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Invited Review

What are the germ cell phenotypes from infertile men telling us about spermatogenesis?

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Summary. *Drosophila* mutants for known genes and those obtained following germline genetic engineering in mice have led to the identification of genes involved in the initiation and the maintenance of spermatogenesis and in the different steps of meiosis. Mutants allow the definition of meiosis-specific checkpoint controls that ensure the transmission of complete and undamaged genetic information. They reveal what spermatogenesis events are interdependent. In the light of these data, an attempt is made to define which events of spermatogenesis could be defective in some well-defined human spermatogenesis failures. They appear to be good models to study the decouplages of spermatogenesis events, the morphogenetic relationships between germ cell structures and the occurrence of pleiotropic sperm phenotypes. It is discussed whether a germ cell with a normal phenotype can transmit a non-functional gene involved in spermatogenesis and how homologous genes can lead to different germ cell phenotypes depending on the species.

Key words: Meiosis, Mutants, Sperm, Spermatogenesis, Transgenic, Testis

Human pathological models revealing some aspects of the spermatogenesis developmental program

New male germ-cell specific genes will be soon identified, particularly genes involved in meiotic divisions and sperm morphogenesis. Disruption of such genes must lead to germ cell phenotypes already known in the human (Holstein et al., 1988). We will consider human pathological models in an attempt to define some determinative events of human spermatogenesis and whether they can be compared, or not, to mouse and *Drosophila* mutants. Concerning targeted mutagenesis in mice, only the major spermatogenesis disturbance obtained is considered in this review. For additional information, see the review on mammalian spermatogenesis investigated by germline genetic engineering (Escalier, 1999).

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Initiation and maintenance of spermatogenesis

Absence of germ cells can be due to failure of their migration in the testis or to factors involved in the control of proliferation and/or in the maintenance of spermatogonia. A large percentage of idiopathic Sertoli cell-only syndrome in men share microdeletions on the Y-chromosome long arm euchromatic region (Yq) which seems to possess one or more still unknown gene essential for spermatogenesis (Foresta et al., 1998). Much information has accumulated in recent years on two gene families of the Yq (*RBM* and *DAZ*) that have been considered as candidates for AZF (Azoospermia factor) (reviewed in Cooke et al., 1998). However, their study is difficult due to the existence of multiple *RBM* genes, each gene generating protein isoforms (Chai et al., 1997) and to the fact that the *DAZ* genes show varying numbers of *DAZ* repeats and polymorphism of these repeats in the population (Yen et al., 1997). Deletions removing some *RBM* copies are associated with male sterility in man but no mutations within members of either the *RBM* or *DAZ* gene family have yet been identified in infertile males. This questions whether an AZF gene really exists (Vogt, 1998) or if the azoospermia phenotypes are only observed after deletion of a set of genes in the AZF region.

Some factors involved in the proliferation and/or maintenance of spermatogonia have been identified by genetic engineering (Sassone-Corsi, 1997). The zinc-finger proteins ZFX and ZFY, which may function as sequence-specific transcription activators, are encoded by genes on the sex chromosomes, *Zfy* being localized to the sex-determining region of the human Y chromosome. *Zfx* null mutant mice provided evidence for a role of *Zfx* in the growth and maintenance of germ cell populations (Luoh et al., 1997). A Sertoli cell-only phenotype has been obtained inactivating the *tiar* gene which encodes an RNA-binding protein expressed in primordial germ cells (PGCs) (Beck et al., 1998). The germ cell defect of $tiar^{-/-}$ mice could be compared to the PGC defects characterizing mutations in the *c-kit*

tyrosine kinase receptor (white spotting) and its ligand, steel factor. A role was suggested for TIAR in growth factor signaling (Beck et al., 1998). The autosomal *Dazla* gene also encodes a protein containing RNA-binding motifs. Disruption of the *Dazla* gene (Ruggiu et al., 1997) has led to loss of germ cells, showing that the mouse *Dazla* gene is essential for the development and survival of germ cells. It is worth noting that a decline in germ cell proliferation can appear only after several generations, as seen for mice lacking telomerase RNA (Lee et al., 1998).

Altered X-chromosome dosage can impair germ cell development. XXY mice show reduced germ cell proliferation in the early stages of testis differentiation. Since the *in vitro* proliferative potential of the fetal germ cells is normal, it was suggested that a somatic/germ cell communication is involved (Hunt et al., 1998). Spontaneous *jsd* (juvenile spermatogonial depletion) mutation in mice suggests that initiation and maintenance of mammal spermatogenesis are under different mechanisms (see discussion in Zhao et al., 1996). This fact is now demonstrated in mice with inactivation of the bone morphogenetic proteins (BMPs) which are secreted molecules of the transforming growth factor-ß (TGFß) superfamily. In the mouse, *Bmp8b* (Zhao et al., 1996) seems to be required for both the initiation and maintenance of spermatogenesis in the mouse and *Bmp8a* in only the maintenance of spermatogenesis (Zhao et al., 1998). Finally, the regulation and maintenance of spermatogenesis through cell signaling factors secreted by Sertoli cells have also been demonstrated inactivating the *Desert hedgehog (Dhh)* gene, a homolog of the *Drosophila* segment polarity gene hedgehog. In *Dhh* null mutants on a *129/Sv* inbred background, germ cells undergo cell death resulting in only a residual lining of Sertoli cells. On a *129/Sv-C57BL/6J* F1 hybrid background, sperm development was blocked in late spermatids, suggesting that *Dhh* regulates both early and late stages of spermatogenesis. The loss of expression of Patched gene (encoding a multiple membrane-spanning protein) in Leydig cells of *Dhh* mutants indicates that Leydig cells may be the direct target of *Dhh* signaling (Bitgood et al., 1996).

