

## 2 Formulation of the problem

Little is known about the genetic mechanisms that govern the processes of spermatogonial stem cell renewal, germ cell differentiation during spermatogenesis or fertilization. Thus far, only few of the genes expressed in germ cells have been identified and characterized, and thus a large number of genes and gene functions remain unknown. Consequently, we do not understand how a male germ cell develops into a mature spermatozoon that is then capable of fertilizing an egg. The advent of DNA microarrays has resulted in a capability to simultaneously analyze gene expression profiles of thousands of genes, thereby providing an ideal tool to define the mRNA expression profiles of developing male germ cells. The identification of all genes that are activated or repressed at each stage of germ cell development would allow us to better understand the complex processes involved in spermatogenesis.

Comprehensive gene expression profiles, in combination with available expression data for other tissues, will allow us to identify genes expressed specifically in post-meiotic male germ cells. A large number of these genes probably play critical roles in fertilization, and some of them may be critical for fertility and thus become targets for male contraceptive development.

A better understanding of the molecular mechanisms of spermatogonial stem cell renewal and spermatogenesis could also lead to the establishment of advanced germ cell culturing methods. Development of technology to expand spermatogonial stem cells in culture would open the door to gene disruption in animals other than the mouse given embryonic stem (ES) cell technology used for gene disruption has so far only been applicable to the mouse. The laboratory rat represents one of the most comprehensively studied mammals, and many physiological studies that are easily accomplished in the rat are very difficult and expensive in the mouse. Direct germ-line transmission, by virtue of the introduction of genetically modified sperm cells, could represent a successful means by which to disrupt genes in the rat and other animals. This would represent a significant advance in biology and medicine. An ability to genetically manipulate spermatogonial stem cells in culture could also lead to methods to correct male infertility and provide a means to rapidly screen for germ cell-directed contraceptives.

The initial aims of the project were:

- Generation and evaluation of mouse testis-specific cDNA microarrays
- Gene expression profiling of the mouse testis at different time points during post-natal development
- Gene expression profiling of mouse and rat germ cells cultured under different conditions

These experiments were expected to yield the most complex description of genes involved in spermatogenesis generated in any lab. By mining the data, the goals of this study were:

- Identification of genes responsible for spermatogonial stem cell renewal and differentiation
- Comparison of genes enriched in spermatogonial stem cells to those enriched in other stem cells
- Identification of genes expressed specifically in post-meiotic germ cells to find key players in spermiogenesis and genes potentially involved in fertilization