, in fetal (13 dpc) mouse testes SCFm is predominant, whereas between 15/18 dpc to 2 dpp SCFs is the majority form (Figure 2). From 6/10 dpp to adulthood, SCFm is predominant (Figure 2; Manova *et al*., 1993; Marziali *et al*., 1993). From these different data, it appears that SCFm is predominantly expressed when germ cells are proliferating. Indeed, in fetal gonads, PGC proliferate from 7.5 to 13 dpc (Eddy *et al*., 1981), and then germ cells are quiescent from 15 dpc to 3 dpp. Initiation of spermatogenesis begins with the growth of spermatogonia at about 6 dpp (Bellvé *et al*., 1977). Although SCFm appeared rapidly as a key form of the cytokine for germ cell development, little was known about the mechanisms of regulation of SCF alternative splicing in favour of this membrane form. Recently we have shown that, in the context of Sertoli cell–germ cell interactions, the acidic pH in the Sertoli cell environment may be involved. Indeed, although Sertoli cells from 18-day-old mice in in-vivo conditions produced predominantly SCFm, after 48 h of culture they chiefly expressed SCFs. These observations suggest that the regulatory mechanisms involved in the predominance of SCFm are lost during Sertoli cell culture. We have shown that the SCFm might be maintained in culture after lowering the pH (Mauduit *et al*., 1999). This change in SCF alternative splicing in culture can be prevented by acidification of culture medium at pH 6.3. As Sertoli cells produce a very large amount of an acid metabolite, lactate (which is known to be used as an energy substrate), we hypothesize that lactate or another acid metabolite might be involved in the regulating mechanism of SCF alternative splicing. Indeed, the presence of lactate in Sertoli cell culture medium maintained the SCFm messenger ribonucleic acid (mRNA). Based on the observations that, in acidic conditions, Sertoli cells preferentially express the SCFm, we hypothesize that at certain specific stages during the seminiferous epithelium cycle which particularly associate Sertoli cells and spermatids, an acidic microenvironment might be generated by an elevated concentration of lactate. Therefore, lactate synthesis—in addition to supplying spermatids with their energy substrate—can also, by lowering the pH, allow the synthesis of SCFm which stimulates the proliferation of spermatogonia through c-kit and therefore triggers a new spermatogenic cycle. The hypothesis that terminally differentiated germ cells (spermatids) might send signals to stem germ cells to initiate a new spermatogenic cycle was proposed over 40 years ago (Roosen-Runge, 1952). Although other candidates (e.g. residual bodies; Roosen-Runge, 1952) have been suggested as the source of such signals between differentiated and stem germ cells, the precise determining factor remains unknown. We propose that an increased acidity through an increase in lactate production at the level of Sertoli cells in contact with spermatids may represent the triggering signal for SCFm expression (Figure 3). It is of interest to note that the lactate concentrations in Sertoli cells are probably under the control of local signalling factors. Indeed, we have shown that tumour necrosis factor α (TNFα) stimulates lactate production in cultured Sertoli cells through the regulation of lactate dehydrogenase A expression (Nehar *et al*., 1997; Boussouar *et al*., 1999).

The regulation of alternative splicing of SCF in favour of SCFm seems under the control of an RNA binding protein. Indeed, no *cis*-acting elements can explain the splicing of SCF in favour of the membrane-bound form. Moreover, UV cross-linking of RNA–protein interactions show the presence of a complex between exon 6 SCF RNA and a Sertoli cell

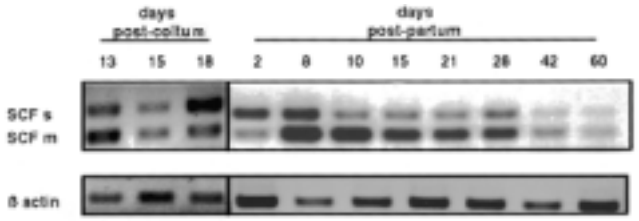

Figure 2. SCF alternative splicing pattern during development. Expression of SCF mRNAs was analysed by RT–PCR in testis from fetus (13, 15, 18 days post-coitum; dpc) and from 2- to 60-day-old mice. β actin mRNA was measured as an internal control (Mauduit *et al*., 1999).