Spermatogenesis arrests reveal meiotic checkpoint controls

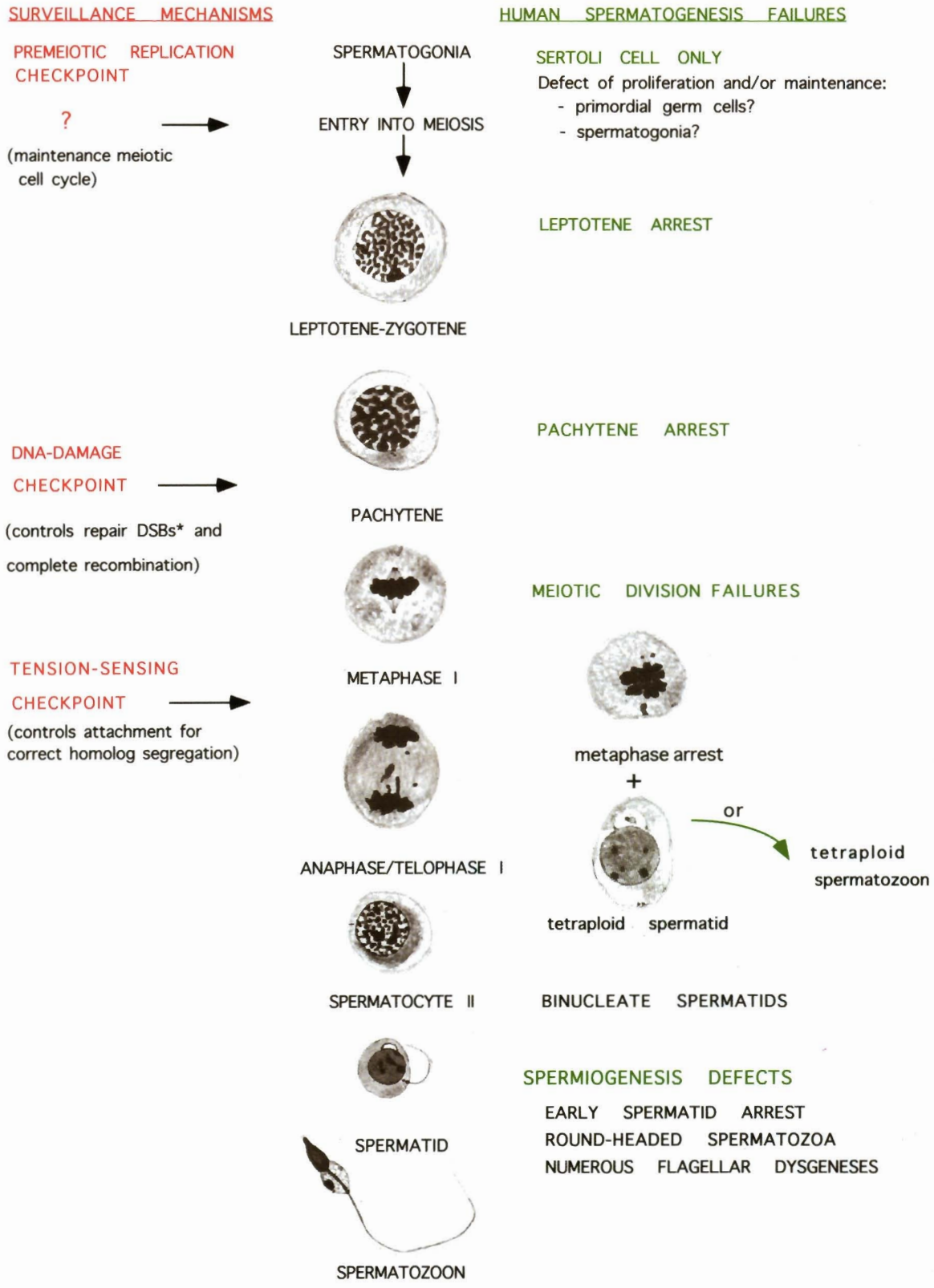
Checkpoints maintain the interdependency of cell cycle events by permitting the onset of an event only after the completion of the preceding event (reviewed in Kitazono and Matsumoto, 1998). Meiotic checkpoint controls (meiotic surveillance mechanisms) (Fig. 1) and the genes involved have been identified in yeast and *Drosophila* from mutants with checkpoint arrests (Roeder, 1995; Page and Orr-Weaver, 1997). One checkpoint ensures that meiotic recombination is finished (with double-strand breaks repaired) before the meiosis I spindle is set up (DNA-damage sensing mechanism). Another forces anaphase to wait until homologs are attached to the spindle (tension sensing checkpoint; i.e., requirement of tension for stable attachment to the spindle). Recent data from yeast mutants suggest the existence of a premeiotic DNA replication checkpoint activated by E-type cyclins (Stuart and Wittenberg, 1998).

In the human, various chromosomal abnormalies have been described that lead to an arrest at the meiotic prophase I (Martin-du-Pan and Campana, 1993; Lange et al., 1997), the pachytene stage being the most frequent arrest point in human spermatogenesis. This suggests that the DNA-damage sensing mechanism is conserved in the human. Anomalies induced by an antimitotic agent in the mouse also suggest a checkpoint at early pachytene in mammals (Tepperberg et al., 1997).

