Every sperm is sacred—or is it?

Robert E. Braun

Department of Genetics, Box 357360, 1959 NE Pacific, University of Washington, Seattle, Washington 98195, USA. e-mail: braun@genetics.washington.edu

The average human male produces approximately 200 million sperm each day—equivalent to 1,500 sperm per heart beat. Why so many? In humans, and to a lesser extent in other mammals, a significant percentage of mature spermatozoa stored in the epididymis have obvious structural abnormalities (Fig. 1). Defects include sperm with more than one head, structurally abnormal flagella and defective acrosomes. One might conclude that the strategy employed is to sacrifice quality over quantity, to produce as many sperm as possible and let them compete for those few precious eggs. The journey of a sperm from ejaculation to fertilization is an arduous one, and many sperm will fall by the wayside.

It has long been known, however, that sperm quality is monitored during the terminal stages of fruit-fly spermatogenesis and those that receive a 'seal of approval' are culled from inferior sperm which end up in a biological waste bag[1]. Several studies, some old and some new, suggest that mammals also have a quality-control system for sperm production. Long before the discovery of apoptosis, testis morphologists described the death and subsequent removal of a sizeable fraction of germinal cells (primarily diploid spermatogonia) during normal spermatogenesis[2]. The cause of cell death is unknown, but it is presumably in response to differentiation gone awry and an attempt to integrate germ-cell numbers with the number of support cells. George Miklos[3], and later, Paul Burgyone and Terry Baker[4], proposed that there might be a quality-control system in mouse

meiotic spermatocytes that detects unsynapsed chromosomes. In this issue of *Nature Genetics*, Burgoyne and colleagues[5] dissect the genetic control of this system and conclude that p53, a major apoptosis inducer in somatic cells[6], is not involved in the meiotic arrest and subsequent apoptosis observed in cells that have incompletely-synapsed chromosomes. Also in this issue, Andrea Ross and colleagues[7] describe a mutation in *Bclw*, an anti-apoptotic member of the *Bcl2* gene family, that causes male sterility. Mutant animals have a block in the later stages of spermatogenesis and exhibit progressive degeneration of the germ line and supporting Sertoli cells.

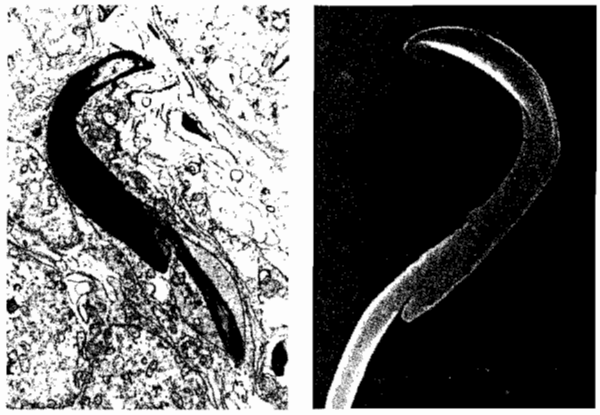


Fig. 1 In sickness and in health. Defective mouse sperm are engulfed and degraded by a Sertoli cell (left panel) while healthy sperm (right panel) tend to escape this fate. *Micrographs kindly provided by Lonnie Russell.*

Spermatogenesis in mammals is a dynamic process that occurs continuously during the reproductive lifetime of the individual. In the mouse, sperm production starts a few days after birth and lasts throughout the animal's adult life. The production time for an individual spermatozoon is approximately five weeks—a remarkably long time given that all of embryonic development occurs in less than three weeks. Differentiating spermatogenic cells develop in a syncytium, sometimes consisting of several hundred cells within an individual clone. Spermatogenesis is divided into three phases: the mitotic proliferation of differentiating diploid spermatogonia from a self-renewing pool of stem cells, meiosis, and the elaborate differentiation of haploid spermatids into mature spermatozoa (Fig. 2). Spermatogenesis occurs within a tubular seminiferous epithelium, consisting of germ cells at different stages and supporting Sertoli cells. Outside of the seminiferous tubules lie the steroidigenic Leydig cells, whose main function is to synthesize testosterone, and the peritubular myoid cells that induce a peristalsis-like action on the tubules, facilitating movement of mature sperm through the lumen of the epithelium. Spermatogenesis represents a wonderful developmental paradigm: it is an ongoing process that can be studied in the adult, and although essential for the species, it is dispensible for the individual.