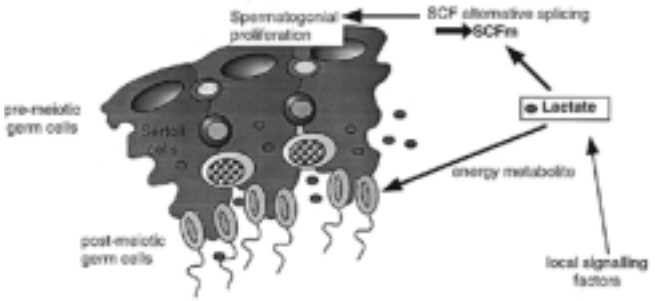

Figure 3. The lactate hypothesis of the SCF alternative splicing control. Lactate is produced by Sertoli cells and is used as an energy metabolite for the post-meiotic germ cells, but may also trigger the preferential expression of membrane-bound SCF which induced the spermatogonial proliferation and protected spermatogonia against apoptosis.

nuclear protein about 69 kDa in size. These observations suggest that RNA binding protein(s) are involved in the regulation of alternative splicing of SCF (Mauduit *et al*., 1999).

Sertoli cell SCF expression has been shown to be regulated hormonally by growth hormone regulating hormone (GHRH) (Breyer *et al*., 1996) and follicle stimulating hormone (FSH) (Rossi *et al*., 1993). These hormones have been shown to enhance SCF expression in cultured Sertoli cells. Moreover, induction by FSH of SCF mRNAs is maximal at stages II–VI (Yan *et al*., 1999). These observations are consistent with those reported previously (Jiang *et al*., 1997), which showed that SCF transcription in Sertoli cells is regulated by a cAMP-dependent pathway in the proximal promoter region.

Action of SCF/c-kit system

SCF/c-kit has been shown to be involved in different functions, including germ cell migration, cell adhesion, cellular proliferation and anti-apoptotic actions in the testis (Table II).

Table II. Actions of SCF/c-kit in the testis

	Actions
SCF/c-kit	Promotes PGC migration
	Anti-apoptotic factor for PGC
	Enhance proliferation of PGC and spermatogonia (A1–A4), particularly SCFm
Truncated c-kit	Role in capacitation and/or the acrosome reaction

SCF/c-kit in mutant mice and spermatogenesis

Spontaneous or induced mutations at the W and the Sl locus (summarized in Table III) provided mice with varying degrees of spermatogenic, haematopoietic and fur colour disorders (for reviews see Morrison and Takahashi, 1993; Loveland and Schlatt, 1997). These mutations suggest different roles for the SCF/c-kit system during spermatogenesis from the fetus until adulthood. Indeed, W and Sl mutations, which resulted in the absence of c-kit or SCF production respectively, are associated in homozygous mice with the absence of germ cells in post-natal testis. Such alterations of spermatogenesis might be related to defects in PGC migration and/or apoptosis. Point mutations in c-kit tyrosine kinase domain involving reduction of kinase activity decrease the fertility in male mice. With regard to the steel locus mutations, spermatogenesis defects in mutant mice models suggested that soluble and membrane-bound SCF do not have the same role. Deletion of transmembrane domain (Steel-Dickie mutation) induced the production of the SCF soluble form only. Those mice showed, at puberty, an absence of germ cell proliferation. Moreover, the Sl^{17H} mutant mice (absence of SCF cytoplasmic tail, leading to a membrane-bound SCF abnormality) showed a proliferation of germ cells at puberty, but the adult mice were sterile. These data suggested a role of SCFm in the renewal of germ cells in adult spermatogenesis. Finally, one of the lessons from the SCF/c-kit mutant mice is related to the possible different role of this signalling molecule in the male and in the female. Indeed, it is noteworthy that female Steel panda or Steel contrasted mutant mice are sterile, whereas males are fertile. In contrast, male $Steel^{17H}$ mutant mice are sterile, whereas females are fertile.