That the pachytene stage is a prominent commitment point for meiotic cell cycle progression is now demonstrated by germ line genetic targeting. The pachytene stage comprises several determinative chromosomal events involving a great number of genes. Depending on the gene disrupted, the arrested spermatocytes exhibited different subcellular anomalies. Null mutation in the *mlh1* gene (encoding a protein required for DNA mismatch repair) has led to a blockage of pachytene spermatocytes I (Baker et al., 1996; Edelmann et al., 1996). On a different genetic background, spermatocytes deficient in MLH1 arrested at metaphase I (Baker et al., 1996), suggesting a conserved tension-sensing checkpoint in the mouse (Page and Orr-Weaver, 1997). The *pms2* gene also encodes a protein required for mismatch repair. In *Pms2*$^{-/-}$ males (Baker et al., 1995), spermatocytes I exhibited various anomalies of synapsis at the stage of homologue searching. The ATM protein is involved in sensing double-stranded breaks in DNA and signaling cell-cycle arrest after this type of DNA damage. Spermatocytes in *Atm*$^{-/-}$ mice (Barlow et al., 1996; Elson et al., 1996; Xu et al., 1996) appeared to halt development between the zygotene and pachytene stages of meiosis prophase. ATM interacts with c-Abl. In *Abl*$^{-/-}$ mice, defects in meiotic progression beyond the pachytene stage were found (Kharbanda et al., 1998).

A synapsis checkpoint is suggested in mice with chromosome translocations leading to unsynapsed chromosomal regions. In such a case, spermatocytes do not trigger the synapsis checkpoint (Burgoyne and Mahadevaiah, 1998). Autosomal translocation and faulty chromosome synapsis have been obtained by transfer of foreign DNA in mice (Gordon et al., 1989). The *Dmc1* gene (a RecA-like gene) is required for homologous synapsis in mice (Pittmann et al., 1998; Yoshida et al., 1998). Null mutation in the *Dmc1* resulted in an impairment of synapsis, of the formation of the synaptonemal complexes and arrest at the early zygotene stage. Synaptonemal complex desynapsis requires HSP70-2, and desynapsis impairment in Hsp70-2$^{-/-}$ mice leads to apoptosis of mid-late pachytene spermatocyte (Dix et al., 1997; Zhu et al., 1997).

Human spermatogenesis arrest affects the primary



* double strand breaks

Fig. 1. Examples of human spermato-genesis arrest or derailment.

spermatocytes at the leptotene step (Fig. 2A) (see also next section). A similar germ cell henotype has not yet been found in other mammalian species. The arrested spermatocytes exhibit a dilated nucleus containing numerous thin chromatin strands, suggesting that the chromosomes have undergone replication (Holstein and Eckmann, 1986a). The maintenance of protein synthesis suggested a blockage restricted to nuclear event(s) and specific of the meiotic prophase (Escalier et al., 1992). The spermatocytes are arrested just before the formation of the "bouquet" characterizing the zygotene stage. This transient chromosome arrangement enables pairing and synapsis. It is preceded by movements of the centromeres at the preleptotene stage and accomplished at the zygotene stage by displacement of the telomeres along the nuclear envelope where telomeres cluster at one nuclear pole (Scherthan et al., 1996). Yeast strains with meiosis anomalies suggest that the telomere clustering promotes homolog pairing (Rockmill and Roeder, 1998). Proteins implicated in this process have been recently identified in yeast, particularly Taz1: in the absence of Taz1, the telomere clustering is disrupted. Taz1 is a telomere-binding protein containing a Myb-like motif similar to the two human telomere-binding proteins TRF1 and TRF2 (see discussion in Cooper et al., 1998; Nimmo et al., 1998).

Failures to exit from the metaphase of the first meiotic division are known in the human but with an unexpected germ cell phenotype: they can perform some or all the events of the spermiogenesis program (see their analysis in the next section). In one case, this leads to spermatids with a large nucleus that arrest at the round head step (Fig. 2B). In the other case, the spermiogenesis events occur normally, leading to spermatozoa with a large head (Escalier, 1983). This suggests that human spermatocyte division is controled by the tension-sensing checkpoint but that it can escape to the arrest. Similar mutants, whose genes are known, have been found in *Drosophila* (see next section).

Two examples of meiotic division failures have been obtained after germ line genetic engineering. First, null mutation in basigin (a member of the immunoglobulin superfamily possibly involved in cell-surface recognition) has led to germ cells arrested at the metaphase of the first meiotic division (Igakura et al., 1998). Second, overexpression of v-*Mos* in primary spermatocytes has led to arrest of spermatogenesis at the metaphase plate of the first meiotic division, the metaphase spindles bearing anomalies of α, ß and γ tubulins and of phosphoproteins (Rosenberg et al., 1995). In addition, the defects in the first meiotic division found in mouse sterile hybrids suggest alleles of Tcte2 (t complex testes expressed 2) as candidates for loci which contribute to meiotic drive (Braidotti and Barlow, 1997).

In addition to arrests related to the meiosis checkpoint control(s), male germ cells can stop differentiation during spermiogenesis. Human spermatids arrested at the early spermatid stage have been found in two brothers. Neither the acrosome nor the sperm tail initiates differentiation. An intriguing observation is the complete absence of the centrioles (Aumuller et al., 1987). CREM-deficient mice show that arrest at the round spermatid step can be due to blockage of nuclear protein synthesis and that CREM (a transcriptional activator) may be involved in the activation of protamine

