All living things share the basic biological principal that tens of thousands of genes encoded in the DNA (deoxyribonucleic acid) are used at specific developmental stages in response to hormones, nutrients, infections, and other important physiological stimuli to make proteins that determine an animal’s functional status. This genomic response (i.e., gene-use response) of relevant cells to such stimuli thus underlies an animal’s overall physiology and significantly influences economically important traits (phenotypes) of interest in dairy cattle. Thus, individual cells’ usage of various genes at any point in time determines the physiological responses of each dairy cow.

Some of the most important phenotypes that affect dairy farm performance are disease resistance, feed intake, mammary development and milk production, and reproductive efficiency. Great strides have been made by producers to enhance these performance phenotypes through management strategies that presumably optimize immunity, rations, pregnancies, and genetic improvement. However, we have almost no understanding of what the various cells of a cow’s body really need for optimal performance. We also have no idea of what genes are influenced by our management practices, or how we can regulate expression of the most important genes in relevant cell types for improved performance. To change this lack of understanding we need to study all genes potentially expressed in bovine cells under scenarios that are important to dairy production. This new line of study is called animal functional genomics. The function of given cell types are examined according to the variety of genes they express in response to their blood and tissue environments.

The Center for Animal Functional Genomics (CAFG) at Michigan State University is comprised of researchers in the Department of Animal Science. They have collaborated with other laboratories on campus, nationally, and internationally to create bovine-specific functional genomics tools. These tools are used to identify patterns of expressed genes in key cells exposed to relevant stimuli that underlie some of the best and worst phenotypes in dairy cows. Examples are immune...
defense responses of white blood cells during stress and infection, responses of mammary and liver cells to nutrients and growth hormones, and responses of ovarian cells to estrous synchronization and aging. The goals of this article are to introduce readers to functional genomics tools and to show how researchers are beginning to use these tools to identify key phenotype-changing genes in the immune system, digestive system, mammary glands, and ovaries of dairy cows.

cDNA Microarrays (Gene Chips)

The principal tenant of functional genomics research is that a cell’s phenotype is determined by the pattern of genes expressed as messenger ribonucleic acid (mRNA) molecules when the cell responds to various stimuli (top part of Figure 1). To detect these mRNA pattern changes requires access to all possible genes used by the cells of interest. In the laboratory, these genes are attached to a solid material so they can bind complementary mRNA molecules in test cells and be easily visualized. The material of choice in animal science work is a specially treated glass microscope slide spotted with thousands of copied DNA (cDNA) sequences that represent most or all genes potentially used by the cell under various scenarios. The cDNA collections used to spot these so-called cDNA microarrays, or gene chips, are derived from “libraries” of all mRNA molecules present in the cells of interest (see lower part of Figure 1).

To date, the CAFG has created cDNA libraries and microarrays for studies using bovine immune cells, mammary cells, ovarian cells, and cells from all other organ systems. Also, CAFG is the hub for activities of the National Bovine Functional Genomics Consortium (NBFGC) comprised of over 20 researchers from seven US institutions. Recently, CAFG and the NBFGC developed the largest bovine cDNA microarray ever made, containing approximately one third of all genes (over 18,000) present in the bovine genome. The genes included on this and other cDNA microarrays can be viewed at the CAFG web site (http://nbfgc.msu.edu) under the “Search Libraries” bullet, and the cDNA libraries used to create them are described on the Department of Animal Science web site (http://www.ans.msu.edu/research/genomics.html) and elsewhere (1-5).

Faculty and Students Study Physiological Systems

Disease Susceptibility in Newly Calved Dairy Cows - Figure 2 shows an example of how Dr. Jeanne Burton’s laboratory is using CAFG’s microarrays to study gene expression patterns in white blood cells of disease-susceptible cows during the transition from the dry period into early lactation. The cells were purified from blood of cows collected before, at, and after calving and their mRNA molecules were converted to cDNA and stained with red or green fluorescent dyes. The stained cDNA molecules were then mixed together and allowed to compete for binding to their complementary gene sequences spotted on immune cell microarrays. In the example shown in

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**Figure 2.** spots with red fluorescence (shown as solid black spots) highlighted genes that were expressed in the cells at but not before calving, and were thus “turned on” by calving. Green spots (shown as solid gray spots) highlighted genes that were expressed before but not at calving, and were thus “turned off” by calving. When red and green fluorescent dyes are in equal amounts on a gene spot they fluoresce yellow (shown as black and white speckled spots in Figure 2). Thus, yellow spots on the microarray represented genes that were not changed in expression before versus at calving. While these unchanged genes are undoubtedly important to the cells’ disease fighting function they are not as interesting as the affected red and green fluorescing genes because these differentially expressed genes may underlie susceptibility to mastitis and other important peripartum diseases.