SCF promotes germ cell migration

Different data suggest a role for SCF/c-kit in the migration of PGC. Indeed, c-kit is present in PGC (from 7.0 dpc), and SCF is expressed along their migratory pathway (Matsui *et al*., 1990; Motro *et al*., 1991). Moreover, the testes of mice bearing a W/W mutation (where c-kit tyrosine kinase domain is inactive) or a Sl/Sl mutation (null mutation, no SCF produced) present at 8 dpc a normal number of PGC, but they fail to increase in number. At 14 dpc, the gonads are devoid of germ cells (Mintz and Russell, 1957), suggesting that SCF/c-kit has a role in PGC migration.

Table III. Examples of SCF/c-*kit* mutations in mice

	Phenotype of heterozygous	Phenotype of homozygous	Mutation	Reference(s)
W (white spotting)	Fertile	Embryonic lethal, absence of PGC at 14 dpc in the fetal gonad, sterile	78 aa deletion, no kinase activity, no cell surface c-kit	Nocka *et al*. (1990)
W^{v} (dominant white spotting)	Fertile	Death by birth, sterile	Point mutation at aa660 (Thr → Met), reduced kinase activity	Nocka *et al*. (1990); Reith *et al*. (1990)
W^{37}	Fertile	Embryonic lethal, sterile	Point mutation at aa582 (Glu → Lys), reduced kinase activity	Geissler *et al*. (1981); Nocka *et al*. (1990); Reith *et al*. (1990)
W^{42} (1990)	Fertility affected	Embryonic lethal, sterile	Point mutation at aa790 (Asp → Asn), reduced kinase activity	Geissler *et al*. (1981); Tan *et al*.
W^{41}	Fertile	Fertile	Point mutation at aa831 (Val → Met), reduced kinase activity	Nocka *et al*. (1990); Reith *et al*. (1990); Geissler *et al.* (1981)
W^{44}	Fertile	Fertility affected	Rearrangement of the genome	Geissler *et al*. (1988)
W^{57}	Fertile	Fertile	Reduced mRNA level, protein kinase active	Reith *et al*. (1990)
W^{19H}	Fertile	Embryonic lethal	Large deletion, no kinase activity, no cell surface c-kit	Lyon *et al*. (1984); Chabot *et al*. (1988); Geissler *et al.* (1988)
W	–	–	Point mutation, reduced kinase activity	Chabot *et al*. (1988)
Sl (Steel)	Fertile	Embryonic lethal, absence of PGC at 14 dpc in the fetal gonad, sterile	Null mutation, no production of SCF	McCoshen and McCallion (1975)
Sl^{d} (Steel-Dickie)	Fertile	Presence of germ cells in post-natal gonad, but no proliferation; male sterile	Deletion of transmembrane domain, production of SCFs form only	Brannan *et al*. (1991); Flanagan *et al*. (1991)
Sl^{17H} (Steel-17H)	Fertile	Female fertile, male sterile (reduction in PGC number, later, after the first wave of spermatogenesis was complete, a near-cessation of sperm development was observed)	T → A transversion resulting in skipping of exon 8 (cytoplasmic tail), the mutant protein may not be well anchored in the cell membrane	Brannan *et al*. (1992)
Sl^{J} (Steel J) (1990)	Fertile	Embryonic lethal	Large deletion	Copeland *et al*.
Sl^{gb} (Steel grizzle belly) (1990)	Fertile	Embryonic lethal	Deletion	Copeland *et al*.
Sl^{8H} (Steel 8H) (1990)	Fertile	Embryonic lethal	Deletion	Copeland *et al*.
Sl^{10H} (1990)	Fertile	Embryonic lethal	Deletion	Copeland *et al*.
Sl^{pan} (Steel panda)	Fertile	Male fertile, female sterile	Mutation in non-coding region, decreased mRNA level	Bedell *et al*. (1995)
Sl^{con} (Steel contrasted)	Fertile	Male fertile, female sterile	Mutation in non-coding region, decreased mRNA level	Bedell *et al*. (1995)
Sl^{18H}	Fertile	Embryonic lethal	Deletion	Copeland *et al*. (1990)
W/W^{v}	Few germ cells at the most primitive stage	0		For review, see Sar vella and Russell (1956)
Sl/Sl^{d}	Sterile, migration of few PGC, no proliferation of spermatogonia	0		For review, see Sar vella and Russell (1956)