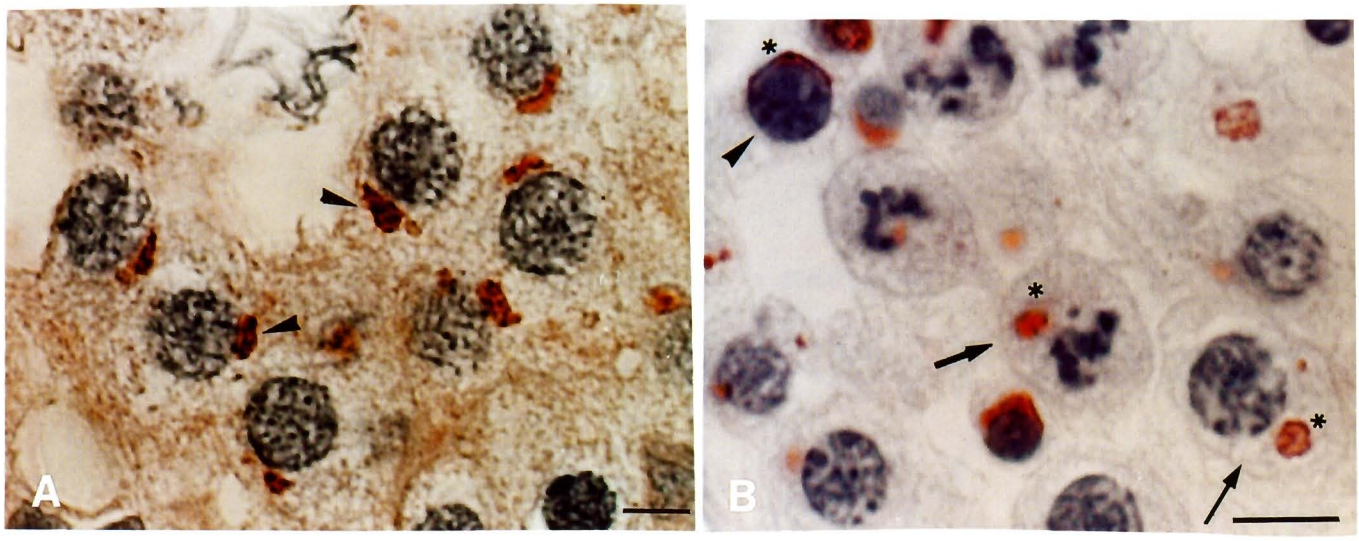


Fig. 2. Immunolabelling of human spermatogenesis pathological models. **A.** Human spermatogenesis arrested at the leptotene stage of primary spermatocytes. Unexpected labelling of proacrosin (arrowheads) (normally first expressed later, at the mid-pachytene stage) reveals heterochrony of the meiotic developmental program. **B.** Human spermatogenesis events decouplage leading to a pleiotropic phenotype. Failure of spermatocytes I (thin arrow) to perform the meiotic divisions (thick arrow) is bypassed, the spermiogenesis developmental program initiates leading to tetraploid spermatids (arrowhead). Labelling of proacrosin using Mab 4D4 monoclonal antibody (stars) from the spermatocyte I pachytene step (in the Golgi complex) to the spermatid step (in the acrosome). Bar: 10μm.

1 and 2, and transition proteins 1 and 2 (Blendy et al., 1996; Nantel et al., 1996).

Spermatogenesis events can be decoupled

Human binucleate spermatids constitute an example of ability to perform spermiogenesis events in spite of incomplete meiosis (see mutinucleated sperm cells section). Also human tetraploid spermatozoa (also named large headed) reveal that meiotic divisions are not an indispensable prerequisite for spermatid development. In one type, germ cells start the spermatid-specific program in spite of absence of meiotic divisions but do not undergo nuclear elongation and fail to form a flagellum (tetraploid round spermatid arrest; Fig. 2B). In another type, meiotic division failure appears permissive for complete differentiation of tetraploid spermatozoa. Several cellular anomalies can be observed throughout spermatogenesis in these cases, such as unipolar meiotic spindles, unilateral microtubular manchette, and in some cases of the second type, poorly elongated axoneme (Escalier, 1983, 1985). The early blockage of Golgi movements around the nucleus suggests that the Golgi complex partitioning during meiosis depends on the function of the microtubules (Escalier et al., 1991, 1992) (it is known that the Golgi partitioning during mitosis is dependent on the microtubules).

Therefore, these two types of human meiotic division failure exhibit a phenotype which can be used as landmarks for microtubule-related events at various steps of spermatogenesis. Moreover, they can be compared to the *Drosophila* B2tD mutation affecting a testis-specific ß2 tubulin subunit which causes disruption of microtubule function in all stages of spermatogenesis, beginning with meiosis (Kemphues et al., 1980, 1982; Hoyle et al., 1995). Other *Drosophila* mutants cannot enter meiosis divisions but continue to differentiate leading to tetraploid round spermatids with more or less further development (Page and Orr-Weaver, 1997). The *mgr* (merry-go-round) mutant exhibits tetraploid spermatids due to failure of both the nuclear and the cytoplasmic divisions. The chromosomes are arranged in a plate but no structure resembling a meiotic spindle could be found. Nevertheless, the first steps of spermatid morphogenesis take place although spermatid degenerate during the stage of elongation. In spermatids, flagellar basal bodies are either absent or unrecognizable (Gonzalez et al., 1988). Other mutations in *Drosophila* are characterized by a failure of both meiotic divisions and absence of spindle (reviewed in Maines and Wasserman, 1998). They fall into two classes. The *twine* locus class (*twine*, meiosis-specific cdc25 homologue, *Dmcdc2*, *boule* and *pelota*) comprises male mutants which are able to perform many aspects of postmeiotic differentiation. The Spermatocyte Arrest class comprises *meiotic I arrest (mia)*, *cannonball (can)*, *spermatocyte arrest (sa)* and *always early (aly)*, all with blockage of both the meiotic divisions and the postmeiotic differentiation. In *Dub* (Double or nothing), non-disjunction in meiosis I appears to be a consequence of precocious separation of the sister chromatids at the prometaphase I (Moore et al., 1994). In *ord*, the chromosomes cosegregate to the same pole resulting in nondisjunction of all chromosomes in meiosis I (Miyazaki and Orr-Weaver, 1992).