Dr. Burton’s group has identified about 300 immune cell genes with changed expression during calving, and has determined that fluctuations in the hormones that regulate calving are responsible for expression changes in approximately 25% of these genes. Cow, nutritional, and management factors...
responsible for the changed expression of remaining genes are still unknown but are being investigated. The ultimate goal of this work is to use the identified immune cell genes as targets for development of new nutritional supplements, drug therapies, and management practices that producers could use to sustain critical infection-fighting functions of the cells during calving stress. Two management practices that have been identified by this work as potentially deleterious to disease resistance in newly calved cows are the early pulling and surgical removal of calves before cows are fully ready to give birth. These practices do not allow the normal changes in blood steroid hormones to occur and as such negatively affect gene expression patterns in immune cells normally needed to protect the cow’s reproductive tract and mammary glands from infection. As a result, affected cows often come down with metritis and (or) mastitis. Ultimately, Dr. Burton’s group also will explore these genes for DNA mutations that may help explain an animal’s inherent ability to resist metritis and mastitis. Such mutations could then serve as molecular genetic markers to guide producers’ selection of bulls used to produce daughters with superior disease-fighting capacity during the transition period.

Pathogenesis of Johne’s Disease – Johne’s disease is of paramount concern to US dairy producers. Johne’s disease is caused by the bacterium, Mycobacterium paratuberculosis (M. paratuberculosis). How the bovine immune system responds to M. paratuberculosis has a profound effect on the outcome of infection and expression of clinical disease. In fact, infected animals begin to exhibit clinical signs of disease when the bacterium causes a detrimental shift in the cow’s immune response away from a protective “killer cell” response to a non-protective antibody response. It is this antibody response that is used in the field to diagnose sick animals. The underlying mechanisms of how M. paratuberculosis inhibits the cow’s killer cell response over time are poorly understood, a fact that limits early diagnosis and control of this fastidious pathogen. In an effort to understand these interactions, Dr. Paul Cousens’ laboratory recently used CAFG’s immune cell cDNA microarrays to study white blood cells, infected gut tissues, and affected lymph nodes from Johne’s disease negative and positive cows. The goal of this work is to identify which genes (and subsequently what proteins) are operating properly and improperly during infection and subsequent tissue damage. Results from these studies have led to the development of a logical model that describes how M. paratuberculosis and the bovine immune system become locked in battle of defense versus disease, a battle that the cow all too often loses. This model opens the door for continued research into mechanisms responsible for the symptoms of Johne’s disease and the shifting immune response to this pathogen. Hopefully, this will lead to better diagnostics, vaccines, and therapies to help fight and control the spread of Johne’s disease in dairy farms.

Mammary Development - Dairy heifers account for 20% of the expenses associated with dairy farming. One way to decrease the cost of raising heifers is to feed them for faster growth and earlier breeding and calving. However, rapid body growth can impair mammary development and reduce future milk production. Dr. Mike VandeHaar and co-workers are using traditional nutritional and endocrinology studies combined with functional genomics to understand the mechanisms whereby high energy intake depresses mammary development. Researchers in his laboratory have shown that the hormone, leptin, produced by fat cells, decreases creation of bovine mammary epithelial cells in culture and in heifers after infusion of leptin into the teat canal. Ongoing studies utilizing mammary and immune cell cDNA microarrays indicate that leptin alters expression of genes that encode key intracellular proteins governing cell creation and thus may be important in reduced mammary development of rapidly growing heifers. Understanding the basic mechanisms governing growth of the mammary system was used to read the microarray a final microarray image was produced. From the color of the spots on this image it was easy to see that expressions of over 300 genes were either turned on (solid black spots) or turned off (solid gray spots) by calving. Black and white speckled spots indicated genes that were unchanged by calving stress; these spots bound equal amounts of both fluorescently stained cDNA molecules.