This table shows a variety of SCF/c-*kit* mutations described in mice. The SCF/c-*kit* mutations affect the development of three stem cells: melanocytes, germ cells and haematopoietic precursors cells. Homozygous mutations in mice lead to the lack in pigmented coat (neural crest-derived melanocyte migration defect), infertility (germ cell dysfunction) and deficiency in erythrocytes (that leads to death by anaemia) and in mast cells.
aa = amino acid; embryonic lethal = death by anaemia.

SCFm also participates in the adhesion of germ cells to Sertoli cells. Indeed, Sertoli cells from Sl/Sl^d mutant mice, which express only soluble SCF, are unable to bind germ cells. Transfection with a plasmid, expressing only the membrane-bound form of SCF, restores the ability of Sertoli cells from Sl/Sl^d mutant mice to bind germ cells (Marziali *et al*., 1993).

SCF is an anti-apoptotic factor

SCF has been shown to protect cells (bone marrow-derived mast cells) against apoptosis generated by serum depletion or gamma irradiation (Yee *et al*., 1994). SCF inhibits apoptosis by enhancing Bcl-2 expression (Carson *et al*., 1994) in lymphocytes. The two forms of SCF (soluble and membrane-bound) seem not to have the same action on apoptosis. Indeed, soluble SCF (which is the unique form of SCF produced by Sertoli cells in Sl/Sl^d mice) is able to support only short-term PGC survival, but is unable to support their long-term survival (Dolci *et al*., 1991). By contrast, SCFm supports the long-term survival of germ cells (Dolci *et al*., 1991). The reduced ability of SCFs to support long-term survival may reflect a requirement for localized, high concentrations of SCF (Dolci *et al*., 1991) which seem to be exerted by SCFm. Finally, intravenous injection of ACK2 (a neutralizing monoclonal antibody raised against c-kit) suggests that the SCF/c-kit system promotes survival of differentiating type A spermatogonia (Yoshinaga *et al*., 1991).

SCF as a germ cell growth factor

SCF/c-kit has been shown to be involved in germ cell proliferation at the different stages of testicular development. Indeed, SCF is involved in PGC growth (8.5–10.5 dpc embryos; Dolci *et al*., 1991; Godin *et al*., 1991; Matsui *et al*., 1991). Different reports suggest that: (i) in the absence of SCF, no proliferation of PGC was observed, as Sl/Sl^4 cells (cells derived from mouse embryo where the SCF gene is deleted) do not support an increase in PGC number; and (ii) the presence of SCFs alone is not sufficient for an optimal proliferation of PGC, as culture of PGC in the presence of saturating amounts of SCFs shows, on Sl/Sl^4 cell feeder (that do not express SCF), a PGC number lower than on Sl^4-m220 cell feeder (that express only SCFm). Therefore, SCFm is required in addition to SCFs for optimal effects on PGC growth (Matsui *et al*., 1991). By contrast, PGC from 12.5 dpc embryos do not respond to SCFs, suggesting a loss of responsiveness of those cells which are in mitotic arrest between 13/15 dpc and 3 dpp (Matsui *et al*., 1991). While c-*kit* is still expressed on gonocytes (from 0 to 4 dpp) (Orth *et al*., 1996), these cells seem to be c-kit-independent, as shown by experiments with ACK2 antibodies (Yoshinaga *et al*., 1991). Indeed, injection of ACK2 antibodies to prepubertal animals (0–4 dpp) has no effect on spermatogonia number. By contrast, administration of ACK2 between 6–12 dpp, or in adult animals, blocks the mitosis (and decreases the number) of type A spermatogonia (Yoshinaga *et al*., 1991). Thus, proliferation of spermatogonia depends upon SCF only before 12.5 dpc and after 4/6 dpp, while germ cells express c-*kit* and Sertoli cells express SCF from 7 dpc to adulthood. So how can the lack of responsiveness of spermatogonia between 12.5 dpc to 4 dpp to SCF/c-kit be explained? The study of the relative expression of SCFs and SCFm may shed light on this matter. Indeed, in fetal and postnatal mice, SCFs is expressed predominantly (from 15 dpc to 2 dpp), and from 6/10 dpp to adulthood SCFm is expressed predominantly in the testis (Mauduit *et al*., 1999). These observations suggest that germ cell arrest could in part be due to a relative decrease in SCFm expression in the spermatogonial environment between 15 dpc and 4 dpp. The enhancement of SCFm (which occurs at about 6 dpp) could induce spermatogonial proliferation.