Decouplage of the spermatogenesis events can affect the post-meiotic events. Human round-headed spermatozoa reveals the existence of separate controls of many sperm head differentiation events. They are devoid of nuclear elongation and of head components such as the acrosome and the post-acrosomal cap (Kullander and Rausing, 1975; Nistal et al., 1978). All these defects are due to attachment failure of the head components, as seen by anomalies of the nucleoskeleton, the nuclear envelope and the cytoplasmic matrix (Escalier, 1990). Nevertheless, other spermiogenesis events occur normally: the chromatin condenses, the spermatid cytoplasm is eliminated, the acrosomal granule forms apart from the nucleus and undergoes its typical flattening before its elimination. An insertional mutation induced in the mouse has led to anomalies that confirm the role of the nuclear envelope in acrosomal morphogenesis and nuclear shaping (Russell et al., 1994). The analysis of mice with karyotypic anomalies has suggested that the Y-specific region of the Y chromosome long arm includes information essential for the normal development of the sperm head (Burgoyne et al., 1992).

In human round-headed spermatozoa, all the head components fail to be linked to the nucleus, except the flagellum which is normally attached to the nuclear basis by cytoplasmic linkages that can be seen in the implantation fossa. In that region, the nuclear envelope leaflets are normally apposed between them and to the chromatin, which is not the case in the other nuclear regions (Escalier, 1990). This sperm phenotype reveals that the head-tail attachment is a process distinct from attachment of the head structures to each other and suggests the existence of specific component(s) controlling the formation of tail-head linkages. This hypothesis is supported by the human case exhibiting separation of the sperm head from its tail due to failure to differentiate the implantation fossa (Perotti et al., 1981). The contribution of nuclear proteins in the head-tail attachment is suggested in mice expressing the galline protamine (Maleszewski et al., 1998). In this case, apparently normal spermatozoa are vulnerable to mechanical handling, as about 40% of heads and tails were separated after gentle pipetting. These anomalies should be distinguished from failure of the flagellum to dispose at the implantation fossa owing to an irregular nuclear outline, as may be the case for the spermatozoa from the ebouriffe (ebo) mouse mutation (Lalouette et al, 1996).

A meiotic prophase stop may lead to heterochrony of the developmental events. This is the case for human spermatocytes arrested at the leptotene stage (see preceding section). These large spermatocytes (megalo-

spermatocytes) exhibit no pairing of the chromosomes, no synaptonemal complex, and small nucleoli. Widened cisternae of the endoplasmic reticulum and of the perinuclear cisternae have suggested that they either produce more proteins or are unable to utilize synthesized proteins (Holstein and Eckmann, 1986a). Immunocytochemistry (Escalier et al., 1992) has revealed asynchrony of the protein synthesis; a protein which normally appears at the mid-pachytene stage is present in these "leptotene" spermatocytes (Fig. 2A). Normally, mRNA related to this protein also appears later at the mid-pachytene stage. This suggests that the nuclear events directly related to the meiotic processes are blocked, but not the transcriptional activity and the protein biogenesis. Other patients exhibit a similar spermatocyte nuclear arrest but the protein synthesis seems also to be blocked. The asynchrony of these developmental events has thus revealed the existence, for this meiotic arrest step, of two distinct human syndromes in spite of similar cytological characteristics.

Cascade of defects leads to pleiotropic sperm phenotype

The term pleiotropic is used in the sense that several organelles or several developmental stages of a germ cell are affected. The pathological syndrome is due to a cascade of consequences of the primary lesion, this latter acting on one or several structures. The resulting sperm phenotypes are spectacular due to the normally high organization of the mammalian spermatozoa.

Human syndromes exhibiting decouplage of spermatogenesis events (see preceding section) lead to pleiotropic sperm phenotypes. In the case of failure of meiotic divisions, the sperm nucleus is about four times increased in size and irregularly shaped, disturbing several spermiogenetic events: cytoplasmic elimination, acrosome morphogenesis and implantation of the flagella (Escalier, 1983, 1985). Similar spermiogenesis anomalies are found in *Drosophila* lacking meiotic divisions (Casal et al., 1990).

Another example is round-headed spermatozoa showing a defect in the cohesiveness of several sperm head structures (see preceding section). The factor involved in the formation of these spermatozoa is not yet identified. The observed absence of calicin (Escalier, 1990), a perinuclear protein related to the sperm-specific cytoskeleton, may be the cause of failure of linkage of the head components. Another possibility is that the calicin may be unable to keep aside the nucleus due to the absence of another factor (of the cytoplasmic matrix and/or the nuclear matrix) and is eliminated with the cytoplasm. Nevertheless, the lack of linkages leads to a cascade of sperm head anomalies, particularly a failure of nuclear elongation, leakage of the acrosome and absence of the post-acrosomal sheath. A mouse insertional mutation inducing a spermatid nuclear envelope anomaly also has led to a cascade of defects. In that case, spermatids present anomalies of the acrosome spreading over the nucleus, of the nuclear envelope and of nuclear elongation due to manchette anomalies. It has been suggested that modification of the nuclear envelope may be the primary event that leads to the other anomalies of morphogenesis of the spermatid head structures (Russell et al., 1994). A distinct phenotype is spontaneous acrosomeless mouse spermatozoa without evident nuclear differentiation defects (Sotomayor and Handel, 1986; Fouquet et al., 1992).

Human sperm commonly contains abnormally-shaped spermatozoa which are not related to a genetic cause. This phenomenon requires quantitative determinations of sperm anomalies. Nevertheless, when an anomaly is present in a relatively high incidence but is not representative of an anomaly known in familial cases, a more or less penetrating genetic cause due to non-germ cell factors cannot be determined.

