Y chromosome and male infertility

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Recent genome analysis of the Y chromosome has increased the number of genes found on this chromosome markedly. Many of these genes in the part of the Y chromosome that does not undergo recombination with the X chromosome are members of gene families. Evolutionary considerations imply that genes on the Y chromosome will degenerate unless they have male advantageous or female deleterious functions. Spermatogenesis is an example of a male advantageous function and genes in three regions of the human Y chromosome have been promoted as candidate male fertility factors.

Long considered a genetic wasteland, the mammalian Y chromosome is now thought to harbour a number of genes, including several implicated in male fertility. Strong circumstantial evidence supports this view but formal proof of the action of any gene in spermatogenesis is as yet lacking because of the genomic complexities of this part of the human genome.

The Y chromosome in mammals carries the gene that switches the development of the indifferent gonad from the default female pathway to the male pathway and results in the switches the development of the indifferent gonad from the de-generate manner. For evolutionary reasons, the Y chromosome had been thought to be a pseudoautosomal region. Isolation resulted in the loss of DNA that encodes a ubiquitin-activating enzyme with an advantageous function and genes in three regions of the human Y chromosome have been promoted as candidate spermatogenesis genes (Mitchell et al., 1998).

Mutant forms of the mouse Y chromosome have arisen that provide deletion intervals of the short arm of the Y chromosome (Fig. 1). The Sxra region of the mouse Y chromosome contains genes that are sufficient for male sex determination and that can support spermatogenesis up to the late stages of spermiogenesis (Burgoyne et al., 1992). A naturally occurring deletion within the Sxra region between the two copies of Zfy removes a gene or genes required for spermatogenesis. A number of genes are known to lie in this interval, for example Uby which encodes a ubiquitin-activating enzyme with a function in spermatogenesis.

Y chromosome genes in humans and mice

In meiosis in humans and mice, an obligate recombination occurs between the X and Y chromosomes in a region of sequence exchange, and hence identity, between these chromosomes, the pseudoautosomal region (Rappold, 1993). In humans, this region is close to the sex-determining locus SRY but, in mouse, it is on the other arm of the chromosome. Multiple genes have been mapped to this region in humans but are outside the scope of this review since they are shared by females and none are thought to be involved in spermatogenesis. In both humans and mice, there are a number of genes present as homologous copies on both the X and the Y chromosomes and some of these remain candidate spermatogenesis genes (Mitchell et al., 1998).

Mice can be manipulated genetically in ways that are not currently possible for other mammals and it is, in principle,
possible to reconstitute the genic components of various deletions to test which genes are required for spermatogenesis. This is an area being investigated actively but which has yet to provide answers. In some aspects, the human Y chromosome is even more problematic because of its repetitive nature. However, a combination of clinical resources and concerted efforts on the genomic mapping of this chromosome have complemented the mouse genetic approaches.

Ma et al. (1992) located several regions of the human Y chromosome that were expected to contain a gene or genes required for spermatogenesis by assembling a set of patients with impaired fertility. Positional cloning strategies have revealed two gene families within these regions. The first to be identified was the RBM gene family, the members of which encode a protein with RNA-binding properties (Ma et al., 1993). Refinement of the deletion intervals has allowed the expressed copies of this gene to be mapped to the AZFb region (Fig. 1) (Elliott et al., 1997), although there are other copies in other parts of the Y chromosome (Prosser et al., 1996) and the protein is closely related to an autosomally encoded protein, hnRNPg (Delbridge et al., 1998). The RBM genes are found on the Y chromosomes of all mammals tested, including the marsupials. In mice, the gene is again present as a multicopy family and is found between the centromere and the Tdy gene on the short arm of the chromosome (Laval et al., 1995).

When the AZFb region is deleted and RBMP is missing from the tests, germ cells show a pachytene arrest (Elliott et al., 1997). As with all large deletions, it is currently impossible to exclude the possibility that genes other than the candidate are also within the deletion and are, in fact, the critical genes. This caveat also applies to the second gene family isolated by positional cloning, the DAZ gene family (Reijo et al., 1995). These genes map to the AZFc region of the human Y chromosome and, again, encode a protein with a putative RNA-binding capacity. This gene family is found on the Y chromosome only in old world monkeys and great apes (Shan et al., 1996). All other mammals tested (including old world monkeys and apes) have a single copy of an autosomal gene DAZL and

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**Fig. 1.** A comparison of the mouse and human Y chromosomes and their genes. Genes that are the same colour are homologues. (Redrawn from Vogt et al., 1997).
homologues have been found in Drosophila (Eberhart et al., 1996) and Xenopus (Houston et al., 1998). P-element mutagenesis of Boule, the Dazl homologue, results in male sterility with a block in the first meiotic division.