The SCF/c-kit system also promotes the growth of post-natal germ cells. Whereas primitive type A spermatogonia and type A_0 spermatogonia (for self-renewal of testicular stem cells) are independent of c-kit, spermatogonia types A1 to A4 are c-kit-dependent. It has been demonstrated (Yoshinaga *et al*., 1991) that type A_0 spermatogonia, c-kit-independent cells, repopulate aplastic seminiferous tubules obtained after a long-term treatment by ACK2. In contrast, mitosis of differentiating type A spermatogonia (A1–A4) is absolutely dependent on c-kit/SCF, but differentiation of type B spermatogonia into preleptotene spermatocytes is independent of c-kit (Yoshinaga *et al*., 1991). A recent study has shown that SCF expression follows a stage-specific regulation. The highest mRNA level is detected in stages II–VI (Harkovirta *et al*., 1999); moreover, these authors have shown that SCF induces thymidine incorporation at stages VIII–IX and that spermatogonia type A and intermediate spermatogonia are the cellular site of the increased DNA synthesis (Hakovirta *et al*., 1999). Finally, the role of SCFm in the post-natal growth of spermatogonia was confirmed by the developmental abnormalities observed in Sl^{17H} mutant mice. Sl^{17H} mutation results in a splicing defect due to exon skipping in the SCF cytoplasmic tail encoded by exon 8. The mature mutant protein may not be well anchored in the cell membrane (Brannan *et al*., 1992). In Sl^{17H} mutant mice at 11.5/12.5 dpc, a decrease in the number of PGC was observed in the gonad (Brannan *et al*., 1992). Moreover, after the first wave of spermatogenesis was complete, a near cessation of spermatogenesis was observed in these mutant mice, leading to a depletion in differentiated germ cells by 8 weeks of age (Brannan *et al*., 1992). These observations reinforce the hypothesis that SCFm stimulates the proliferation of post-natal germ cells, and that SCFm could be involved in proliferation/recruitment of the renewing testicular stem cells (Figure 4).

SCF has also been suggested to regulate other germ cell processes such as meiosis and the acrosome reaction. A c-*kit* mRNA has been identified in purified pachytene spermatocytes (Vincent *et al*., 1998). By using a Sertoli cell