Abnormal phenotypes reveal morphogenetic relationships between sperm structures

Cascades of defects leading to pleiotropic germ cell phenotypes (see preceding section) can be due to morphogenetic relationships between structures. In cases of failure of the meiotic divisions in man, the irregulary-shaped sperm head impairs implantation of some of the multiple flagella and the cytoplasmic elimination. In cases of round-headed spermatozoa, anomalies of the linkages impairs the nuclear elongation, the acrosome anchorage and the formation of membrane-associated structures.

The highly organized sperm flagellar structures and the various flagellar disturbances encountered in the human have led to demonstration of morphogenetic relationships between sperm structures. A classification of human flagellar dysmorphies has been proposed (Escalier and David, 1984) and these phenotypes have been compared to those of *Drosophila* sterile mutants and to anomalies induced by microtubular inhibitors (Escalier, 1985). Human pathological models have allowed the definition of morphogenetic factors that must control mammalian flagellar morphogenesis; the molecular organization of axonemal microtubules should control the differential arrangement of the peri-axonemal structures (Escalier and Serres, 1985). A similar hypothesis is now postulated from mice homozygous for *Hst-6* at the *t* complex region of chromosome 17 (Phillips et al., 1993). Other pathological human flagella have suggested that absence of dynein arms or of microtubule linkages may be due to anomalies of the molecular organization of the microtubules (Escalier, 1985). The same conclusion is made from mutants in *Chlamydomonas* suggesting that axonemal components, in addition to tubulin, direct dynein arms to their proper location (Wilkerson et al., 1995). The finding of a differential distribution of tubulins along the human axoneme supports these notions (Gallo et al., 1986). Other human flagellar pathological models reveal morphogenetic relationships between a fibrous sheath protein and the flagellar actin (Escalier et al., 1997). A

high incidence of simultaneous absence of axonemal doublets and dense fibers can be found in spermatozoa from infertile men (Escalier and David, 1984). A similar anomaly has been observed following an insertional mutation in a transgenic line overexpressing the human epidermal growth factor receptor (EGF receptor). An average of 71% sperm were missing axonemal outer doublets resulting in a 5+2 pattern in the middle piece. Interestingly, this anomaly was observed in the vas deferens and the epididymis but not in the testis, suggesting extensive microtubule disruption and/or disassembly after spermiation. It was proposed that the transgene was integrated into an endogenous gene which could encode a highly expressed, haploid-specific axonemal protein or, alternatively, a protein that indirectly dictates axonemal stability (Merlino et al., 1991). Also, spermatids of *Drosophila* sharing mutations of the testis-specific centrosomin isoform (Li et al., 1998) can be compared to this mammalian flagellar phenotype (see section: germ cells with a normal phenotype can share an anomaly of a gene involved in spermatogenesis). A human flagellar abnormal phenotype is characterized by accumulation of unassembled flagellar components which impairs elimination of the spermatid flagellar cytoplasm (Escalier, 1985) and can be compared to the anomaly induced in mice with an insertional mutation following genetic engineering with a tyrosinase minigene. The flagellum fails to develop normally and appears as a round process containing disorganized microtubules and crystalline tubulin (Russell et al., 1994).

Multinucleated sperm cells

Human binucleate spermatids (Matano, 1971) constitute an example of occurrence of spermiogenesis events in spite of incomplete meiosis. In the mouse, this sperm phenotype is characterized by two haploid nuclei separately packaged within the sperm head (Burkhart and Malling, 1989). However, the mechanism resulting in this phenotype remains unknown. A possiblity may be that the chromosome segregation is normal owing to functional microtubules of the spindles. In contrast, cytokinesis may be blocked as suggested by the characteristics of the acrosome (single and enlarged). This phenotype is distinct from the diploid spermatids in XOSxr mice which should be due to impairment of meiotic chromosome pairing leading to a failure to reach metaphase II (Burgoyne and Baker, 1984).

Sperm polyploidy with a normal chromosome segregation but a failure of cytokinesis is known in *Drosophila* for the mutations *diaphanous (dia)* and *peanut (pnut.)* (reviewed in Maines and Wasserman, 1998). Absence of cleavage furrows leads to spermatids containing two or four nuclei of normal size. Diaphanous and peanut (septin protein family) localize to the contractile ring and intercellular bridge of dividing cells. Human *DIA* and *Drosophila* dia have 39.3% identical and 66.2% conserved residues. A testis-specific form of *DIA* has been detected, but men with anomalies of spermatogenesis have not yet been investigated for a possible mutation in *DIA*. Disruption of *DIA* has been found in a woman with premature ovarian failure, suggesting that *DIA* may affect cytokinesis of the follicular cells (Bione et al., 1998). However, this disruption of *DIA* was due to a balanced X;12 translocation and it remains to be ascertained that another gene critical for female reproduction was not disrupted. Another *Drosophila* mutation leads to spermatids with two equally-sized nuclei and is related to an anomaly of the contractile ring of the division apparatus. This mutation concerns the gene for the kinesin-like-protein-at-3A (KLP3A) which normally is highly concentrated in the midbody and males carrying a null mutation in KLP3A are defective late in anaphase of meiosis I, the midbody being absent (reviewed in Maines and Wasserman, 1998).

Polyploidy can be due to defects of the intercellular bridges, as documented in the case of human symplasm containing more than two spermatid nuclei (Holstein and Eckmann, 1986b). In mice, symplastic spermatid is a recessive insertional mutation affecting an unidentified gene on chromosome 14 (MacGregor et al., 1990). Spermatids from homozygous *(sys/sys)* males form multinucleated syncytia (symplasts) and do not complete maturation leading to azoospermia. Symplasts result from premature opening of the intercellular bridges that connect round spermatids. It is noteworthy that spermatids in symplasts (Figure 6B in Russell et al., 1991) have initiated nuclear elongation and formed an acrosome while chromatin condensation and flagellar growth did not occur.

