7 Summary

Knowledge of mammalian spermatogenesis and the events surrounding fertilization has advanced slowly due in part to uncertainty about the number and identity of the cellular components involved. Some of the genes responsible for differentiation have been characterized, but a greater number of genes have not yet been identified. Critical to our understanding the processes of stem cell renewal and spermatogenesis is an initial description of those genes that are activated or repressed at each stage of germ cell development. Such a description would greatly facilitate the establishment of an *in vitro* culture system that would allow manipulation of spermatogonial stem cells. Targeted gene disruption is so far only possible in the mouse, because of the inability to culture pluripotent embryonic stem cells in other species. Genetically modified sperm cells could allow gene disruption in the rat and other animals. Generating a gene expression profile of developing germ cells would also lead to the identification of gene products that may play a critical role in fertilization. Determination of genes expressed by germ cells during spermatogenesis, their expression patterns during differentiation *in vivo* and *in vitro*, and their cell type specificity should provide an inclusive list of probable critical proteins.

Here, DNA microarrays were used to identify genes potentially involved in the different phases of spermatogenesis. DNA microarrays allow the reliable analysis of the expression levels of tens of thousands of genes simultaneously and therefore are an excellent tool to comprehensively identify and study the genes involved in spermatogenesis. The initial approach of generating mouse testis-specific cDNA microarrays yielded promising preliminary results. However, when compared to the Affymetrix gene expression platform, these custom-made microarrays turned out to be less reliable, less sensitive, less efficient, and overall more cost-intensive. Through the use of Affymetrix oligonucleotide microarrays, we analyzed mouse and rat gene expression during germ cell differentiation *in vivo* and *in vitro*. The results provide an invaluable depository of genes that could be specifically involved in spermatogenesis and fertilization.

In order to identify genes enriched in spermatogonial stem cells, we determined gene expression profiles of cultured rat germ cells using culture conditions under which male germ cells lose (on STO fibroblasts) or maintain (on MSC-1 Sertoli cells) stem cell activity. Numerous germ cell transcripts strikingly decrease in relative abundance as a function of testis age or culture time on STO feeder cells, but only a subset of approximately 250 genes remain elevated when cultured on MSC-1 feeder cells. If specific gene expression regulates stem cell activity, some or all of these transcripts are candidates to be such regulators. Based on this list of marker genes we established a spermatogonial stem cell index that reliably predicts relative stem cell activity in rat and mouse testis cell cultures. These molecular markers now provide a reliable and rapid means by which to

define spermatogonial stem cells in culture and could alleviate the need for laborious testicular transfers, the current means by which to define spermatogonial stem cell character. This will most certainly accelerate the development of advanced culture conditions. Expansion of spermatogonial stem cells *in vitro* would allow genomic modifications of germ cells and could make targeted gene disruption possible in animals other than the mouse.

Interestingly, when comparing the genes enriched in spermatogonial stem cells to genes that were identified to be commonly enriched in other types of stem cells, e.g. embryonic, neural, or hematopoietic, we found very little overlap. Part of the discrepancies may be explained by technical differences or insufficient purity of the individual stem cell populations. Other studies however suggest that there may be very few commonalities in gene expression between different types of stem cells. Whether there actually is a common set of genes enriched in all stem cells remains questionable at this point.

Furthermore, we gathered expression data of approximately 20,000 genes in the mouse testis at several time points during postnatal development. Removing transcripts found in cultures enriched in Sertoli or testicular interstitial cells yielded a germ cell-enriched transcript profile. Cluster analysis of these transcripts revealed more than 1500 genes whose transcript abundance increased markedly during and after meiosis. Remarkably, nearly 20% of these genes appear to be expressed only in the male germ line. Germ cell-specific transcripts are much less common earlier in germ cell development. Further analysis of the NCBI Unigene database coupled with quantitative real-time PCR indicates that approximately 4% of the mouse genome is dedicated to expression in postmeiotic male germ cells. Approximately two thirds of these genes are still fully uncharacterized. Most or many of the protein products of these transcripts are probably retained in mature spermatozoa. Targeted disruption of 19 of these genes has indicated that a majority has roles critical for normal fertility. Thus, we find an astonishing number of genes expressed specifically by male germ cells late in development. This extensive group provides a plethora of potential targets for germ cell-directed contraception and a staggering number of candidate proteins that could be critical for fertilization.

Oligonucleotide microarrays proved to be the ideal tool to study gene expression of spermatogonial stem cells and differentiating germ cells. Compared to similar studies performed with different methods, e.g. cDNA microarrays, differential display, or SAGE, we generated by far the most comprehensive collection of gene expression data. These data will prove extremely valuable in the quest for a spermatogonial culture system and in the unraveling of spermatogenesis and fertilization.