Asynapsis and apoptosis

Not all germ cells within a syncytium achieve maturity. Indeed, elimination of cells occurs at all three phases of spermatogenesis[2]. Burgoyne and colleagues have characterized mice that carry a single translocation sex chromosome (comprised of an X and a Y attached by a shared pseudoautosomal region) which is subject to incomplete synapsis in pachytene cells —thus extending their earlier work that suggested a quality-control system for monitoring chromosome synapsis during meiosis. Interestingly, several mouse mutants with defects in meiosis (for example, those with defective Mlh1; refs 8,9) also have a block in meiotic metaphase and exhibit subsequent germ-cell loss through apoptosis. Perhaps the same meiotic quality control system is being induced in these meiotic mutants. Surprisingly, there appears to be no equivalent meiotic quality-control system at work in females during oogenesis[10].

What signal triggers the removal of meiotic cells with unsynapsed chromosomes and how is it accomplished? Extrapolating from studies of meiotic checkpoints in yeast[11,12], the authors suggest that the meiotic quality-control system recognizes unrepaired double-strand DNA breaks, presumably those that initiate or arise from the process of recombination in the unsynapsed chromosomes. Recognition of the unrepaired DNA by some unknown factor is thought to induce cell-cycle arrest at meiotic metaphase I and subsequent loss of germ cells through apoptosis. But unrepaired DNA breaks in unsynapsed chromosomes are only one possible signal for such a checkpoint; it remains to be determined whether they are indeed the inducer. A principal finding is that the quality-control system monitoring synapsis does not require p53, a surprising result given that p53 is a major regulator of apoptosis in response to DNA damage in somatic cells[13] and is highly expressed during the pachytene stage of meiosis[14].

To be or not to be?

Do the haploid spermatids also have a quality-control system? Ross *et al.*[7] show that *Bclw* is expressed in late-stage elongating spermatids and in Sertoli cells. Studying the first wave of spermatogenesis in pre-pubertal animals with mutated *Bclw*, they discovered an elevated level of apoptotic spermatocytes and an arrest in spermatid differentiation. Mature adult mutants eventually lose all germ-line cells, followed by a loss of somatic Sertoli cells. It is likely that death of late-stage spermatids is due to absence of Bclw function

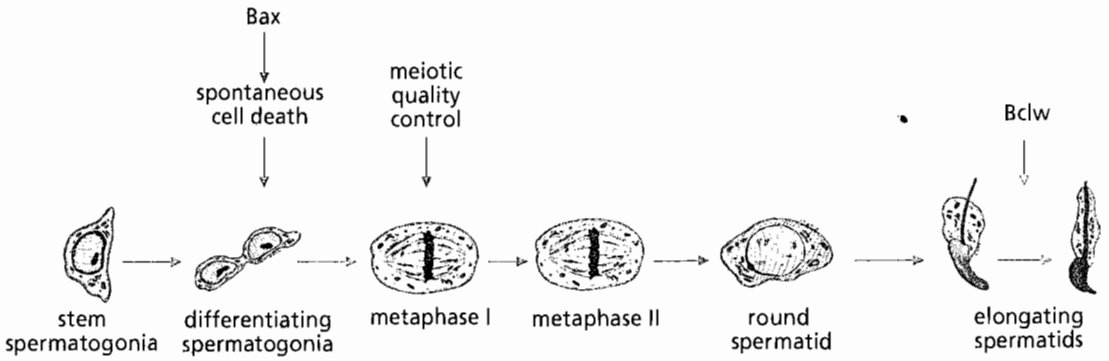
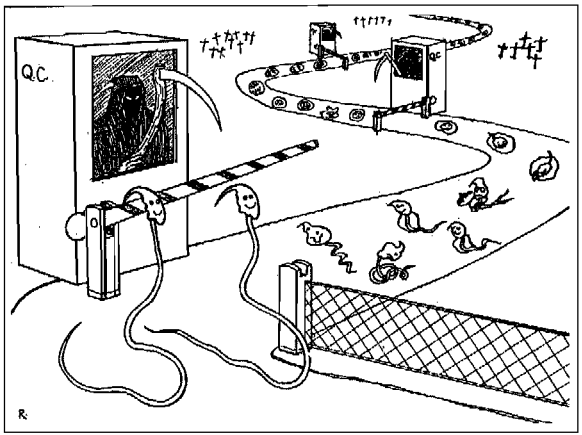