In conclusion, several human spermatogenesis failures reveal that, as in *Drosophila* (Hackstein, 1991), mammal spermatogenesis is a concerted, but not interdependent, execution of separate developmental programs for the particular components of the male germ cells. This is well illustrated by the different types of meiotic division failures encountered in the human which lead to the conclusions already made in *Drosophila*. Indeed in flies, male mutants have revealed that the meiotic divisions constitute a program that is in large measure independent of the developmental events that either precede or follow MI and MII. Similarly, the initial phase of the program for spermatid differentiation is unaffected by mutations that disrupt chromosome segregation or that block the meiotic divisions altogether (Maines and Wasserman, 1998).

From genotype to phenotype: germ cells with a normal phenotype can share an anomally of a gene involved in spermatogenesis

A genetic origin of an abnormal germ cell phenotype is suspected when all the spermatozoa of an individual share a homogeneous phenotype and when this phenotype has familial occurrence. It is the case for tetraploid spermatozoa (umpublished data), round-

headed spermatozoa (Kullander and Rausing, 1975; Nistal et al., 1978) and some flagellar dysmorphies (Alexandre et al., 1975; Bisson et al., 1979). However, spermatogenesis disturbances can lead to different destinies of germ cells, some of them escaping to blockage. Consequently, spermatozoa with an apparently normal phenotype can be found in semen containing a substantial number of spermatozoa with a phenotype characteristic of a genetic disorder. This may be the case of semen that contain spermatozoa with either a diploid or a haploid chromosome number (Yurov et al., 1996) or spermatozoa with various head sizes and flagellar numbers (Pieters et al., 1998). This leads to questions as to whether the fraction of spermatozoa with an apparently normal phenotype share, or not, the genetic defect. The possible genetic origin of a heterogeneous phenotype is revealed in the case of infertile brothers with the typical syndrome of round-headed spermatozoa whose father showed only 30% of abnormal sperm heads (Flörke-Gerloff et al., 1984).

The question of heterogeneous phenotypes has been enlightened by *Drosophila* male steriles. They show that mutation of a given gene can lead to heterogeneous anomalies even in the homozygous state. Weaker classes of *pelota* mutations (see section: spermatogenesis events can be decoupled) lead to cells with either the *pelota* phenotype and or a wild type (Eberhart and Wasserman, 1995). The mutation B2tD (affecting a gene of the testis-specific ß2-tubulin locus) causes disruption of microtubule function to an extent depending on the dosage of the B2tD mutation (Kemphues et al., 1980).

More mysterious is the case of more heterogeneous sperm phenotypes due to the presence of different abnormal phenotypes in a single subject. This is found in men who have spermatozoa lacking various subsets of flagellar microtubules: one or two central microtubules associated, or not, with an absence of doublets (Escalier and David, 1984). *Drosophila* mutants for the centrosomin have demonstrated that these fully heterogeneous phenotypes can have a genetic origin. The testis-specific centrosomin isoform normally associates with the centrosomes of the spermatocytes and the basal body of the spermatids. The presence of several nuclei of different sizes due to anomalies of the meiotic spindles (monopolar or multipolar) is observed in the mutants, indicating disturbed cytokinesis and karyokinesis. Despite the meiotic defects, aberrant spermatids undergo morphogenesis but their flagellar axoneme is affected as follows: 40% of the axoneme have no central pair, 20% have only one central microtubule and very occasional axonemes lack some outer microtubules (Li et al., 1998).

On the contrary, an apparently normal sperm can hide a genetic defect in germ cells. Most of the gene disruptions that lead to sterility in homozygous male mice preserve fertility in heterozygous males (Escalier, 1999). This phenomenon can be easily explained considering that germ cells develop within a common syncytium allowing circulation of gene products (mRNA and proteins) through intercellular bridges. This fact has been demonstrated for a transgene expressed post-meiotically (Braun et al., 1989). It is noteworthy that this implies that nonfunctional alleles carried by heterozygous individuals could be transmitted to their offspring (see discussion in Hagaman et al., 1998). In addition, the mode of transmission of a genetic defect is complex: the penetrance may depend on some factor(s) and a chromosomal microdeletion may concern more or fewer genes. Yet a fertile man with a Y chromosome deletion (comprising *DAZ*/SPGY) has been described; his son, who inherited the Y microdeletion, was azoospermic (reviewed in Cooke et al., 1998).

Germ cell phenotypes and gene homologies

Genes important in regulating meiosis in eukaryotes might have evolutionarily-conserved counterparts in higher organisms (Wolgemuth et al., 1995). This fact is fully demonstrated concerning yeast mutants that are the source of identification of genes involved in meiotic recombination in mammals (Roeder, 1995). This must also be the case for cell cycle determinative events of male germ cells. Yet a murine homologue of the cdc-like *Aspergillus* Ser/Thr protein kinase has been isolated and found to be highly expressed during the meiotic prophase I (Rhee and Wolgemuth, 1997). New cell cycle effectors of the cdc-cyclin complex should be identified from *Drosophila* mutants with G2/M transition anomalies (Lin et al., 1996). Their identification should lead to the finding of homologous genes between *Drosophila* and the human, and should provide biological probes to investigate meiotic events and thus contribute identifying relationships between the genome and the resulting phenotype.

