

## Candidacy Projects

### Project 1

Recent studies have provided a database (GEISHA) of the microRNAs (miRNAs) expressed in the chicken embryo. These findings have given a starting point for investigating early embryonic miRNA function and expression profiling. We will attempt to gain an understanding of the biological role of miRNAs expressed during embryogenesis by way of antisense 2' OM-ORN-mediated depletion and loss-of-function phenotype analysis *in vivo*. MiRNAs are non-coding RNAs that play a role in the regulation of gene expression, particularly during different developmental stages. MiRNAs regulate gene expression at the post-transcriptional level and have been found to be important for cell proliferation and differentiation in early developmental stages. We will examine a few selected miRNAs from the 135 miRNAs mapped for expression in the first 5 days of embryogenesis. Results from these experiments will add to the growing knowledge on the presence and function of miRNAs in avian embryogenesis.

### Project 2

Both male and female embryos express chicken estrogen receptor (cER) transcripts before differentiation of the gonads. It has been demonstrated that the cER transcript is subject to repression in the male gonad at the onset of sexual differentiation in the chicken embryo. The proposed project is to determine if the silencing of cER in the male gonad is achieved through epigenetic alterations. We want to investigate the possible role of DNA methylation and histone acetylation. This can be done by transfecting cells with an estrogen receptor promoter/luciferase expression vector and dosing with Sssl used as a CpG methylase, 5-azacytidine which causes DNA demethylation, and TSA which is a histone acetylation inhibitor. After administration of reagents, levels of luciferase expression/repression can be observed. A large number of studies are finding that epigenetic alterations such as DNA methylation, histone modifications, and chromatin remodeling during development are important in the control of gene expression and tissue differentiation. However, very little has been done to investigate DNA modifications in the chicken. It seems reasonable to think, based on the changes in cER expression just before sexual differentiation, that the cER in the chicken could be regulated in such a manner.

### Project 3

Recent studies are suggesting that under certain conditions spermatozoa contain specific mRNAs and that these RNAs are transferred to the egg. It is thought that these RNAs affect the development of the offspring or play an unknown role in fertilization. RNAs present in sperm cells are of interest because it has been established that male gametes are transcriptionally silent cells. Residual RNA could be a common feature in the spermatozoa of mammals and the pollen of plants. Spermatozoal RNA has been found in many species including the *Mus* (mouse and murine), and the human. However there is no information on the chicken or any avian model for comparison. This proposal aims to identify the RNA transcripts resident in chicken sperm by a serial analysis of gene expression (SAGE) digital approach. The results will address the universality of male gamete RNAs in vertebrates.