Fig. 2 Stages of spontaneuos and induced cell death during mammaliam spermatogenesis. Spontaneous cell death is frequently observed in differentiating spermatogonia and is elevated in *Bax* mutants[15]. Meiotic spermatocytes have a quality-control system which recognizes unpaired chromosomes and induces apoptosis in meiotic metaphase I. Burgoyne and colleagues[5] show that meiotic quality control-induced apoptosis is p53-independent. Disruption of the *Bclw* gene blocks spermatid generation in young adults and causes progressive degeneration of the testis in older males.



"We made it!
Now, I wonder what chance we have of fertilizing an egg?"

in the germ cells, whereas depletion of the entire germline in adults reflects the loss of Bclw function in the Sertoli cell. The eventual loss of Sertoli cells is unusual, as studies of other mouse mutants that lack germ cells suggest that Sertoli-cell survival is not dependent on germ cells. Bclw must therefore act to promote Sertoli-cell survival as well as germ-cell survival. Does Bclw perform an essential function in spermatogenesis, or is it simply inhibiting cell-death? Perhaps spermatids also have an inducible cell death pathway used in quality control. If so, what are the signals within defective spermatids that are recognized by the quality-control system? Does degradation of Bclw in defective spermatids trigger spermatid suicide?

Loss of function of other Bcl2 family members also disrupts male germ-cell differentiation. Mutations in *Bax*, whose product heterodimerizes with Bcl2, causes male sterility[15]. Pre-meiotic spermatogonial cells and early meiotic cells are affected, resulting in a complicated phenotype that appears to include both an accumulation of spermatogonial cells and increased cell death. The study of the initiation of spermatogenesis in pre-pubertal animals and the maintenance of spermatogenesis in adults should elucidate the opposing roles of the Bcl2 family members in male germ-cell differentiation, cell death and quality control.

Germ-cell heaven

Where do germ cells go after they die? Dying cells are usually phagocytosed by macrophages in somatic tissues. The seminiferous epithelium, however, is an immunologically privileged hide-a-way for germ cells, protected by tight junctions between adjacent Sertoli cells that form a blood-testis barrier to macrophage entry. The Sertoli cell, however, compensates for its 'anti-social' behaviour by acting as the phagocytic cell of the testis (Fig. 2), removing residual cytoplasm left behind by spermatozoa when they exit the epithelium and engulfing defective and degenerating spermatids. Like a loving mother, the Sertoli cell not only nurtures its progeny—it also cleans up the mess when things go wrong. □

1. Tokuyasu, K.T., Peacock, W.J. & Hardy R.W.Z. *Zellforsch. Mikrosk. Anat.* **127**, 492–525 (1972).
2. Roosen Runge, E.C. *J. Reprod. Fertil.* **35**, 339–348 (1973).
3. Miklos, G.L. *Cytogenet. Cell. Genet.* **13**, 558–577 (1974).
4. Burgoyne, P.S. & Baker, T.G. *Symp. Soc. Exp. Biol.* **38**, 349–362 (1984).
5. Odorisio, T. *et al. Nature Genet.* **18**, 257–261 (1998).
6. Ko, L.J. & Prives, C. *Genes Dev.* **10**,1054–1072 (1996).
7. Ross, J.R, *et al. Nature Genet.* **18**, 251–256 (1998).
8. Baker, S.M. *et al. Nature Genet.* **13**, 336–342 (1996).
9. Edelmann, W. *et al. Cell* **85**, 1125–1134 (1996).
10. LeMaire-Adkins, R., Radke, K. & Hunt, P.A. *J. Cell. Biol.* **139**, 1611–1619 (1997).
11. Lydall, D., Nikolsky, Y., Bishop, D.K. & Weinert, T. *Nature* **383**, 840–843 (1996).
12. Xu, L., Weiner, B.M. & Kleckner, N. *Genes Dev.* **11**, 106–118 (1997).
13. Nelson, W.G. & Kastan, M.B. *Mol. Cell. Biol.* **14**, 1815–1823 (1994).
14. Schwartz, D., Goldfinger, N. & Rotter, V. *Oncogene* **8**, 1487–1494 (1993).
15. Knudson, C.M. *et al Science* **270**, 96–99 (1995).