RBM and DAZ provide strong candidate genes for involvement in infertility caused by micro-deletions of the Y chromosome in two of the three regions previously defined. In the AZFa region, a number of genes have been mapped (Fig. 1) and deletion break points have been located within one gene, DFFRY, which encodes a de-ubiquitinating enzyme, but the extent of the deletion remains unknown (Brown et al., 1998). Again, this makes it impossible to exclude the involvement of other genes.

A cDNA selection-based search for genes has revealed twelve new Y chromosome genes or gene families, of which seven have no X chromosome homologue (Lahn and Page, 1997). This finding strengthens the correlation previously established by RBM and DAZ that genes found only on the Y chromosome have a preferential expression in the testis, and those with X chromosome homologies tend to be expressed more widely. The function of these genes and their significance for spermatogenesis remains unknown but, given their expression patterns, they represent strong candidates for genes with functions important for spermatogenesis. Many of these genes are members of gene families rather than single copy genes.

Y chromosome gene function in spermatogenesis

Relatively little is known about the function of the Y chromosome genes implicated in spermatogenesis. Although genes such as Ube1Y1 and Dffry can be implicated in proteolysis by homology, their targets are unknown. This is similar to the position for the RNA-binding proteins RBM and DAZ, but in these cases a little more is known. Antibody studies show that RBMp is expressed only in the germ cell component of the testis from fetal stages of testis development onwards. In adult mouse testis, it is predominantly found in premeiotic cell types with a decreased concentration in spermatid stages. As a nuclear RNA-binding protein, RBMp could be involved in RNA packaging, transport or localization, but immunocytochemical data suggest that it may be involved in modulating the splicing of pre-mRNA molecules. This hypothesis is based on the observation that, at specific stages of spermatogenesis, RBMp is co-localized with a number of known splicing factors (Elliott et al., 1998).

Alternative splicing is a potentially powerful mechanism for regulating gene expression and developmental pathways, for example, Drosophila sex determination is based on alternative splice site choices (Hager et al., 1997).

Although Dazlp is found only in the germ cells of both the ovary and testis, its function must be completely different in these organs, since it is excluded from the nucleus and occurs in a particulate distribution in the cytoplasm (Ruggiu et al., 1997). The Dazl gene may have a role in the positioning of RNA, in stabilizing RNA by protecting it from nucleases or in translational control by sequestering the RNA from the translation machinery. As is the case for RBMp, the binding targets are unknown but, because of the absence of the gene from the Y chromosome of mice and its representation by a single autosomal locus, it has been possible to test the consequences of the deletion of Dazlp.

Both male and female mice without functional Dazl genes are infertile (Ruggiu et al., 1997). This is in contrast to the situation in Drosophila, where only male fertility is affected. The gonads of adult mice Dazl−/−Dazl− are devoid of germ cells and, as a result, are minute. Consistent with the expression pattern of the gene, no other gross effects are apparent elsewhere in the animals. Since the mouse provides an accessible source of material, it has been possible to look at the expression of Dazl during development, when it is expressed in the gonia from at least 11 days after mating. The effects of the knockout become histologically apparent by 19 days after mating, a time at which female germ cells have entered meiosis and have arrested but when male germ cells are quiescent but still mitotic. Again, this is in contrast to the situation in Drosophila, where mutations in the homologue Boule have effects apparent in meiosis.

Evolution of genes and functions on the Y chromosome

The RBM and DAZL genes provide interesting time points in what seems to be one common mode of evolution of genes on the Y chromosome: the arrival of genes on the non-recombining part (NRY) from an autosome. In the case of the RBM genes, this must have predated the divergence between the eutherian and metatherian mammals (Delbridge et al., 1997) some 130 × 10⁶ years ago. It has survived in various copy numbers on the Y chromosome of all mammals tested, which implies that it must have rapidly acquired and maintained a function or expression pattern distinct from the hnRNP-G from which it most probably originated.

DAZ has probably followed a similar route to the great ape and old world Y chromosomes but much more recently (approximately 30 × 10⁶ years ago) from the DAZL gene found...
in species as far apart as *Drosophila* and *Xenopus*. The relatively recent divergence of these genes may not yet have resulted in completely distinct functions and so partial complementation may occur, confusing the interpretation of genotype–phenotype correlations in patients with *DAZ* deletions (see below).

The other common mode of evolution for genes on the Y chromosome is descent from genes present on the original homologous pair that gave rise to the sex chromosomes. Genes taking this route have been mentioned above, the best studied of which is *Ube1Y*.