Figure 2. cDNA microarrays spotted with thousands of immune cell genes were used to study effects of calving stress on gene expression in white blood cells of dairy cows. The mRNA molecules from immune cells of test cows collected before and at calving were stained differentially with fluorescent dyes [shown as Green cDNA (gray above) or Red cDNA (black above) squiggle lines], mixed, and allowed to bind competitively to their complementary gene spots on the microarrays. After a specialized fluorescence detection system was used to read the microarray a final microarray image was produced. From the color of the spots on this image it was easy to see that expressions of over 300 genes were either turned on (solid black spots) or turned off (solid gray spots) by calving. Black and white speckled spots indicated genes that were unchanged by calving stress; these spots bound equal amounts of both fluorescently stained cDNA molecules.
mammary gland prior to puberty will provide the foundation for development of novel nutritional, pharmacological, or transgenic approaches to increase the lifetime productivity and efficiency of dairy cows.

Regulation of Feed Intake - Energy intake is a primary limitation on milk yield, reproduction, and health for genetically superior dairy cows. Dr. Mike Allen and co-workers are using functional genomics to understand how propionate oxidation in the liver regulates feed intake. Propionate, produced from fermentation of feeds by microbes in the rumen, can reduce energy intake compared with other absorbed fuels. Propionate production increases when more starch is fermented in the rumen. Although highly fermentable diets can decrease feed intake, the response is not consistent among cows. In addition, increased diet fermentability can sometimes improve energy intake by increasing energy density in the diet. Two groups of cows that differ markedly in feed intake response to increased diet fermentability have been identified. Liver mRNA from these cows is being used along with CAFG’s large bovine cDNA microarray to compare expression of over 18,000 genes in the test samples. The goal is to detect potential pathways within individual cells related to satisfaction of hunger and feed intake regulation during propionate metabolism. Identification of the key genes related to the regulation of feed intake by propionate is important for development of feeding and grouping strategies to increase energy intake of dairy cows, improving their health and productivity.

Oocyte Development and Reproduction - Reproductive efficiency is a major factor in the economic success of the dairy industry. The ovarian cycle is central to the reproductive process because only mature ovarian follicles release eggs (oocytes) to be fertilized. Functional studies indicate that the oocyte itself plays a key regulatory role in control of ovarian follicle development and the early stages of embryonic development after the oocyte is fertilized. Furthermore, the unique content of the oocyte’s internal fluid (cytoplasm) is absolutely required for successful nuclear transfer (cloning) procedures. Ongoing functional genomics studies in Dr. George Smith’s laboratory are using CAFG’s ovarian cell cDNA microarrays to characterize bovine oocyte expressed genes and identify the as yet unknown genes that regulate folliculogenesis and early embryonic development in cattle. This cDNA microarray technology also is being used to identify molecular markers that are predictive of oocyte quality/competence and ultimately potential for successful pregnancy. Identification of oocyte-expressed genes with important regulatory roles and reliable markers of oocyte competence will be important for development of future technologies to improve reproductive efficiency in dairy cows.

Reproductive Ageing and Fertility in Cows – Decline in fertility as dairy cows age contributes to costs associated with reproductive management and culling for reproductive problems. The decline in fertility as cows age is associated with a rapid loss of ovarian follicles and oocytes within each follicle. Over 90% of the original number of follicles and oocytes present in heifers at birth have degenerated before animals reach 2 to 3 years of age. Whether this rapid loss in number of follicles and oocytes after birth explains the decline in fertility of dairy cows is currently unknown, but suspected. The research program of Dr. Jim Ireland’s laboratory is using CAFG’s ovarian cell and immune cell cDNA microarrays to identify expressed genes in ovaries that regulate the rapid degeneration of ovarian follicles during ageing. Once these death genes are identified and it is understood how they are regulated by factors such as hormones, nutrition, and the ageing process, new therapeutic methods could be developed to improve reproductive life span, and thus fertility, of dairy cows.

Summary
The bovine cDNA libraries and microarrays developed in Michigan State University’s CAFG have enabled animal science researchers to gain a more sophisticated view of the molecules responsible for orchestrating development and function of organs and cells critical to optimal immune defense, mammary development, feed intake, and reproductive performance in dairy cows. There potentially are great practical consequences of applying microarray information to production animal agriculture.

References and Web Resources