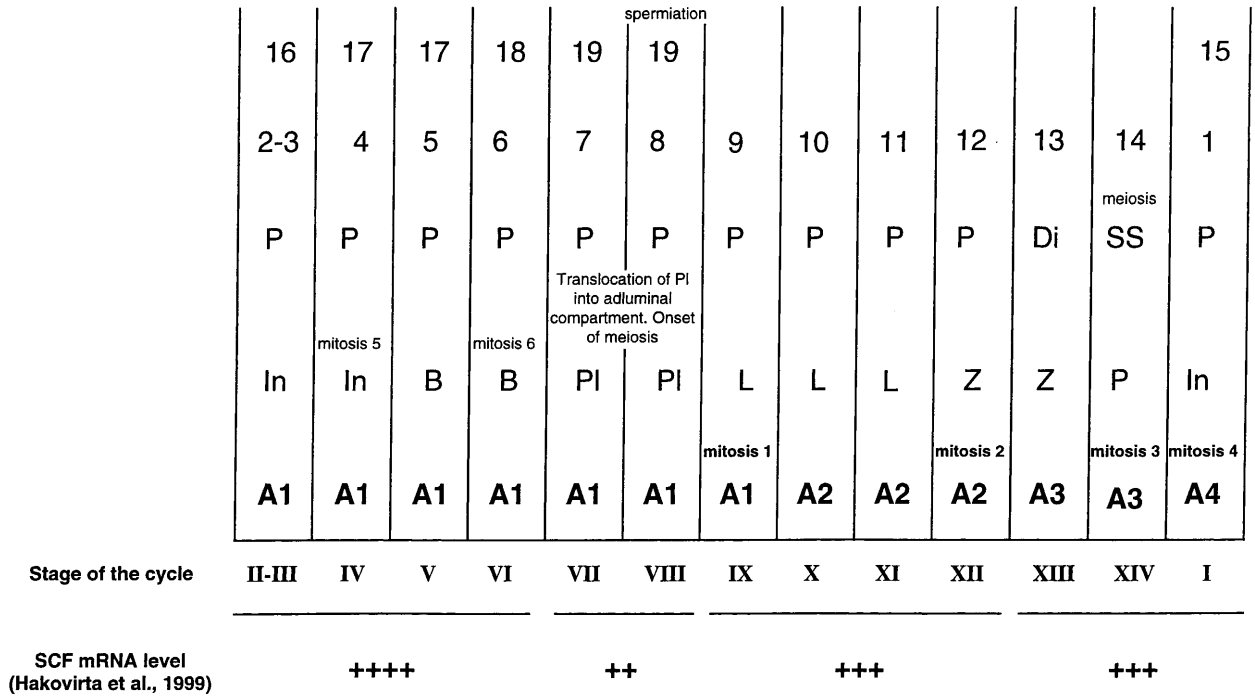

Figure 4. SCF mRNA concentration according to the stage of the seminiferous epithelial cycle (in rat). Type A1, A2, A3 and A4 spermatogonia mitosis are under SCF/c-kit control. A1, A2, A3, A4 = type A spermatogonia; B = type B spermatogonia; In = intermediate spermatogonia; Di = diplotene spermatocyte; L = leptotene spermatocyte; P = pachytene spermatocyte; Pl = preleptotene spermatocyte; SS = secondary spermatocyte; Z = zygotene spermatocyte; 1 to 19 = spermatids.

line–germ cell co-culture, these authors suggested that the SCF/c-kit system has a role in the meiotic progression of spermatocytes, while in haploid germ cells, a truncated c-kit seems to be involved. C-kit is present in mature spermatozoa, and may play a role in capacitation and/or the acrosome reaction (Feng *et al*., 1997).

Finally, the SCF could regulate testosterone production by Leydig cells, though reports are contradictory. Injections of blocking antibodies raised against c-kit show a decrease in testosterone levels, suggesting that SCF enhances androgen production (Yoshinaga *et al*., 1991). In contrast, it has been shown in cultured Leydig cells that SCF decreases testosterone secretion (Verhoeven and Cailleau, 1991), though further investigations are needed in this regard.

SCF/c-kit and human testicular pathology

Since their first discovery, SCF and c-*kit* genes have been shown to be important for germ cell development. The actions and their mechanisms—as well as the potential role of the SCF/c-kit system—have been largely clarified through the use of genetically manipulated murine models. Nonetheless, we have not as yet identified similar events in human pathology. Piebaldism—an autosomal dominant genetic disorder—has been shown to be a human homologue to white spotting (W) defect of the mouse with the same mutation, namely a Gly → Arg substitution at the codon 664 within the tyrosine kinase domain (Giebel and Spritz, 1991; Giebel *et al*., 1992). Interestingly, the three lineages (haematopoetic, germ cells and melanocytes) affected by SCF/c-kit mutations seem to have a different degree of sensitivity to SCF/c-kit withdrawal in mice. Indeed, for a complete arrest of spermatogenesis, a 10-fold lower dose of antibody (ACK2 raised against c-kit) was required than for blocking adult haematopoiesis (Ogawa *et al*., 1991; Yoshinaga *et al*., 1991). These results suggest that severe germ cell defects with mild/no anaemia might be observable in the presence of SCF/c-*kit* expression defects. Moreover, an SCFm/SCFs ratio alteration could be involved in infertility. Indeed, administration of 2,5-hexanedione induced testicular atrophy in rat, and the following infertility was associated with a decrease in the expression of SCFm. Administration of SCFs promoted a modest recovery of the testicular atrophy (Allard *et al*., 1996). This atrophy was partly corrected by gonadotrophin releasing hormone (GnRH) agonist (leuprolide) therapy (Blanchard *et al*., 1998). GnRH agonist is believed to increase the expression of SCFm compared with that of SCFs (Blanchard *et al*., 1998).