However, it is likely that, once genes have arrived on the Y chromosome, they are subject to the pressures of Muller’s ratchet (Muller, 1964). This process applies to genes in non-recombining regions of the genome and implies that mutations cannot be repaired by intergenic recombination (Fig. 2a) and, therefore, must lead inexorably to loss of function. Muller’s ratchet may also provide a clue to another frequent occurrence on the Y chromosome: repetition. On the human Y chromosome, *RBM, DAZ, Ube1Y* and *TSPY* are all multi-copy gene families rather than single genes, unlike their autosomal ancestors (where these are known) (Vogt et al., 1997). The newly described genes also have more than their share of gene families. In the case of *RBM* and *DAZ*, the genes are themselves internally repetitive with an intron–exon unit amplified a variable number of times. *TSPY* in humans is present in approximately 30 copies, in rats in approximately three copies and, in mice, it is represented by a non-functional gene. *Ube1Y* is present in about six copies on the mouse Y chromosome but is absent from two branches of the primate lineage, suggesting two independent loss events. How can this be linked to Muller’s Ratchet? One possibility is that intragenic, unequal sister chromatid exchange (Fig. 2b) provides a means of reconstituting a functional gene from two mutation-damaged copies and that the outcome of this would be selection for gene duplication on the Y chromosome. Single copy genes in the NRY would become non-functional and ultimately lost. *Ube1Y* in primates and *TSPY* in mice are examples of this (Fig. 3).

A consequence of the impact of Muller’s ratchet on the Y chromosome is that most genes will be relatively transient inhabitants. Only those such as *Sry*, the sex determining gene, for which selective pressure is presumably intense, will be found on the Y chromosomes of a majority of species. Other genes that are more transient may also have functions less critical to spermatogenesis, or not critical at all, as they can be substituted by autosomal or X-linked copies.

**Clinical aspects of Y chromosome genes**

Many laboratories have reported micro-deletions of the Y chromosome (Barbaux et al., 1995; Nakahori et al., 1996; Quereshi et al., 1996; Stuppa et al., 1996; Yen et al., 1996; Bhasin et al., 1997; Kremer et al., 1997; Simoni et al., 1997; Vereb et al., 1997; Vogt et al., 1997) but the frequency reported varies substantially.

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**Fig. 3.** The probable patterns of loss and gain of three genes, *DAZ* (black), *Ube1Y* (red) and *RBM* (blue) from the Y chromosome during mammalian evolution. The presence of the gene on the Y chromosome is indicated by a line of the appropriate colour. Note that *DAZ* only appears in the human–chimp and rhesus–macaque lineages.
Foresta and co-workers (1997) have reported the greatest proportion of azoospermic men with micro-deletions of the Y chromosome (37%). The lower amounts of deletion found are difficult to estimate given the bias against publication but the average deletion frequency in azoospermic males is probably 5–10%. The region of the Y chromosome most commonly deleted is the AZFc region, but other deletions may cumulatively account for as many or more deleted chromosomes. There are a number of problems in interpreting these findings.

First, the phenotype of individuals with apparently the same deletion is variable. In the case of DAZ deletions, cases are known where deletions have been transmitted from father to son and, in general, the precise type of spermatogenic arrest is variable. This is also true for deletions in other regions of the Y chromosome, such as those involving RBM. The implication is that the penetrance of the mutation is affected by genetic or environmental background. Second, the genes known to lie within the deleted regions remain merely candidate genes; no mutations have been found in infertile patients and it remains possible that other genes are present within these intervals. Third, the frequency of these deletions is relatively high and, given their association with infertility, they must most commonly occur de novo. This raises the possibility that germ-line mosaicism or deletions occurring during spermatogenesis result in apparently normal fathers having sons with deleted Y chromosomes.

It is not known what other variables are involved with the repeated genes on the Y chromosome. Copy number may vary either from individual to individual or from population to population, and an effect of paternal age on the frequency of deletions has been postulated.

Conclusion

The past few years has seen an explosion in our knowledge of genes on the Y chromosome in men, driven by a combination of the availability of large collections of clinical samples and by the advances in genome analysis technology. It is now clear that deletions of the Y chromosome are not infrequent in sub-fertile or infertile men and some genes have been implicated as contained within these deletions. Despite these findings, little is known about the precise significance of individual deletions in terms of the fertility of an individual patient, and only in the case of Dazl and Rbm do we have information about the sub-cellular location of the protein that points towards possible functions.

In the overall context of infertility, the Y chromosome needs to be put into a wider perspective. The Y chromosome contains < 1% of the genome, and any mutations affecting fertility that it carries will be lost rapidly from the population, as this is a haploid region of the genome. Even though the small size and vulnerability to mutation are likely to be counterbalanced, at least in part, by evolutionary pressures for genes involved in spermatogenesis to relocate to the Y chromosome, the majority of genes affecting male fertility will be autosomal and mutations in them will be recessive in effect. The apparent focus on the Y chromosome is in effect ascertainment bias, as genes with effects on fertility elsewhere in the genome are difficult to identify using current methods.

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Key references are indicated by asterisks.


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