However, recent data on homologous genes between *Drosophila* and mammals lead us to ponder the comparisons between species. The mammalian *Daz* (Deleted in AZoospermia) genes share homology with the *boule* gene in *Drosophila* (Seboun et al., 1997). Like *Daz*, the *boule* gene contains an RNA binding motif (42% aa identity). A second region of *Daz* shows 33% aa identity with *boule*. Nevertheless, the expression patterns appear different between species. The human *Daz* gene product is found in the cytoplasm of late spermatids and in the tail of mature spermatozoa (Habermann et al., 1998). In contrast, the homologous autosomal mouse *Dazla* is expressed before germ cell sex differentiation in male and female embryonic gonads (Seligman and Page, 1998), in spermatogonia before and after puberty (Reijo et al., 1996) and in the cytoplasm of preleptotene and zygotene spermatocytes (Ruggiu et al., 1997). Disruption of the mouse *Dazla* gene (Ruggiu et al., 1997) has led to loss of germ cells in both males and females. Testes from homozygous mice at 9 weeks showed an almost complete absence of germ cells. By contrast, *Drosophila* male mutants for the *boule* gene are sterile due to a blockage of meiotic divisions although spermiogenesis can lead to aberrant spermatids (see discussion in Lin et al., 1996). In addition, disruption of

the *Dazla* gene affects female mice which are infertile owing to a complete absence of follicles and ova (Ruggiu et al., 1997) while female *Drosophila* mutants for the *boule* gene are fertile (Eberhart et al., 1996). These data suggest that these homologous genes can either have different functions or that their inactivation can have markedly different phenotypes when placed on different genetic backgrounds (Erickson, 1996).

Even with genes such as *RBM/Rbm* (RNA-binding motif), that are present on the Y chromosome of a wide range of mammals, there may be substantial differences in function betwen species (Mahadevaiah et al., 1998). In man, *RBM* is expressed in spermatogonia, spermatocytes and round spermatids. Deletions removing functional copies of *RBM* lead to azoospermia due to spermatocyte arrest in man (Elliott et al., 1997). In the mouse, *RBM* is expressed in spermatogonia and elongating spermatids, and mouse males with a marked reduction in *RBM* expression (absent in spermatogonia and residual in spermatids) are fertile but exhibit 32% of spermatozoa with distorted heads (Mahadevaiah et al., 1998). In addition, *RBM* shows sequence homology (76% similarity and 60% identity at the amino acid level) to the heterogeneous ribonucleoproein (hnRNP)G. However, *RBM* colocalizes with pre-mRNA splicing components but not with hnRNP proteins suggesting that, despite its sequence similarity to hnRNPG, *RBM* is likely to have a different function (Elliott et al., 1998). Targeted mutagenesis of the Y chromosomal genes in the mouse should not provide pointers to the function of human homologues due to their different expression patterns between species.

Other disparities between species in the function of a given gene involved in spermatogenesis have already been noted (Maines and Wasserman, 1998). The cdc25 and cdc2 activities are thought to be necessary for chromosome condensation at the G2/M transition in yeast and frogs, but not in *Drosophila* male meiosis. In *Drosophila pelota* mutants, the meiotic divisions are blocked, but in *S. cerevisiae*, mutation of the *pelota* homolog dom34 accelerates the meiotic divisions.

The behaviour of the products of homologous genes has been considered regarding biological structures (Bolker and Raff, 1996) and it appears that homologous genes may be involved in disparate and non-homologous developmental processes, and expressed in non-homologous structures. One of the mechanisms is the co-option of conserved regulatory genes for new developmental functions as a general feature of evolution. In such a case, co-opted genes remain homologous to each other, as genes, but the structures in which they are expressed are not necessarily homologous at higher levels (Bolker and Raff, 1996).

Another example is the homology between rodents of proteins expressed during the prophase of male meiosis. SCP3 and COR1 (reviewed in Heyting, 1996) are designed as homologs to the Xlr (X-chromosome linked-lymphocyte regulated) and thus to Xmr (94% Xlr homology). Sequence analysis of SCP3, COR1 and Xlr shows that homology concerns the coiled-coil region. However, Xmr (Calenda et al., 1994), SCP3 (Yuan et al., 1998) and COR1 (Dobson et al., 1994) did not support comparison when their expression timing and their nuclear localization was considered. SCP3 and COR1 are proteins located on the synaptonemal complexes (SCs). Xmr is expressed in the whole autosomal compartment before the formation of SCs, then in the XY body. In fact, these proteins share homology with intermediate filament proteins, such as lamins and cytokeratins owing to a similar coiled-coil region. Proteins with homology limited to a domain are considered to constitute protein superfamilies. Besides widespread functional relationships, their non-homolog domains should be important for a specific function. Moreover, their activity may be specified through interactions with other proteins.

Future directions

The demand for assisted human reproduction strengthens the need to identify gene(s) involved in the numerous failures of spermatogenesis known in the human. However, in no case of morphological and/or functional sperm defect has the molecular nature of the defect been demonstrated at the DNA level in the human (Nayernia et al., 1996).

This review has shown that well-defined human spermatogenesis failures can be compared to some failures in *Drosophila* mutants and knockout mice. From this comparison, some genes can be retained as possibly involved in these types of infertility in man. Based on gene homologies as target candidate genes, searches by genetic linkage for mutations in these men would allow the identification of genes involved in human male reproduction. However, data in the literature show that differences in expression of homologous proteins between species could be more extensive than expected, including proteins involved in ancestral events such as the meiotic machinery.

Other genetic engineering strategies (se also in Escalier, 1999) could emerge with the availability of germ cell lines. They should allow transfection of genes (or their products) in mammal germ cells and will be the source of important and direct information on factors involved in spermatogenesis. Yet the study of the effect of drugs on germ cells in culture has demonstrated the role of proteins involved in chromatin metabolism during male mammal meiosis (Cobb et al., 1997). An increasingly comprehensive view of spermatogenesis could also result from the development of new fields in cell biology technology. Among them, germ cell markers of proteins, mRNAs and chromosomes, allow investigations on the developmental program of spermatogenesis for an interpretative analysis of mutant phenotypes.

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