With regard to human pathology, certain testicular dysfunctions, gonadal defects, infertilities and testicular tumours could be associated with SCF and/or c-kit disorders.

Indeed, fetal testicular germ cells of intersex subjects express c-*kit* at a later developmental age than controls. These observations could indicate a disturbance of the germ cell development chronology and/or a dysregulation of c-*kit* expression in gonadal development disorders (Meyts *et al*., 1996). In adult testes, immunoreactive SCF and c-kit were identified in somatic cells, germ cells and also in seminal plasma. Immunoreactive c-kit was identified in human germ cells (early spermatogenic cells, acrosomal granules of the round spermatids and acrosome of the spermatozoa) and in Leydig cells, whereas immunoreactive SCF was identified in Sertoli cells (Sandlow *et al*., 1996). Both soluble and membrane-bound forms of SCF were identified in normal and oligozoospermic/azoospermic men. The concentration of SCF was significantly higher in normospermic men than in infertile men; thus, the SCF concentration in seminal plasma might predicate the ability to produce spermatozoa (Fusigawa *et al*., 1998). More recently, in testis from subfertile men, a decrease in the expression of c-kit receptor was reported to be associated with an increased apoptosis (Tesarik *et al*., 1998; Feng *et al*., 1999). In addition to the potential involvement of SCF/c-kit in infertility, this signalling system has also been implicated in testicular cancers. This system appears as a good marker of testicular seminoma (Izquierdo *et al*., 1995; Strohmeyer *et al*., 1995; Bokemeyer *et al*., 1996), though not of other testicular tumours, because it is accepted that the tumoral cells of testicular seminomas derive from spermatogonia (which express c-kit). In one study (Bokemeyer *et al*., 1996), 78% of the seminomas (7/9) presented a strong immunostaining for SCF/c-kit, while in non-seminoma only 26% of tumours presented a faint positivity for SCF/c-kit, and the others were negative. Thus, the SCF/c-kit system might be implicated in the development of seminoma, but probably not non-seminoma, tumours. In addition, an elevated expression of c-*kit* in carcinoma in-situ, a potential precursor of invasive germ cell tumours, supports the hypothesis that these tumours originate from PGC. It has been suggested (Rajpert-De Meyts and Skakkebaek, 1994) that c-kit protein products might be considered as a marker for carcinoma in-situ of the testis.

The data obtained from animals models (and the few data obtained from humans) suggest that SCF/c-kit disorders could affect testicular functions, but further experiments and more data are required to ascertain SCF/c-kit involvement in human testicular pathologies.

Conclusions

Based on the different reports both in experimental in-vivo systems (mainly in mutant mice) and in in-vitro models, SCF/c-kit appears to represent one of the key regulators of testicular formation, development and function. With regard to human gonadal pathologies, including testicular developmental defects, infertility and testicular cancers, there is now an increasing number of reports that indicate SCF/c-kit also to be a key regulator in the human testis. At present, the great majority of the available data are based mainly on immunohistochemical and radioimmunoassay techniques of the measurement of SCF/c-kit. However, in the near future new approaches—especially with molecular biology techniques [reverse transcriptase–polymerase chain reaction (RT–PCR), gene sequencing]—will permit qualitative evaluation (for example mutations with loss or gain of function, alternative splicing) of SCF/c-*kit* expression in human testicular pathologies.

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